Original Article

SGK1 Kinase Upregulates GLUT1 Activity and Plasma Membrane Expression

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Phosphatidylinositol 3-kinase (PI3 kinase) inhibition disrupts the ability of insulin to stimulate GLUT1 and GLUT4 translocation into the cell membrane and thus glucose transport. The effect on GLUT4 but not on GLUT1 is mediated by activation of protein kinase B (PKB). The serum- and glucocorticoid-inducible kinase SGK1, a further kinase downstream of PI3 kinase, regulates several transporters by enhancing their plasma membrane abundance. GLUT1 contains a consensus site (^{95}Ser) for phosphorylation by SGK1. Thus, the present study investigated whether GLUT1 is regulated by the kinase. Tracer-flux studies in *Xenopus* oocytes and HEK-293 cells demonstrated that GLUT1 transport is enhanced by constitutively active ^{S422D}SGK1. The effect requires the kinase catalytical activity since the inactive mutant ^{K127N}SGK1 failed to modulate GLUT1. GLUT1 stimulation by ^{S422D}SGK1 is not due to de novo protein synthesis but rather to an increase of the transporter's abundance in the plasma membrane. Kinetic analysis revealed that SGK1 enhances maximal transport rate without altering GLUT1 substrate affinity. These observations suggest that SGK1 regulates GLUT1 and may contribute to or account for the PI3 kinase–dependent but PKB-independent stimulation of GLUT1 by insulin. *Diabetes* 55:421–427, 2006

Insulin stimulates glucose transport in hormone-responsive tissues mainly by inducing the redistribution of the facilitated hexose carrier isoforms GLUT1 (SLC2A1) and GLUT4 (SLC2A4) from intracellular compartments to the plasma membrane (1–3). The cascade of signaling events involved in glucose transporter trafficking to the cell surface in response to insulin is triggered by an increase in insulin receptor tyrosine kinase activity followed by tyrosine phosphorylation of insulin receptor substrate proteins and activation of phosphatidylinositol 3-kinase (PI3 kinase). Downstream elements of PI3 kinase include the phosphoinositide-dependent kinase PDK1, which in turn phosphorylates and thus activates the serine/threonine kinase Akt/protein kinase B (PKB) (4–6).

The role of PI3 kinase in insulin-dependent and -independent stimulation of GLUT1 and GLUT4 translocation has been confirmed by several studies using pharmacological (Wortmannin and LY294002) blockade and genetic (PI3 kinase dominant-negative mutants) knockout of the kinase (7–11). The effect of PI3 kinase on GLUT4 trafficking is mediated by PKB (12,13). PKB is, however, at least in some cells, not required for the PI3 kinase–dependent trafficking of GLUT1 (12). Thus, some other PI3 kinase–dependent protein kinases are presumably involved in the regulation of GLUT1.

A further downstream molecule in the PI3 kinase signaling cascade is the serum- and glucocorticoid-inducible kinase SGK1. SGK1 was originally cloned as a glucocorticoid-sensitive gene from rat mammary tumor cells (14) and later as a human cell volume–regulated gene (15). SGK1 shares ~80% homology with its isoforms SGK2 and SGK3 (16) and ~60% homology with PKB (17).

To become catalytically active, SGK1 requires phosphorylation through the PI3 kinase/PDK1 pathway (16–18). Through this pathway, SGK1 and its isoforms are activated by growth factors such as IGF-1 and insulin (19–21), by oxidative stress (18,20), and by exposure to hyperosmolarity (22). The enzymatic activity of SGK1 is disrupted upon inhibition of PI3 kinase (18).

SGKs have been shown to stimulate several transport proteins including the Na^+,K^+,$2Cl^-$ cotransporter NKCC2 (23); the Na^+/K^+ ATPase (24,25); the dicarboxylate transporter NaDCT1 (26); the amino acid transporters SN1 (27), EAAT1 (28), EAAT3 (29), and EAAT4 (30); and the glucose transporter SGLT1 (31). Similar to protein kinase A, the kinases have been suggested to play a broad function in transport regulation (32). The GLUT1 amino acid sequence contains a potential SGK1 phosphorylation site at position ^{95}Ser. Thus, the present work explored whether GLUT1 is regulated by SGK1.

In this study, we show that SGK1 modulates GLUT1 activity by enhancing GLUT1 abundance in the plasma membrane without altering total GLUT1 expression levels. The results suggest that the kinase acts as a signaling molecule downstream of PI3 kinase involved in GLUT1 trafficking to the cell surface.

RESEARCH DESIGN AND METHODS

Constructs and site-directed mutagenesis. The constitutively active human ^{S422D}SGK1 (16) and inactive human ^{K127N}SGK1 (16) were kindly provided by Sir Philip Cohen (University of Dundee, Dundee, Scotland). The mutated human GLUT1 transporter ^{S95A}GLUT1 was generated by site-directed mutagenesis (QuikChange site-directed mutagenesis kit; Stratagene, Heidelberg, Germany) according to the manufacturer's instructions. The following prim-

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2-DOG, 2-deoxy-D-glucose; GADPH, glyceraldehyde-3-phosphate dehydrogenase; HEK, human embryonic kidney; PI3 kinase, phosphatidylinositol 3-kinase; PKB, protein kinase B.

