Contribution of α-Adrenergic and β-Adrenergic Stimulation to Ischemia-Induced Glucose Transporter (GLUT) 4 and GLUT1 Translocation in the Isolated Perfused Rat Heart

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Abstract—The intracellular signaling mechanism of the ischemia-stimulated glucose transporter (GLUT) translocation in the heart is not yet characterized. It has been suggested that catecholamines released during ischemia may be involved in this pathway. The purpose of this study was to evaluate the contribution of α-adrenoceptors and β-adrenoceptors to ischemia-mediated GLUT4 and GLUT1 translocation in the isolated, Langendorff-perfused rat heart. Additionally, GLUT translocation was studied in response to catecholamine stimulation with phenylephrine (Phy) and isoproterenol (Iso). The results were compared with myocardial uptake of glucose analogue [18F]fluorodeoxyglucose (FDG). Subcellular analysis of GLUT4 and GLUT1 protein on plasma membrane vesicles (PM) and intracellular membrane vesicles (IM) using membrane preparation and immunoblotting revealed that α- and β-receptor agonists stimulated GLUT4 translocation from IM to PM (2.5-fold for Phy and 2.1-fold for Iso, P<0.05 versus control), which was completely inhibited by phentolamine (Phe) and propranolol (Pro), respectively. Plasmaemmal GLUT1 moderately rose after Iso exposure, and this was prevented by Pro. In contrast, ischemia-stimulated GLUT4 translocation (2.2-fold, P<0.05 versus control) was only inhibited by α-adrenergic antagonist Phe but not by β-adrenergic antagonist Pro. Similarly, Phe but not Pro inhibited ischemia-stimulated GLUT1 translocation. GLUT data were confirmed by FDG uptake monitored using bismuth germanate detectors. The catecholamine-stimulated FDG uptake (6.9-fold for Phy and 8.9-fold for Iso) was significantly inhibited by Phe and Pro; however, only Phe but not Pro significantly reduced the ischemia-induced 2.5-fold increase in FDG uptake (P<0.05 versus ischemia). This study suggests that α-adrenoceptor stimulation may play a role in the ischemia-mediated increase in glucose transporter trafficking leading to the stimulation of FDG uptake in the isolated, perfused rat heart, whereas β-adrenergic activation does not participate in this signaling pathway. (Circ Res. 1999;84:1407-1415.)

Key Words: glucose transporter ■ ischemia ■ intracellular signaling ■ heart ■ catecholine

The heart uses various substrates, including fatty acids, glucose, and lactate, for the production of high-energy phosphates. During ischemia, glucose becomes the preferred substrate as glycolysis switches from aerobic to anaerobic conditions. Glucose transport into myocardial cells is mediated by the glucose transporter (GLUT) isoforms GLUT4 and GLUT1. GLUT4, which predominates in heart tissue, was shown to be localized mainly on intracellular membrane vesicles (IM) in the basal state and to translocate to the plasma membrane in response to stimuli such as insulin, contraction, ischemia and hypoxia, or anoxia. GLUT1 in heart is found to a great extent on the plasma membrane, and it is generally considered to be responsible for basal glucose transport. Previously, GLUT1 movement from an intracellular storage pool to the sarcolemma was shown to respond to insulin and to low-flow ischemia. However, the signaling mechanism by which insulin, contraction, and ischemia stimulate translocation of GLUTs is not well understood. In a recent study, it was demonstrated that wortmannin, an inhibitor of phosphatidylinositol-3 kinase (PI3-kinase), has no influence on the ischemia-mediated GLUT4 translocation, in contrast to its inhibitory effect on insulin-mediated GLUT4 translocation in the rat heart. Furthermore, studies in skeletal muscle have shown that separate pools of intracellular GLUT4 may exist, which respond differently to insulin and exercise/contraction. These and other findings suggest the existence of various signaling pathways leading to GLUT4 trafficking, 1 for insulin, another for ischemia/hypoxia, and probably a third one for exercise/contraction.

Thus far, the signaling pathway of the ischemia-induced GLUT translocation has not yet been characterized. It has been reported that catecholamines are released during myocardial ischemia and activate the α- and β-adrenergic, G protein–coupled receptors (for review, see References 9–11). Stimulation of the adrenoceptors results in the regulation of various effector systems, including the α-adrenoceptor–me-
Adrenergic Stimulation in GLUT Translocation

diated release of intracellular Ca²⁺ through the activation of phospholipase C and the generation of 2 second-messenger molecules, inositol-1,4,5-trisphosphate and diacylglycerol. β-Adrenoceptors stimulate adenylcyclase, thus leading to the activation of cAMP-dependent protein kinase A. Additionally, by activating α- and β-receptors, catecholamines also regulate myocardial glucose metabolism. Studies from Clark and Patten and Collins-Nakai et al. in perfused rat hearts have demonstrated that glucose uptake is stimulated by epinephrine. Very recently it was shown that intracellular signaling induced by epinephrine seems to be independent of the activation of the PI3-kinase enzyme, because epinephrine-stimulated glucose transport was not inhibitable by wortmannin. Isoproterenol (Iso), a β-agonist, was also found to stimulate myocardial glucose uptake, an effect that could be prevented by propranolol (Pro).

The effects of catecholamines on glucose transport are, at least partially, explained by the stimulation of GLUT4 translocation as shown in epinephrine- and Iso-perfused rat hearts and in isolated rat cardiomyocytes incubated with phenylephrine (Phy). Contrary to the results mentioned above, other studies showed no stimulatory effects of Iso or epinephrine on myocardial glucose transport. These controversial results also reflect the conflicting findings of catecholamine action on glucose transport in skeletal muscles or adipose cells.

In view of this controversy and the paucity of data available about myocardial GLUT recruitment in response to α- and β-adrenergic stimulation, the present study was designed to investigate the effects of adrenergic agonists (Phy and Iso) and antagonists [phenolamine (Phe) and Pro] on subcellular GLUT4 and GLUT1 distribution and [¹⁸F]fluorodeoxyglucose (FDG) uptake under baseline and postischemic conditions in the isolated Langendorff-perfused rat heart.

Materials and Methods

Percoll and density marker beads were purchased from Pharmacia Biotech. Rainbow-colored protein molecular weight markers were purchased from Amersham. All other chemicals, including Phy, Phe, and Pro, were purchased from Sigma or Merck. [¹⁸F]FDG was synthesized as previously described.

Antibodies

The polyclonal antiserum MC2A raised against a peptide corresponding to the GLUT4 carboxyl terminus (kindly provided by Dr. Maureen Charron, Albert Einstein College of Medicine, New York, NY) and a polyclonal rabbit anti-human, anti-mouse, anti-rat GLUT1 antibody were used for the experiments (Diagnostic International). Polyclonal rabbit anti-rat Na⁺/K⁺-ATPase α₁ fusion protein and ¹²⁵I-labeled IgG were purchased from Biomol, and Amersham, respectively.

Control experiments with α-adrenoceptor and β-adrenoceptor agonists/antagonists were carried out in the isolated, Langendorff-perfused rat heart model to investigate the potential of catecholamines to stimulate myocardial GLUT4 and GLUT1 translocation and to test whether the chosen amount of α- and β-receptor antagonists (10 μmol/L Phe and Pro, respectively) leads to a complete inhibition of GLUT translocation induced by Phy and Iso. On the basis of these experiments, we investigated whether an adrenoceptor stimulation induced by a release of endogenous catecholamines is involved in the ischemia-mediated GLUT translocation and the stimulation of FDG uptake in rat heart by selective inhibition of α- and β-receptors with Phe and Pro.

In this study, α-adrenoceptors and β-adrenoceptors were stimulated by the α-agonist Phy and the β-agonist Iso, respectively. A concentration of 10 μmol/L for both agents was found to give maximal adrenoceptor stimulation in Langendorff-perfused rat hearts reflected by (1) a maximal increase in contractility due to incubation with 10 μmol/L Phy and (2) a maximal response in 2-deoxy-¹[H]glucose uptake stimulated by 10 μmol/L Iso. A dose of 10 μmol/L Phe and 10 μmol/L Pro was chosen for inhibition of catecholamine receptors. This dose was based on studies in perfused rat hearts using 10 μmol/L Phe as α-adrenoceptor inhibitor to characterize specific binding of other receptor ligands to α-adrenoceptors by Scatchard analysis and using 10 μmol/L Pro as β-adrenoceptor inhibitor to show complete blocking of epinephrine-mediated glucose uptake by α-antagonists.

Perfused Rat Heart Model

Male Sprague-Dawley rats weighing 250 to 300 g were fed a standard diet and fasted overnight before the experiment. Hearts were rapidly removed from the anesthetized animals, placed in an ice-chilled Krebs-Henseleit bicarbonate buffer, cannulated via the aorta, and perfused at 37°C with constant pressure (60 mm Hg) according to the Langendorff method. The nonrecirculating Krebs-Henseleit buffer system was oxygenated with 95% O₂/5% CO₂, and contained 10 mmol/L glucose as an energy source. Coronary flow was recorded during perfusion and expressed as milliliters perfusate per gram of tissue per minute. Phy, Iso, Phe, and/or Pro (10 μmol/L) were added to the perfusate where indicated.

FDG Uptake Studies

To evaluate the transport and phosphorylation of exogenous glucose, FDG (500 μCi/L) was added to the perfusate as previously described. FDG accumulation was detected using 2 external detectors placed on opposite sides of the heart and interfaced with a computer. Coincident events were monitored as a function of time. Tissue time-activity curves were decay corrected and normalized to the radioactivity in the perfusate. Slopes were calculated from fitted linear curves as an index of rate of tracer accumulation and expressed as milliliters per gram of tissue per minute.