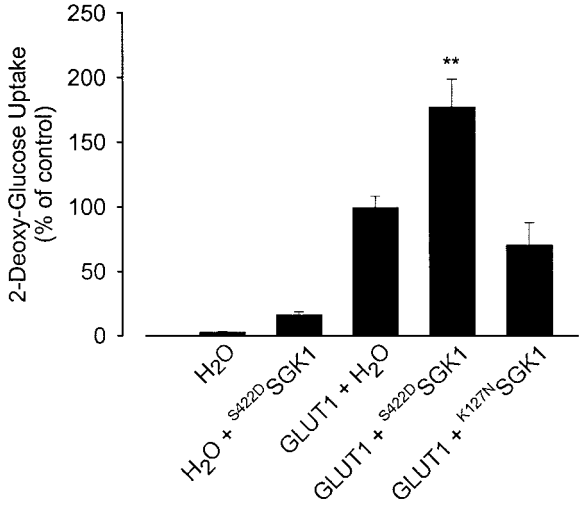

FIG. 1. ^{S422D}SGK1 enhances GLUT1-mediated glucose transport in *Xenopus* oocytes. *Xenopus* oocytes were injected with GLUT1 with or without constitutively active ^{S422D}SGK1 and inactive ^{K127N}SGK1 or the kinase alone. Four days after cRNA injection, labeled 2-DOG uptake was studied as a measure of GLUT1 activity. Arithmetic means ± SE. *Statistically significant difference to uptake in *Xenopus* oocytes expressing GLUT1 alone ($P < 0.01$). Uptake values were normalized in each batch of oocytes to the mean value obtained in oocytes expressing GLUT1 alone.**

ers were used: S95AGLUT1 s: 5′ CTTTGGCCGGCGGAATGCAATGCTGATGA TGAAC 3′ and S95AGLUT1as: 5′ GTTCATCATCAGCATTGCATTCCGCCGGC CAAAG 3′. The ^{S95A}GLUT1 mutant was sequenced to verify the presence of the desired mutation.

Expression in *Xenopus laevis* oocytes. cRNA encoding human GLUT1 (33), ^{S95A}GLUT1, human constitutively active ^{S422D}SGK1, inactive ^{K127N}SGK1 (20), and human Nedd4-2 were synthesized in vitro as described previously (34). Dissection of *Xenopus laevis* ovaries and collection and handling of the oocytes have been described in detail elsewhere (34). Oocytes were injected with 1 ng GLUT1 or ^{S95A}GLUT1 and/or 7.5 ng ^{S422D}SGK1, ^{K127N}SGK1, or Nedd4-2. H_2O-injected oocytes served as control.

Cell transfections. Human embryonic kidney (HEK)-293 cells were incubated in Dulbecco's modified Eagle's medium containing 4.5 g/l glucose, 10% FCS, and 1% penicillin-streptomycin (Invitrogen, Karlsruhe, Germany). Cells were seeded on six-well plates at 0.2×10^6 cells/well. After 24 h, cells were transfected with 2 μg pIRES2EGFP-^{S422D}SGK1 or pIRES2EGFP-^{K127N}SGK1 by using Lipofectamine (Invitrogen) according to the manufacturer's instructions. As a control, cells were transfected with 2 μg pIRES2EGFP empty vector (Clontech, Heidelberg, Germany).

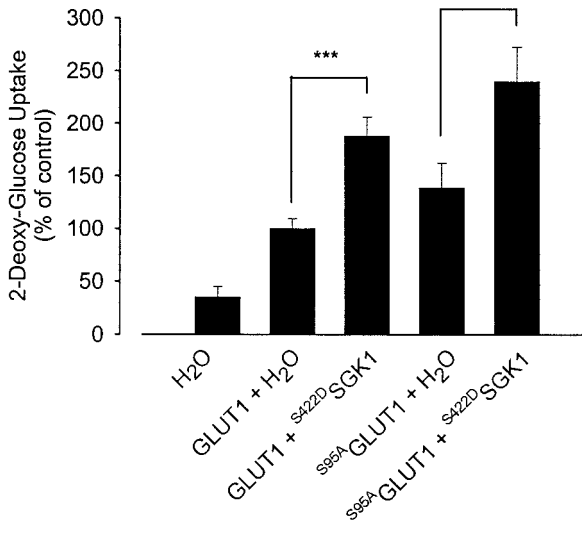

FIG. 2. Disruption of putative SGK phosphorylation site on GLUT1 does not prevent transporter stimulation. *Xenopus* oocytes were injected with wild-type GLUT1 or ^{S95A}GLUT1 with or without constitutively active ^{S422D}SGK1. Four days after cRNA injection, labeled 2-DOG uptake was studied. Arithmetic means ± SE. *Statistically significant difference to uptake in *Xenopus* oocytes expressing wild-type GLUT1 or ^{S95A}GLUT1 alone (*$P < 0.05$, ***$P < 0.005$). Uptake values were normalized to the mean value obtained in oocytes expressing wild-type GLUT1 alone.