Perfusion Protocol

Experiments were performed in 3 subgroups that were perfused according to the protocol shown in Figure 1. Control hearts were perfused without further additives to define baseline conditions. In the catecholamine subgroup, catecholamine agonist and antagonist exposure was studied. For this purpose, hearts were treated with 10 μmol/L of either the α- or the β-adrenergic agonist Phy or Iso. To validate the respective receptor blocker, hearts were exposed to the combination of either Phy and Phe or Iso and Pro. Furthermore, 2 groups of hearts were perfused with buffer containing Phe or Pro alone. In the ischemia subgroup, stimulation of adrenoceptors by endogenous released catecholamines was studied in hearts subjected to 15 minutes of no-flow ischemia with reperfusion. Therefore, hearts were treated with either Phe or Pro before and after ischemia and compared with a group that was subjected to ischemia with reperfusion without additives.

A total duration of 70 minutes of perfusion, all hearts were rapidly frozen in liquid nitrogen and stored at −70°C until further analysis for GLUT protein.

Subcellular Membrane Fractionation

To assess myocardial GLUT4 and GLUT1 distribution between the IM and the plasma membrane (PM), rat hearts were subjected to subcellular membrane fractionation, as recently described. Briefly, hearts were minced in bicarbonate buffer (10 mmol/L NaHCO₃, 5 mmol/L NaCl, pH 7.0) and further homogenized by ultradispersion and a glass-glass homogenizer. A sample of the crude homogenate (CH) was saved for protein determination and the remainder centrifuged (8000g). The supernatant was further centrifuged at 44 000g, the pellet discarded, and the liquid phase pelleted at 200 000g, resulting in an IM-enriched fraction. For PM enrichment, the 8000g
binding to protein was visualized by 2 hours of incubation with filters were washed 6 times in TBS and blocked again. Antibody Antibodies were diluted 1:800 in blocking buffer. After incubation, room temperature with antibodies against GLUT4 and GLUT1. 150 mmol/L NaCl and 1% NP-40) and then incubated for 2 hours at in Tris-buffered saline (TBS; 20 mmol/L Tris-HCl, pH 7.4, with binding, nitrocellulose membranes were blocked with 5% skim milk was confirmed by ponceau S staining. To reduce nonspecific sheets 31 using a tank transfer apparatus (Bio-Rad). Blotting integrity was confirmed by ponceau S staining. To reduce nonspecific Radioactivity of labeled bands always layered in the linear range of performed using a phosphor imager (445 SI, Molecular Dynamics). Gel Electrophoresis and Immunoblotting Procedure SDS-PAGE of membrane proteins was performed with slight modification of a previously described method.30 Samples (50 µg) were separated on 10% acrylamide gels and transferred to nitrocellulose sheets31 using a tank transfer apparatus (Bio-Rad). Blotting integrity was confirmed by ponceau S staining. To reduce nonspecific binding, nitrocellulose membranes were blocked with 5% skim milk in Tris-buffered saline (TBS; 20 mM Tris-HCl, pH 7.4, with 150 mM NaCl and 1% NP-40) and then incubated for 2 hours at room temperature with antibodies against GLUT4 and GLUT1. Antibodies were diluted 1:800 in blocking buffer. After incubation, filters were washed 6 times in TBS and blocked again. Antibody binding to protein was visualized by 2 hours of incubation with 125 I-labeled IgG (1:500 in TBS with 10% rat serum). Documentation and quantification of specific immunoblabeled protein bands was performed using a phosphor imager (445 SI, Molecular Dynamics). Radioactivity of labeled bands always layered in the linear range of the detector.

Membrane Fractionation Data Analysis
Total protein recovery of PM- and IM-enriched fractions was calculated as a percentage of protein compared with the protein content in CH. Total GLUT4 and GLUT1 recovery in PM and IM and relative percentage of GLUT protein in PM and IM was calculated according to Reference 3. For determination of GLUT4 protein, the complete band doublet shown on the gel was taken into account. Na⁺,K⁺-ATPase subunit α₁ enrichment was expressed as the x-fold amount found in PM compared with IM.

Statistical Analysis
All values are expressed as mean±SD. For evaluation of statistical significance for each parameter, the ANOVA F test was used. In case of significance, the Student t test was used for 2-group comparisons. P<0.05, corrected by the Bonferroni-Holm procedure, was considered significant.

Results
Fractionation of Plasma and Intracellular Membrane Vesicles
After tissue homogenization, mean total protein contents of CH ranged from 109.2±12.3 to 119.9±10.0 mg/g heart tissue, reflecting similar heart sizes among all groups (Table 1). Mean total protein yields were 1.0±0.3% for PM and 2.2±0.2% for IM compared with total protein recovery in CH. Total protein recoveries in PM or IM did not differ significantly between groups, as indicated by ANOVA F test (Table 1). Mean recovery of GLUT4 protein in both PM and IM was 22.7±5.6%, and mean recovery of GLUT1 protein was 12.4±2.9% (Table 1). No significant differences of GLUT4 and GLUT1 recoveries, respectively, were found between groups. Mean enrichment of the PM marker Na⁺,K⁺-ATPase subunit α₁ in groups 1 through 10 was 10.3±3.1-fold. The group means differed from 8.77±2.51-fold (Phy+Phe) to 12.38±4.30-fold (ischemia+Pro), but differences did not reach statistical significance (Table 1). Figure 2 shows a representative Western blotting experiment detecting subunit α₁ of Na⁺,K⁺-ATPase in all 3 heart fractions. Specific bands were migrating with an apparent molecular mass of ~97 kDa.