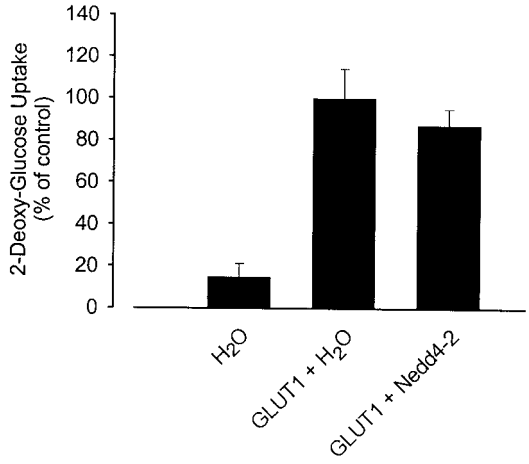

FIG. 3. Glucose transport is unaffected upon Nedd4-2 coexpression. *Xenopus* oocytes were injected with GLUT1 with or without Nedd4-2. Four days after cRNA injection, labeled 2-DOG uptake was studied. Arithmetic means ± SE. Uptake values were normalized in each batch of oocytes to the mean value obtained in oocytes expressing GLUT1 alone.

Isolation of mice adipocytes. Adipocytes were isolated from 129/SvJ mice epididymal fat pads by collagenase digestion as previously described (35). The fat pads were minced and digested with 2 mg/ml type II collagenase for 1 h at 37°C in Krebs Ringer bicarbonate HEPES buffer (120 mmol/l NaCl, 4 mmol/l KH_2PO_4, 1 mmol/l $MgSO_4$, 1 mmol/l $CaCl_2$, 10 mmol/l $NaHCO_3$, 200 nmol/l adenosine, and 30 mmol/l HEPES, pH 7.4) containing 1% fraction V BSA. The resulting cell suspension was filtered through a nylon mesh (250 μm) and washed three times with Krebs Ringer bicarbonate HEPES buffer containg 3% BSA. Then adipocytes were resuspended in Krebs Ringer bicarbonate HEPES buffer with 3% BSA. An aliquot of the final cellular suspension was taken to measure lipocrit and cell number.

Transport studies. Tritium-labeled 2-deoxy-D-glucose (2-DOG) was used as the glucose analog for uptake determination. In *Xenopus* oocytes, the transport assay was performed 4 days after cRNA injection and contained 10–15 single oocytes in 0.25 ml ND96 (96 mmol/l NaCl, 2 mmol/l KCl, 1.8 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, and 5 mmol/l HEPES, pH 7.4) containing 1 μCi ^{3}H 2-DOG and 50 μmol/l (or the indicated amount in the kinetic analysis) of unlabeled 2-DOG. After incubation for 30 min at room temperature with

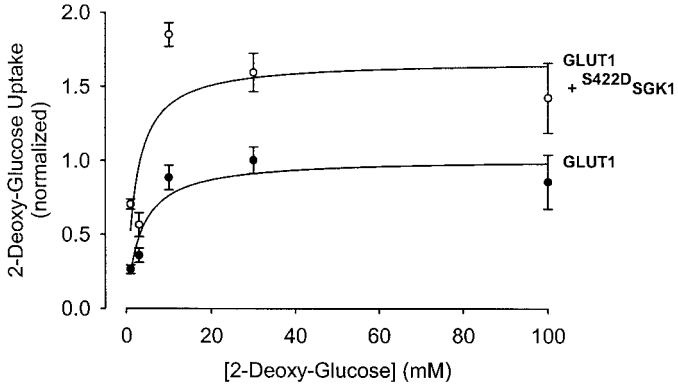

FIG. 4. GLUT1 maximal transport rate is enhanced by ^{S422D}SGK1. *Xenopus* oocytes were injected with water or with GLUT1 with or without constitutively active ^{S422D}SGK1. Four days after cRNA injection, 2-DOG uptake was studied at the indicated substrate concentrations. Uptake values of water-injected *Xenopus* oocytes were substracted and results were normalized in each batch of oocytes to the V_{max} obtained in oocytes expressing GLUT1 alone. Arithmetic means ± SE.

TABLE 1
Kinetic parameters of GLUT1 upon coexpression of ^{S422D}SGK1 in *Xenopus* cocytes

Injected cRNA	K_m (mmol/l)	V_{max} (normalized)
GLUT1	3.13 ± 0.99	1.00 ± 0.01
GLUT1 + ^{S422D}SGK1	2.17 ± 1.00	1.66 ± 0.16 (*$P < 0.05$)

Data are means ± SE.

2-DOG (linear range of uptake), uptake was terminated by washing the oocytes four times with 3 ml ice-cold PBS containing 100 mmol/l unlabeled 2-DOG. Oocytes were individually transferred into scintillation vials and dissolved by adding 200 μl of 10% SDS before the radioactivity was determined. In HEK-293 cells, 2-DOG uptake was measured 2 days after transfection by incubating the cells at 37°C for 5 min (linear range of uptake) in glucose-free Krebs Ringer HEPES buffer containing ^{3}H 2-DOG 0.1 μCi/well and 0.3 mmol/l cold 2-DOG with or without 0.1 mmol/l phloretin. Uptake was terminated by rapid aspiration of uptake solution and washing four times with ice-cold PBS containing 50 mmol/l unlabeled 2-DOG. Thereafter, cells were lysed with 10 mmol/l NaOH/0.1% Triton X-100, and radioactivity incorporated into the cells was measured with a liquid scintillation counter. Protein concentrations were determined by the Bradford method. For kinetic analysis, 2-DOG uptake was measured by incubating the cells with ^{3}H 2-DOG 0.1 μCi/well and various concentrations of unlabeled 2-DOG at 37°C for 5 min. In isolated adipocytes, 2-DOG uptake was measured by incubating the cells with 0.1 μCi ^{3}H 2-DOG and 0.1 mmol/l cold 2-DOG with or without 0.1 mmol/l phloretin. Uptake was terminated after 5 min by centrifugation of cells through dinonylphthalate. The separated cells were removed from the top of the oil layer, and cell-associated radioactivity was quantified.

Detection of cell surface expression by chemiluminescence. Defolliculated oocytes were first injected with constitutively active SGK1 (15 ng/oocyte) and 1 day later with GLUT1 (1 ng/oocyte). Oocytes were incubated with 2 μg/ml primary goat polyclonal anti-GLUT1 antibody (Santa Cruz Biotechnology, Heidelberg, Germany), which recognizes an extracellular GLUT1 sequence, and 4 μg/ml secondary, peroxidase-conjugated donkey anti-goat IgG antibody (Santa Cruz Biotechnology, Heidelberg, Germany) for the analysis of GLUT1 protein abundance in the cell membrane. Individual oocytes were placed in 20 μl of SuperSignal ELISA Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL), and chemiluminescence was quantified by luminometry by integrating the signal over a period of 1 s.

Western blotting. To determine whole-cell GLUT1 and SGK1 expression in oocytes, transfected HEK-293 cells, and adipocytes, cells were homogenized in lysis buffer, and 30 μg protein were separated on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane. After blocking with 5% nonfat dry milk in PBS/0.15% Tween 20 for 1 h at room temperature, blots were incubated overnight at 4°C with a goat anti-GLUT1 antibody (diluted 1:100 in PBS/0.15% Tween 20/5% nonfat dry milk; Santa Cruz Biotechnology), a rabbit anti-SGK1 antibody (diluted 1:1,000 in PBS/0.15% Tween 20/5% nonfat dry milk; Upstate, Dundee, U.K.), or a rabbit anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH) horseradish peroxidase–conjugated antibody (diluted 1:1,000 in PBS/0.15% Tween 20/5% nonfat dry milk). GAPDH was used to demonstrate equal protein loading. Secondary peroxidase-conjugated donkey anti-goat IgG antibody (diluted 1:2000 in PBS/0.15% Tween 20/5% nonfat dry milk; Santa Cruz Biotechnology) or sheep anti-rabbit IgG antibody (diluted 1:1,000 in PBS/0.15% Tween 20/5% nonfat dry milk; Amersham, Freiburg, Germany) was used for chemiluminescent detection of GLUT1 or SGK1 with an enhanced chemiluminescence kit (Amersham), respectively. Band intensities were quantified using Quantity One Analysis software (Biorad, Munich, Germany).

Calculations. Data are provided as means ± SE, and n represents the number of oocytes investigated. All experiments were repeated with at least three batches of oocytes. In all repetitions, qualitatively similar data were obtained. All data were tested for significance using ANOVA, and only results with *$P < 0.05$, **$P < 0.01$, and ***$P < 0.005$ were considered as statistically significant.