Effects of α-Adrenoceptor and β-Adrenoceptor Stimulation on Myocardial GLUT4 and GLUT1 Translocation
GLUT4
Figure 3A demonstrates a representative immunoblotting experiment with relative GLUT4 distribution between the PM- and the IM-enriched fractions after heart treatment with Phy, Iso, Phy+Phe, and Iso+Pro. (Relative GLUT4 distribution on PM and IM of control hearts is shown in Figure 6.) Antibody binding specifically detected GLUT4 protein of average molecular mass ~43 to 50 kDa, which, as previously described,21 often migrates as a double band. This is most likely due to protein phosphorylation.22 Results show that relative GLUT4 content on PM of control hearts was 16.4±1.8% (Figure 4A). Thirty minutes of 10 µmol/L Phy or Iso perfusion resulted in a 2.5- and a 2.1-fold increase in GLUT4 on PM, respectively (Figure 4A), and a concomitant decrease in GLUT4 on IM. The catecholamine-induced increases in myocardial GLUT4 translocation were completely abolished by the α-antagonist Phe (Phy

Other Assays
Membrane protein concentration was assayed using bicinchoninic acid with BSA solution as standard according to the manufacturer’s instructions (Pierce). Enrichment of the fractions for plasma membrane protein Na⁺,K⁺-ATPase α₁ was assessed by immunoblot analysis with a polyclonal antibody against rat Na⁺,K⁺-ATPase α₁ fusion protein (1:500 in blocking buffer) as described above.
Stimulation, Ischemia, and Inhibition With Phentolamine and Propranolol

**Figure 2.** Western blotting experiment showing Na\(^{+}\), K\(^{+}\)-ATPase subunit \(\alpha\). Fifty micrograms of protein from the fraction enriched with CH, PM, and IM was subjected to SDS gel electrophoresis and blotted onto nitrocellulose. Nitrocellulose membranes were incubated with an antibody against Na\(^{+}\), K\(^{+}\)-ATPase subunit \(\alpha\), and a corresponding band was detected at \(\approx 97\) kDa. A representative experiment is shown.

**Figure 3.** Representative autoradiogram showing GLUT4 and GLUT1 protein in PM and IM. Hearts were treated with 10 \(\mu\)mol/L each of Iso and Pro, Iso alone, Phy and Phe, and Phy alone. Membrane fractions were isolated and subjected to Western blotting according to the protocol. Immunolabeled bands were visualized by \(^{125}\)I-labeled IgG. The corresponding GLUT4 bands migrate slightly above 46 kDa (A), GLUT1 protein was detected slightly below 46 kDa (B).

**TABLE 1.** Characteristics of Rat Heart Membrane Preparation in Controls and in Response to \(\alpha\)-Adrenergic and \(\beta\)-Adrenergic Stimulation, Ischemia, and Inhibition With Phentolamine and Propranolol

<table>
<thead>
<tr>
<th>Group, n Hearts</th>
<th>Rat Heart, g</th>
<th>Wet Weight</th>
<th>Protein Yield CH, mg/g</th>
<th>Protein Yield IM, % of CH</th>
<th>Protein Yield PM, % of CH</th>
<th>GLUT4 Recovery, %</th>
<th>GLUT1 Recovery, %</th>
<th>Na(^{+}), K(^{+})-ATPase, Fold</th>
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<tbody>
<tr>
<td>Control (7)</td>
<td>0.750±0.093</td>
<td>118.6±12.5</td>
<td>2.2±0.3</td>
<td>1.1±0.3</td>
<td>21.2±2.7</td>
<td>12.9±4.8</td>
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<td>Phy (5)</td>
<td>0.680±0.083</td>
<td>118.7±8.6</td>
<td>2.3±0.2</td>
<td>0.9±0.3</td>
<td>21.9±5.4</td>
<td>12.5±1.1</td>
<td>9.48±2.10</td>
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<tr>
<td>Iso (4)</td>
<td>0.692±0.082</td>
<td>116.7±8.2</td>
<td>2.3±0.3</td>
<td>0.8±0.2</td>
<td>21.9±3.1</td>
<td>11.3±2.7</td>
<td>9.90±1.56</td>
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<tr>
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<td>0.673±0.022</td>
<td>119.9±10.0</td>
<td>2.2±0.2</td>
<td>1.1±0.1</td>
<td>23.3±7.6</td>
<td>11.4±1.7</td>
<td>9.43±3.42</td>
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<tr>
<td>Pro (4)</td>
<td>0.830±0.055</td>
<td>111.4±7.5</td>
<td>2.3±0.2</td>
<td>1.0±0.3</td>
<td>22.7±13.8</td>
<td>13.4±3.1</td>
<td>9.40±2.80</td>
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<td>Phy+Phe (4)</td>
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<td>112.5±7.8</td>
<td>2.1±0.2</td>
<td>1.0±0.3</td>
<td>22.3±5.0</td>
<td>11.6±2.4</td>
<td>8.77±2.51</td>
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<tr>
<td>Iso+Pro (3)</td>
<td>0.725±0.033</td>
<td>109.2±12.3</td>
<td>2.3±0.2</td>
<td>1.1±0.3</td>
<td>22.8±7.1</td>
<td>12.5±4.8</td>
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<tr>
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<td>0.831±0.078</td>
<td>113.0±12.7</td>
<td>2.1±0.2</td>
<td>0.9±0.2</td>
<td>23.1±8.2</td>
<td>11.7±3.8</td>
<td>10.9±4.8</td>
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<tr>
<td>Ischemia+Phe (4)</td>
<td>0.775±0.024</td>
<td>110.8±6.4</td>
<td>2.2±0.1</td>
<td>1.0±0.3</td>
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<td>111.2±6.8</td>
<td>2.1±0.1</td>
<td>1.1±0.2</td>
<td>21.5±3.9</td>
<td>12.8±1.4</td>
<td>12.38±4.30</td>
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</table>