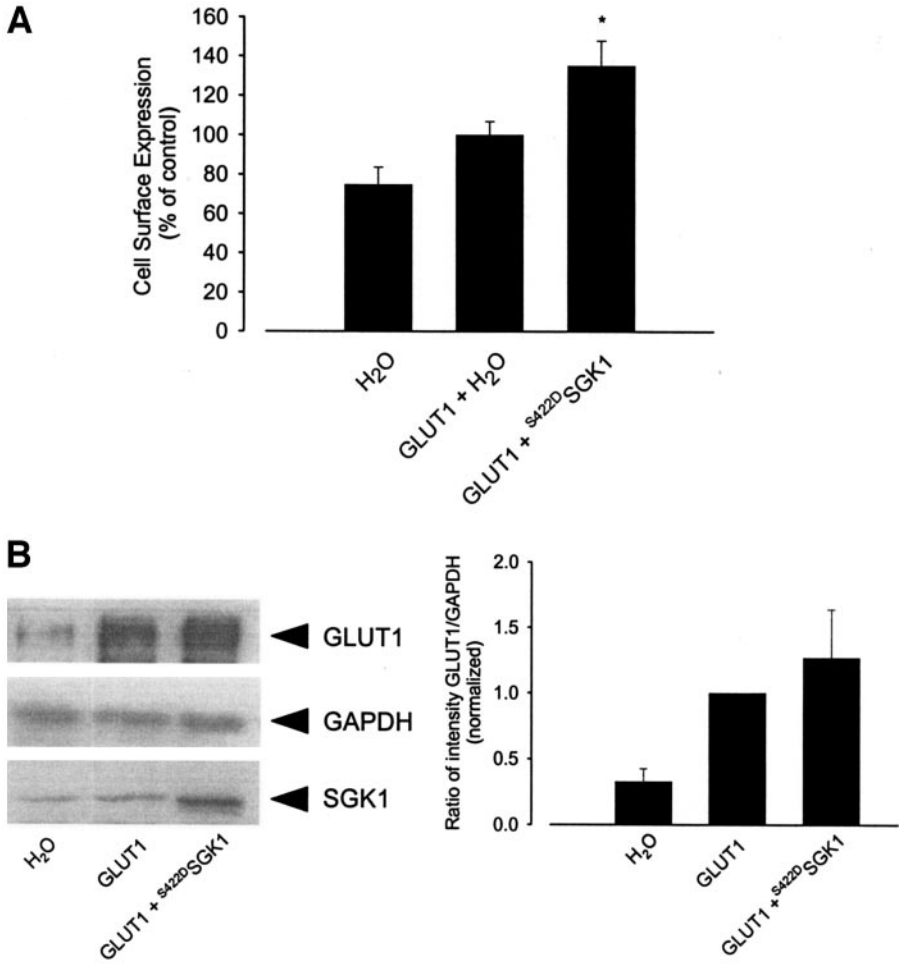

FIG. 5. ^{S422D}SGK1 upregulates GLUT1 plasma membrane abundance without affecting total GLUT1 expression levels. Surface (*A*) and total (*B*) GLUT1 expression was assessed by surface chemiluminescence and whole-cell lysates' western blotting in oocytes expressing GLUT1 alone or GLUT1 together with ^{S422D}SGK1. Additional Western blots were performed to demonstrate proper ^{S422D}SGK1 expression. Arithmetic means ± SE. *Statistically significant difference between oocytes injected with GLUT1 alone and those injected with GLUT1 and ^{S422D}SGK1 (*$P < 0.05$). Cell surface expression was normalized in each batch of oocytes to the mean relative light units value obtained in oocytes expressing GLUT1 alone. For Western blotting, GLUT1/GAPDH band intensities from three independent experiments were normalized in each batch to the mean value of GLUT1/GAPDH band intensity of oocytes expressing GLUT1 alone.

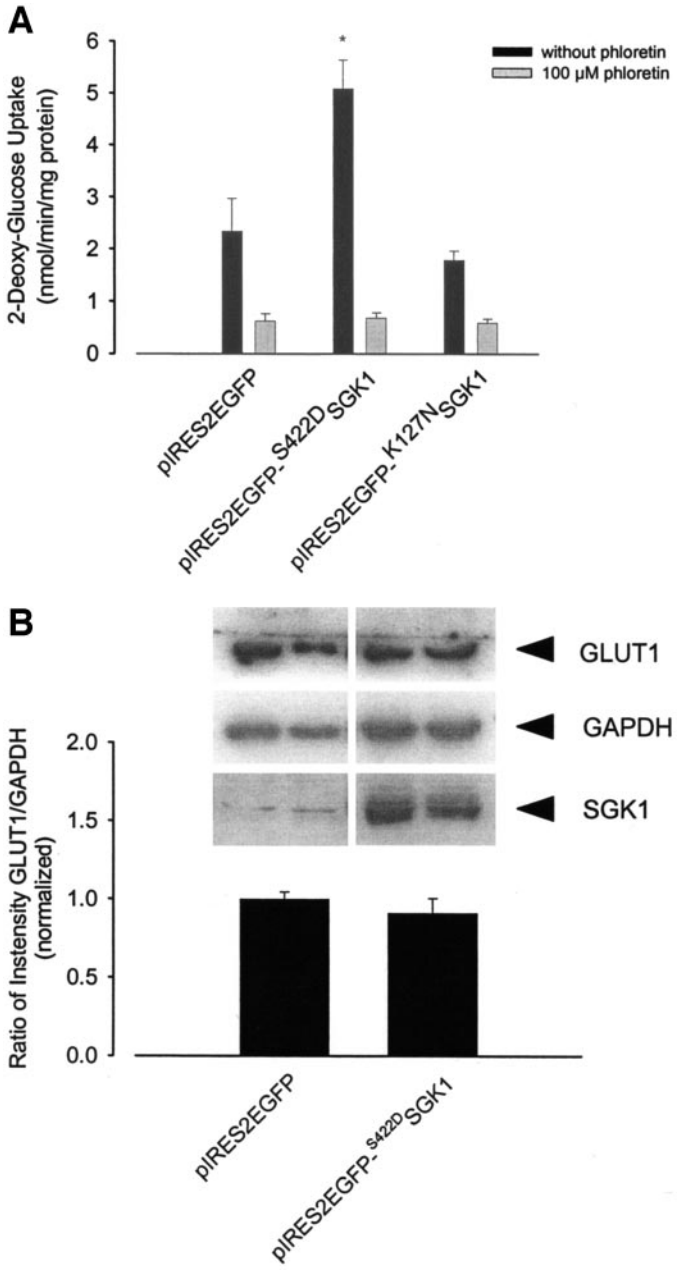

FIG. 6. ^{S422D}SGK1 enhances 2-DOG transport in HEK-293 cells without affecting total GLUT1 expression levels. HEK-293 cells were transfected with pIRES2EGFP-^{S422D}SGK1, pIRES2EGFP-^{K127N}SGK1, or empty vector, and 2 days later labeled 2-DOG uptake was studied in the presence and absence of GLUT1 inhibitor 0.1 mmol/l phloretin (*A*) or Western blots of whole-cell lysates were performed (*B*). Arithmetic means ± SE. *Statistically significant difference to uptake in HEK-293 cells transfected with the empty vector (*$P < 0.05$). For Western blotting, GLUT1/GAPDH band intensities from three independent experiments were normalized in each transfection to the value of GLUT1/GAPDH band intensity of oocytes expressing GLUT1 alone.

RESULTS

To investigate the role of SGK1 in the modulation of GLUT1 activity, GLUT1 was expressed in *Xenopus laevis* oocytes and glucose transport was measured in the presence and absence of the protein kinase. Tracer-flux studies revealed an increase in GLUT1 transport rate (177.1 ± 21.3% of control, $n = 10$, Fig. 1) upon coexpression of ^{S422D}SGK1. Glucose uptake into water-injected oocytes or oocytes expressing constitutively active ^{S422D}SGK1 alone was <6.3 ± 2.3% of control ($n = 26$–37). GLUT1 upregulation by SGK1 was dependent on the catalytic activity of the kinase, as the inactive mutant ^{K127N}SGK1 did not significantly modify the transporter (70.6 ± 17.2% of control, $n = 8$).

TABLE 2
Kinetic parameters of GLUT1 upon transfection of pIRES2EGFP-^{S422D}SGK1, pIRES2EGFP-^{K127N}SGK1, or empty vector into HEK-293 cells

Transfected DNA	K_m (mmol/l)	V_{max} (nmol · min^{-1} · mg protein^{1})
plRES2EGFP	0.95 ± 0.44	17.74 ± 2.31
plRES2EGFP-^{S422D}SGK1	2.89 ± 0.85	44.93 ± 4.40 ($P < 0.05$)
plRES2EGFP-^{K127N}SGK1	2.30 ± 1.38	22.37 ± 4.81

Data are means ± SE.

To test whether SGK1 modulates GLUT1 uptake by phosphorylating GLUT1, the putative phosphorylation site at ^{95}Ser in GLUT1 was mutated into alanine (^{S95A}GLUT1). Disruption of the putative SGK phosphorylation site did not prevent GLUT1 stimulation by ^{S422D}SGK1 (239.8 ± 32.7% of control, $n = 46$), suggesting that the kinase is effective through phosphorylation of an intermediate protein (Fig. 2).

Some of the transporters modulated by SGKs (31,36,37) are indirectly regulated through phosphorylation and thus inhibition of the ubiquitin ligase Nedd4-2, which otherwise tags its target protein for degradation. To assess whether GLUT1 is modulated by Nedd4-2, the transporter was expressed in *Xenopus* oocytes and transport activity was measured in the presence and absence of the ubiquitin ligase. Nedd4-2 coexpression did not significantly affect GLUT1 activity (87.2 ± 7.8% of control, $n = 15$, Fig. 3). Thus, the ubiquitin ligase is apparently not involved in the regulation of GLUT1.

A detailed kinetic analysis of GLUT1 revealed that ^{S422D}SGK1 enhances GLUT1 by modifying the maximal transport rate, V_{max}, without affecting substrate affinity (Fig. 4 and Table 1).

GLUT1 plasma membrane abundance was studied with or without SGK1 coexpression. Chemiluminescence experiments revealed that GLUT1 surface expression was higher in oocytes coexpressing ^{S422D}SGK1 (134.9 ± 12.8%, $n = 50$) than in oocytes expressing GLUT1 alone ($n = 53$, Fig. 5*A*). Thus, GLUT1 modulation by ^{S422D}SGK1 is due to increased transporter abundance in the plasma membrane. Enhanced GLUT1 plasma membrane expression is not due to induced GLUT1 de novo protein synthesis as indicated by western blotting of whole-cell lysates (Fig. 5*B*).

GLUT1 modulation by SGK1 was also observed in mammalian cells (HEK-293). HEK-293 cells were transfected with pIRES2EGFP-^{S422D}SGK1, pIRES2EGFP-^{K127N}SGK1, or with empty vector (as a control), and 2 days later 2-DOG uptake was measured in the presence and absence of GLUT1 inhibitor phloretin (0.1 mmol/l). Tracer-flux studies revealed an increase in GLUT1 transport rate (from 2.3 ± 0.6 [$n = 3$] to 5.1 ± 0.6 nmol · min^{-1} · mg protein^{-1} [$n = 3$], Fig. 6*A*) upon coexpression of ^{S422D}SGK1. As observed in *Xenopus* oocytes, GLUT1 upregulation by SGK1 was dependent on the catalytic activity of the kinase, as the effect was lacking upon expression of the catalytical inactive mutant ^{K127N}SGK1 (from 2.3 ± 0.6 [$n = 3$] to 1.8 ± 0.2 nmol · min^{-1} · mg protein^{-1} [$n = 3$]).