Values are mean±SD of the numbers of measurements in parentheses. Wet weight (g) is related to 1 rat heart. Total protein yield in CH is related to 1 g of heart tissue. Total protein recovery in IM and PM is shown as a percentage of protein compared with the CH. GLUT4 and GLUT1 protein recovery in IM and PM was calculated as described.\(^3\) Na\(^{+}\), K\(^{+}\)-ATPase is expressed as the x-fold enrichment in PM vs IM.

response) or the \(\beta\)-antagonist Pro (Iso response) (18.6±3.7% and 16.8±3.1% GLUT4 on PM). Slightly increased plasma-lemmal GLUT4 levels after treatment with the combination Phy and Phe compared with control hearts did not reach statistical significance.

**GLUT1**

Figure 3B shows an example of an immunoblot against GLUT1 protein migrating at \(\approx 46\) kDa. In the basal state, the relative amount of GLUT1 on the plasma membrane is higher than the relative amount of GLUT4. It accounts for nearly 50% of total cellular GLUT1 (41.6±2.8% GLUT1 on PM; Figure 4B). Perfusion with Phy or Iso resulted in moderate increases on PM. Phy led to a slight but not statistically significant increase on PM (46.8±5.5%) and a concomitant decrease on IM, whereas significant GLUT1 translocation was induced by Iso (52.8±5.0%, \(P=0.0004\) versus control hearts) (Figure 4B). Phy or Iso stimulation of GLUT1 translocation was completely inhibited by Phe and Pro, respectively. GLUT1 levels on PM decreased below control levels of untreated hearts (40.0±2.9% in Phy+Phe and 38.5±0.9% GLUT1 on PM in Iso+Pro hearts) (Figure 4B). However, the differences from control hearts were not significant.

**Effects of \(\alpha\)-Adrenergic and \(\beta\)-Adrenergic Inhibition on Ischemia-Stimulated GLUT4 and GLUT1 Translocation in Rat Myocardium**

The inhibition of \(\alpha\)- and \(\beta\)-adrenergic stimulation by 10 \(\mu\)mol/L Phe and Pro (Figures 3 and 4) served as baseline data for further use of this concentration in experiments investigating the contribution of the \(\alpha\)- and \(\beta\)-adrenergic stimulation to the ischemia-induced increase in GLUT translocation.

**GLUT4**

Figure 5 shows an immunoblot of GLUT4 in untreated rat hearts, in rat hearts subjected to 15 minutes ischemia alone or in combination with Phe/Pro, and in rat hearts perfused with Phe or Pro alone. Subjecting rat hearts to a 15-minute no-flow ischemia produced a 2.2-fold increase in GLUT4 on PM (35.6±3.1%, \(P=0.0002\) versus control) (Figure 6A) and a concomitant decrease on IM compared with controls. Pre- and posts ischemic treatment of hearts with \(\alpha\)-antagonist Phe prevented ischemia-induced GLUT4 translocation to PM almost completely (19.5±3.5%) (Figure 6A). GLUT4 on PM was slightly higher compared with untreated hearts. How-
ever, this difference was not statistically significant. In contrast, blockade of β-adrenoceptors with 10 μmol/L Pro, a concentration that was shown to inhibit GLUT translocation induced by Iso (Figure 4), had no effect on the ischemia-stimulated GLUT1 translocation (44.9±0.6% GLUT1 on PM) (Figure 6B). Both Phe and Pro alone did not significantly affect basal GLUT1 on PM (36.9±2.1% and 39.1±4.2%).

FDG Uptake
In addition to GLUT distribution, the effects of catecholamines and their antagonists on myocardial FDG uptake with and without ischemia were investigated. Results are shown in Table 2. Baseline FDG uptake did not differ significantly among all groups. Coronary flow data, recorded in parallel during baseline conditions in all groups, were not significantly different from coronary flow values of the control group (Table 2).