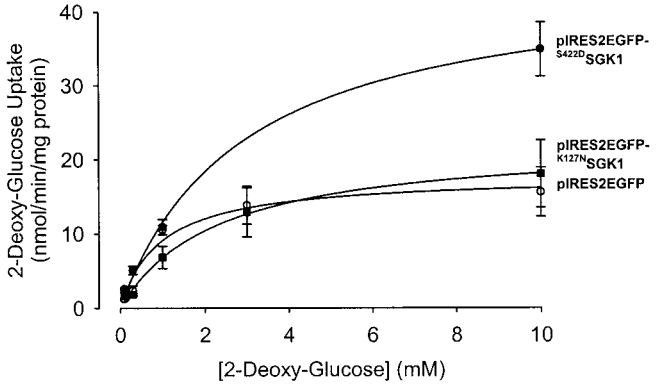

FIG. 7. GLUT1 maximal transport rate is enhanced upon ^{S422D}SGK1 transfection into HEK-293 cells. HEK-293 cells were transfected with pIRES2EGFP-^{S422D}SGK1, pIRES2EGFP-^{K127N}SGK1, or empty vector. Two days after transfection, labeled 2-DOG uptake was studied at the indicated substrate concentrations. Arithmetic means ± SE.

Western blotting of whole-cell lysates indicated that the kinase fails to induce GLUT1 protein synthesis (Fig. 6*B*).

Kinetic analysis of 2-DOG uptake in transfected HEK-293 cells revealed that ^{S422D}SGK1 enhances GLUT1 by modifying the maximal transport rate, V_{max} (from 17.7 ± 2.3 [n = 3] to 44.9 ± 4.4 nmol · min^{-1} · mg protein^{-1} [n = 3]), thereby corroborating the results obtained in the *Xenopus* oocyte expression system (Fig. 7 and Table 2).

Tracer-flux studies in adipocytes isolated from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice demonstrated the role of SGK1 in glucose transport regulation. As depicted in Fig. 8*A*, 2-DOG uptake was lower (55.67 ± 16.46%) in adipocytes isolated from *sgk1*$^{-/-}$ mice compared with *sgk1*$^{+/+}$ mice despite similar total GLUT1 expression levels (Fig. 8*B*).

DISCUSSION

Similar to what has been shown for the Na^+-coupled glucose transporter 1 (SGLT1) (31), SGK1 stimulates the facilitated glucose transporter GLUT1 by enhancing transporter abundance in the plasma membrane. The effect requires the catalytical activity of the kinase. SGK1 shuttles between cytoplasm and nucleus in a stimulus-dependent manner (38). The serum-induced nuclear import of SGK1 suggests that SGK1 acts at a transcriptional level. In fact, SGK1 has been shown to modulate the forkhead transcription factor FKHRL1 (39). Thus, SGK1 might regulate glucose transport in part by increasing GLUT1 transcript levels. However, the stimulation of glucose transport in *Xenopus* oocytes point to posttranscriptional regulation of GLUT1, as GLUT1 mRNA has been injected thus circumventing transcription. In addition, Western blotting of whole-cell lysates exclude GLUT1 regulation at a translational level.

SGK1 is not effective through phosphorylation of GLUT1, since the Ser-to-Ala mutation in the SGK consensus site failed to prevent SGK-mediated stimulation of glucose transport. Several channels and transporters are indirectly regulated through phosphorylation of the ubiquitin ligase Nedd4-2, which decreases the affinity of the enzyme to its target molecules and thus delays the clearance of the transport proteins from the cell membrane (31,36,37). As coexpression of Nedd4-2 did not alter GLUT1 activity, we exclude this mechanism of SGK action.