FDG uptake was stimulated by Phy 6.9-fold and by Iso 8.9-fold compared with untreated controls (0.028±0.018 mL·g⁻¹·min⁻¹) (Table 2). The catecholamine-induced increases in FDG uptake were inhibited by Phe and Pro, respectively [P=0.003 (Phy+Phe versus Phy) and P=0.002 (Iso+Pro versus Iso)]. The uptake rates after inhibition were not significantly different from the FDG uptake in the control group. Treatment of hearts with the inhibitors alone did not change FDG uptake (Table 2). The ischemia-mediated 2.5-fold stimulation of FDG utilization was significantly diminished by the α-adrenoceptor antagonist (P=0.002), whereas it was not altered by the β-adrenoceptor antagonist (Table 2), thus confirming the effects of these antagonists on ischemia-induced subcellular GLUT redistribution in this study (Figure 6).

Discussion
The purpose of this study was to investigate the catecholamine- and ischemia-induced signaling mechanisms leading to GLUT translocation in Langendorff-perfused rat hearts. The results indicate that both α-agonist Phy and β-agonist Iso mediate GLUT4 and, in part, GLUT1 translocation. Furthermore, α-antagonist Phe and β-antagonist Pro completely blocked the Phy- and Iso-stimulated GLUT translocation,
GLUT4 translocation and FDG uptake in perfused rat myocardium comparable with that observed after ischemia (Figure 4 and Table 2). This stimulatory effect was inhibited by α-antagonist Phe, which confirms the results of Rattigan et al.17 However, this group published only qualitative, not quantitative, GLUT4 results because of a relatively large cross-contamination in their membrane fractions. The present study used a modified membrane preparation protocol,1 which has been optimized to provide high enrichments and low cross-contamination in both membrane fractions, indicated by the ≈10.3-fold enriched Na⁺,K⁺-ATPase in PM and the comparatively very low detectable Na⁺,K⁺-ATPase in IM fractions (Table 1 and Figure 2). Furthermore, the consistent recovery of GLUT protein among all groups indicates that the effects on translocation shown in this study are not due to variations in GLUT recoveries (Table 1). Thus, with this method, quantification of relative GLUT amounts on PM and IM was highly reproducible, as data of our previous published work3,7 and Figures 4 and 6 show. However, some over- or underestimation of relative GLUT amount on PM cannot be completely excluded, because Na⁺,K⁺-ATPase is still detectable in IM.

Results of cell culture experiments with Phy in isolated cardiomyocytes of Fischer et al18 are in agreement with those of this study. Using the quantification of GLUT4 and GLUT1 on the plasma membrane with the nonpermeant photoaffinity label 2-N-[4-(1-azi-2,2,2-trifluoroethyl)-benzoyl]-1,3-bis(α-mannos-4-yloxy)propyl-2-amine technique, the investigators showed that Phy induced a 1.8- and 1.5-fold increase of GLUT4 and GLUT1 on PM, respectively, compared with baseline levels. Phy stimulation in our study led to no significant increase in GLUT1 on the plasma membrane (Figure 4B). The discrepancies between these results may be explained by a lower baseline level of GLUT1 on PM in their isolated cardiomyocytes, which could be more susceptible to stimulation. However, it is difficult to compare the 2 baseline GLUT1 levels, because Fischer et al18 normalized their GLUT values to baseline values (arbitrarily set at 1) and did not present relative distribution of subcellular GLUT protein.

Regarding the effect of β-agonist Iso on GLUT4 and GLUT1 translocation and FDG uptake, a stimulatory influence on GLUT trafficking to PM and on FDG utilization was shown and was inhibited by the addition of β-antagonist Pro (Figures 3 and 4 and Table 2). This is in agreement with the GLUT4 translocation and the 2.1-fold increase in 2-deoxy[3H]glucose uptake in isolated rat hearts induced with 10 μmol/L Iso described by Rattigan et al.17 Others16,33 have also demonstrated an increase in glucose- and 3-O-[14C]methylglucose uptake on stimulation with 1 or 100 μmol/L Iso in perfused rat hearts or isolated cardiomyocytes. Although Fischer et al18 suggested that Iso concentrations higher than 1 μmol/L may act on glucose transport via α-adrenergic receptor stimulation, the present study showed a complete inhibition of the Iso-induced GLUT translocation by the β-antagonist Pro (Figure 4). Therefore, the present data do not confirm α-adrenergic stimulation by Iso even with concentrations higher than 1 μmol/L.

In summary, our results confirm the notion that glucose transport in the perfused rat heart is stimulated by α- and β-adrenergic stimulation.

**Figure 6.** Effect of Phe and Pro on GLUT translocation in response to ischemia. Rat hearts subjected to 15 minutes of ischemia (I) combined with 10 μmol/L each of Phe (I+Phe) and Pro (I+Pro) or either inhibitor alone (Phe or Pro) were analyzed for subcellular GLUT distribution as described in Materials and Methods. Relative amounts of GLUT4 (A) and GLUT1 (B) protein found in the fraction enriched in plasma membranes is shown. Values are mean ± SD of number of measurements in parentheses. *Significantly different from the control group (C) (P<0.05).
β-adrenergic mechanisms. Furthermore, it was demonstrated that 10 μmol/L Phe and 10 μmol/L Pro provided selective and complete inhibition of the α- and β-adrenergic stimulation of GLUT translocation in the isolated rat heart.

Involvement of an Endogenous Catecholamine Release and Subsequent Adrenergic Stimulation in Ischemia-Triggered GLUT Translocation and FDG Uptake

We have reported that ischemia and insulin induce translocation of GLUT4 in rat myocardium.3 To investigate whether insulin and ischemia share a common signaling mechanism leading to GLUT4 translocation, PI3-kinase, a key enzyme in the insulin-induced signaling pathway, was inhibited using wortmannin. It was shown that in contrast to insulin, GLUT4 translocation and FDG uptake were stimulated by ischemia in rat heart independently of PI3-kinase.7 Additionally, in a canine heart study, GLUT1 was also shown to translocate to the sarcolemma in response to low-flow ischemia,6 which supports our findings that GLUT1 translocation is increased by 15-minute no-flow ischemia (Figure 6B). The responsible signals in the ischemia-induced pathway are not known. Because catecholamines have been shown to stimulate glucose uptake and translocation of GLUT, it is conceivable that the catecholamine released by the myocardium during ischemia is responsible for the ischemia-induced effects on GLUT translocation and glucose transport.

Although the inhibition of β-receptors with Pro prevented the Iso-stimulated increase in GLUT translocation to PM (Figure 4), it did not have a significant effect on the ischemia-stimulated GLUT translocation (Figure 6) and FDG uptake (Table 2), which suggests no involvement of β-adrenergic receptors in the ischemia signaling pathway. Stimulation of β-adrenoceptors is known to increase cAMP, which further leads to activation of the cAMP-dependent protein kinase A. This kinase phosphorylates proteins that are involved in positive chronotropic and inotropic mechanisms of the myocardium.11 Thus, our results with Pro suggest that the mechanisms involved in ischemia-mediated GLUT translocation and FDG uptake are independent of cAMP.

In contrast to Pro, Phe inhibited the ischemia-induced increase in myocardial GLUT translocation and FDG uptake, indicating for the first time the role of α-adrenergic stimulation in the ischemia-mediated effects on GLUT4 translocation (Figures 5 and 6A) and FDG utilization (Table 2). However, on the basis of the data presented, it is not yet clear what role α-adrenoceptors have in the translocation process of GLUT1.

The signaling pathway by which α-adrenergic activation stimulates GLUT4 translocation and glucose transport is not known. Recently, it was shown that α-adrenergic mechanisms in cardiomyocytes involve the activation of ras, a small GTP-binding protein, which stimulates the mitogen-activated protein kinase cascade.34 It is possible that the ras/mitogen-activated protein kinase pathway is involved in the ischemia-mediated GLUT translocation, because ras overexpression in adipocytes led to a partial translocation of GLUT4 in the absence of insulin.35 Also, 3T3-L1 fibroblasts expressing an active mutant (Lys-61) N-ras protein exhibited a 3-fold

### TABLE 2. Coronary Flow and FDG Uptake in Perfused Rat Hearts Before and After Intervention

<table>
<thead>
<tr>
<th>Catecholamine Exposure</th>
<th>Group, n</th>
<th>Baseline coronary flow</th>
<th>Baseline FDG uptake</th>
<th>Postintervention FDG uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control (4)</td>
<td>Phy (5)</td>
<td>Phy + Phe (5)</td>
</tr>
<tr>
<td>Control coronary flow</td>
<td></td>
<td>10.20 ± 1.21</td>
<td>12.46 ± 1.65</td>
<td>10.90 ± 2.38</td>
</tr>
<tr>
<td>Baseline FDG uptake</td>
<td></td>
<td>0.021 ± 0.014</td>
<td>0.029 ± 0.017</td>
<td>0.018 ± 0.011</td>
</tr>
<tr>
<td>Postintervention FDG uptake</td>
<td></td>
<td>0.028 ± 0.018</td>
<td>0.193 ± 0.044*</td>
<td>0.044 ± 0.018</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ischemia With Reperfusion</th>
<th>Group, n</th>
<th>Ischemia (5)</th>
<th>Ischemia + Phe (5)</th>
<th>Ischemia + Pro (4)</th>
<th>Phe (5)</th>
<th>Pro (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline coronary flow</td>
<td></td>
<td>9.20 ± 0.85</td>
<td>10.62 ± 1.26</td>
<td>9.88 ± 1.51</td>
<td>11.10 ± 0.97</td>
<td>10.82 ± 1.13</td>
</tr>
<tr>
<td>Baseline FDG uptake</td>
<td></td>
<td>0.022 ± 0.012</td>
<td>0.016 ± 0.002</td>
<td>0.017 ± 0.005</td>
<td>0.031 ± 0.019</td>
<td>0.023 ± 0.012</td>
</tr>
<tr>
<td>Postintervention FDG uptake</td>
<td></td>
<td>0.070 ± 0.012</td>
<td>0.038 ± 0.012$</td>
<td>0.060 ± 0.004</td>
<td>0.020 ± 0.004</td>
<td>0.022 ± 0.010</td>
</tr>
</tbody>
</table>

*P=0.003, †P=0.002, ‡P=0.002, §P=0.007, vs control; $P=0.0016 vs ischemia.