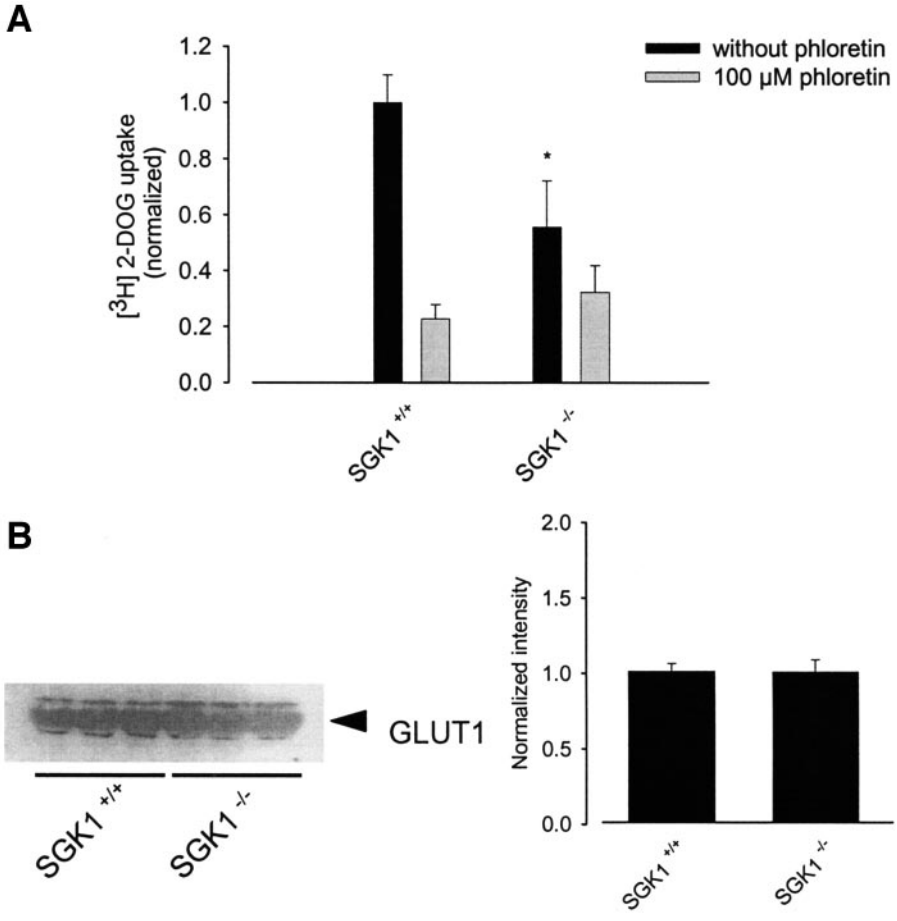

FIG. 8. Glucose transport is reduced in adipocytes isolated from *sgk1*$^{-/-}$ mice. Adipocytes were isolated from *sgk1*$^{+/+}$ and *sgk1*$^{-/-}$ mice and 2-DOG uptake studied in the presence and absence of 0.1 mmol/l phloretin (*A*) or Western blots of whole-cell lysates were performed (*B*). Arithmetic means ± SE. *Statistically significant difference to uptake in adipocytes isolated from *sgk1*$^{+/+}$ mice (P* < 0.05). For Western blotting, GLUT1 band intensities from three independent experiments were normalized to the value of GLUT1 intensity in adipocytes isolated from *sgk1*$^{+/+}$ mice.**

Phosphorylation-dependent events have previously been shown to modulate GLUT1 activity. Protein kinase C isozymes PKCβII, -δ, and -ϵ stimulate GLUT1 by recruiting GLUT1 transporter to the cell membrane (40), and the AMP-activated protein kinase stimulates GLUT1 without affecting the total amount of GLUT1 protein at the cell surface (41). The AMP-activated protein kinase downstream target(s) have not yet been identified.

According to our results, SGK1 could play a similar role in the regulation of GLUT1 as PKB plays in the regulation of GLUT4. In some studies on adipocytes, constitutively active PKB has been able to upregulate GLUT4 but not GLUT1 (12). Insulin, however, upregulated both GLUT1 and GLUT4, and both effects were abrogated by inhibition of PI3 kinase (12). On the other hand, dominant inhibitory PKB has been shown to inhibit the insulin-mediated increase in GLUT1 protein abundance (42), and PKB has been reported to stimulate GLUT1 translocation to the cell membrane (43), suggesting a role of PKB in the regulation of GLUT1. Moreover, insulin may (12) or may not (44) stimulate GLUT1. Thus, the regulation of GLUT1 may depend on the experimental condition and may be supported by PKB and/or SGK1. It should be kept in mind that the transcription of SGK1 is highly variable and subject to a number of regulators (5), and thus SGK1-dependent effects may be similarly variable. GLUT1 modulation by SGK1 reported in this study is not restricted to the *Xenopus* oocyte expression system, as similar results were obtained in cultured mammalian cells. The observed reduced glucose transport in adipocytes isolated from *sgk1*$^{-/-}$ mice points to a physiological role of the kinase in glucose transport regulation.

SGK-dependent stimulation of GLUT1 transporters may be of relevance in the cellular uptake of glucose into several tissues including the brain and defective SGK dependent regulation of GLUT1 may contribute to the GLUT1 deficiency syndrome characterized by a defect in GLUT1 translocation to the plasma membrane (45). Notably, SGK1 expression is upregulated by cerebral ischemia (46) and may help to enhance cellular glucose uptake into the ischemic tissue. Moreover, the enhanced cerebral SGK1 expression in enriched environment (47) may serve to adjust glucose uptake to the enhanced demand following stimulation of neuronal activity. As a matter of fact, SGK1-dependent regulation of glucose transport is reflected by delayed glucose uptake into several tissues in the SGK1 knockout mouse following an intraperitoneal glucose tolerance test (K.M. Boini, A.M. Hennige, D.Y. Huang, B. Friedrich, M.P., C.B., F. Grahammer, F. Artunc, H. Osswald, P. Wulff, D. Kuhl, V. Vallon, H.U. Hăring, F.L., unpublished observations).

In conclusion, the facilitated glucose transporter GLUT1 is modulated by the serum- and glucocorticoid-inducible kinase SGK1. The kinase stimulates GLUT1 by enhancing the transporter plasma membrane abundance and is effective through phosphorylation of a hitherto unknown intermediate protein. The SGK1-dependent regulation of GLUT1 may participate in the adjustment of cellular glucose uptake to the demand.

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