Coronary flow during baseline conditions, baseline FDG uptake, and postintervention FDG uptake are shown as milliliters of perfusate per gram rat heart tissue per minute (mL · g⁻¹ · min⁻¹). Values are mean ± SD of the numbers of measurements in parentheses.
increase in 2-deoxyglucose uptake compared with nontransfected cells.

Another mechanism possibly involved in the ischemia-mediated GLUT4 translocation is the increased production of 1,4,5-inositol triphosphate, found in response to α-adrenergic G protein activation and leading to the liberation of diacylglycerol. Diacylglycerol is known to activate protein kinase C, which was suggested to induce GLUT4 translocation independent of the insulin-stimulated pathway. In this context, it is of interest to note that the stimulation of α-adrenoceptors by endogenous catecholamines through the activation of protein kinase C is also suggested to be involved in ischemic preconditioning in the heart. This was shown by blockade of α1-adrenoceptors with prazosin or pretreatment with pertussis toxin, which abolished the effects of ischemic preconditioning in isolated perfused rat hearts. Another study with protein kinase C antagonists, which inhibited α1-adrenoceptor–induced preconditioning, confirmed this result. The data suggest an important role for α1-adrenoceptors in the cardioprotective effects of preconditioning at least in the rat heart, and it is conceivable that the increased glucose transport, which is part of the beneficial effect of ischemic preconditioning, is mediated by the ischemia-induced α1-adrenoceptor stimulation.

Limitations

The isolated rat heart model used did not allow direct measurements of left ventricular pressure. There was no balloon inserted in the left ventricle to properly assess contractile function. Because no preload exists in these experimental hearts, the Starling curve is shifted to the left. Therefore, this condition cannot be compared with the physiological working state in vivo, because the energy requirements are likely to differ substantially, especially in the setting of ischemia and reperfusion.

In this study, 10 μmol/L Phe was used to achieve complete inhibition of α1-adrenoceptors, and the question may arise whether Phe at that dose affects effector systems other than α1-adrenoceptors. However, perfusion with Phe alone did not alter GLUT and FDG data compared with controls. This observation and the frequent use of 10 μmol/L Phe or even higher doses (up to 100 μmol/L) for selective inhibition of α-adrenergic responses in heart justify the application of this concentration in the experiments.

Catecholamines are known to cause positive chronotropic and inotropic effects in the myocardium. Changes in inotropic state can increase GLUT4 translocation. Thus, it cannot be excluded that the exogenous administered or the endogenous released (during ischemia) catecholamines stimulate GLUT translocation or FDG uptake through increasing contractile activity. Because the contractile function was not assessed in our model, possible influences of changes in the inotropic state cannot be excluded. Moreover, Bihler and Sawh showed that adrenaline increases the uptake of [14C]-labeled 3-O-methylglucose in arrested rat left atria, and Fischer et al reported a catecholamine-mediated [3H]deoxyglucose uptake in quiescent, isolated rat cardiomyocytes independent of a contraction-evoked stimulus. These observations demonstrated that the catecholamine stimulation of glucose uptake may not necessarily depend on changes in contractility. However, direct and indirect effects of catecholamines on translocation are difficult to separate in this model.

The results of this study were obtained in the Langendorff rat heart model perfused with glucose as a sole substrate. Therefore, the results may not be extended to conditions existing in working heart models or to in vivo conditions. The Langendorff-perfused heart is a well-established low-workload model, which is hemodynamically stable with glucose as the sole energy source (Table 2). In the glucose-perfused rat heart model, competition with other substrates such as fatty acids or lactate is avoided. Previous work in our laboratory has reproducibly demonstrated the enhanced glucose utilization in the ischemia-reperfusion protocol using this model.

Although an established isolated rat heart perfusion model was used, the observations were obtained under artificial conditions with respect to the low cardiac work and the perfusate substrate composition. The advantages of this model include standardization of hemodynamic and metabolic conditions before and after intervention (glucose as the only substrate), but this model clearly limits the extrapolation of the observed findings to the in vivo setting and the more physiological situation of patients with ischemic heart disease.

Conclusion

Our results confirm findings of earlier studies that myocardial GLUT translocation and FDG uptake is stimulated by α- and β-adrenergic agonists. Furthermore, the findings suggest, for the first time, that α1-adrenoceptor stimulation may be involved in ischemia-mediated signaling leading to increased FDG uptake, GLUT4, and possibly GLUT1 translocation, whereas β-adrenergic mechanisms seem to not have an effect on FDG uptake and GLUT translocation stimulated by ischemia. The activation of G proteins coupled to α1-adrenoceptors may probably be the first step in the signaling cascade mediating GLUT translocation and glucose transport. However, the molecular mechanism, by which ischemia-activated α1-adrenoceptors trigger redistribution of GLUTs and thus increase glucose uptake, remains to be elucidated.

Acknowledgments

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Contribution of α-Adrenergic and β-Adrenergic Stimulation to Ischemia-Induced Glucose Transporter (GLUT) 4 and GLUT1 Translocation in the Isolated Perfused Rat Heart
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