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Yacir Benomar, Nadia Naour, Alain Aubourg, Virginie Bailleux, Arieh Gertler, et al.. Insulin and leptin induce Glut4 plasma membrane translocation and glucose uptake in a human neuronal cell line by a phosphatidylinositol 3-kinase-dependent mechanism. *Endocrinology*, 2006, 147 (5), pp.2550-2556. 10.1210/en.2005-1464 . hal-02656878

HAL Id: hal-02656878

<https://hal.inrae.fr/hal-02656878>

Submitted on 30 May 2020

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Insulin and Leptin Induce Glut4 Plasma Membrane Translocation and Glucose Uptake in a Human Neuronal Cell Line by a Phosphatidylinositol 3-Kinase-Dependent Mechanism

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The insulin-sensitive glucose transporter Glut4 is expressed in brain areas that regulate energy homeostasis and body adiposity. In contrast with peripheral tissues, however, the impact of insulin on Glut4 plasma membrane (PM) translocation in neurons is not known. In this study, we examined the role of two anorexic hormones (leptin and insulin) on Glut4 translocation in a human neuronal cell line that express endogenous insulin and leptin receptors. We show that insulin and leptin both induce Glut4 translocation to the PM of neuronal cells and activate glucose uptake. Wortmannin, a specific inhibitor of phosphatidylinositol 3-kinase, totally abolished insulin- and leptin-dependent Glut4 translocation and stimulation of glucose uptake. Thus, Glut4 translocation is a phosphatidylinositol 3-kinase-dependent mechanism in neuronal cells. Next, we investigated the impact of chronic insulin and leptin treatments on Glut4 expression and translocation.

Chronic exposure of neuronal cells to insulin or leptin down-regulates Glut4 proteins and mRNA levels and abolishes the acute stimulation of glucose uptake in response to acute insulin or leptin. In addition, chronic treatment with either insulin or leptin impaired Glut4 translocation. A cross-desensitization between insulin and leptin was apparent, where exposure to insulin affects leptin-dependent Glut4 translocation and *vice versa*. This cross-desensitization could be attributed to the increase in suppressor of cytokine signaling-3 expression, which was demonstrated in response to each hormone. These results provide evidence to suggest that Glut4 translocation to neuronal PM is regulated by both insulin and leptin signaling pathways. These pathways might contribute to an *in vivo* glucoregulatory reflex involving a neuronal network and to the anorectic effect of insulin and leptin. (*Endocrinology* 147: 2550–2556, 2006)

GLUCOSE IS AN essential substrate for cerebral oxidative metabolism and is transported into neurons and glial cells via facilitative glucose transporters (1–4). Glucose transporters are membrane-spanning proteins constituted, so far, by 14 isoforms (Glut1–Glut14), and at least three isoforms are expressed in neuronal cells. The predominant isoforms in neuronal cells are Glut3 (5, 6), the insulin-responsive Glut4 (7–10), and the recently cloned Glut8 (11–13). Glut 2 has been also identified in several brain nuclei and was mostly located on glial cells and to a lesser extent in neurons (14, 15).

Glut3 and Glut1 are considered the main glucose transporter isoforms in the brain. Glut1 is mostly located at the blood-brain barrier, including in the choroids plexus and in

microvessels (16). Glut3 is mainly expressed in neurons of the cortex, hippocampus, and cerebellum and facilitates a continuous supply of glucose to neurons (17).

Glut4 and Glut8 isoforms are insulin-responsive glucose transporters in the peripheral tissues (11, 18). Their expression was also reported in the brain, but their plasma membrane (PM) translocation in response to insulin is still a matter of controversy. Glut8 is localized in the intracellular cytosolic compartment, and glucose or insulin stimulation induced its translocation to endoplasmic reticulum but not to PM (19, 20). It has been also recently demonstrated that Glut8 translocation is not responsive to insulin in neuronal cells (21).

Several studies have reported Glut4 mRNA and protein expression in various regions of the brain including the olfactory bulb, hippocampus, cortex, cerebellum, and hypothalamus (8, 22, 23). Glut4 translocation to the PM of neuronal cells in response to insulin is not yet demonstrated.

Glucose uptake from blood to the brain parenchyma involves Glut1 (24, 25) and enters neuron through Glut3 and Glut4 (and may be Glut8). Glut3 transporter is essential for providing the glucose to the brain in a hormonal-independent manner to ensure its metabolic function, and this has been largely documented (5, 6). In contrast, the role of Glut4,

First Published Online February 23, 2006

Abbreviations: 2-DOG, 2-Deoxyglucose; ECL, enhanced chemiluminescence; GK, glucokinase; Glut, glucose transporter; IRS, insulin receptor substrate; JAK, Janus kinase; PI 3-kinase, phosphatidylinositol 3-kinase; PM, plasma membrane; Q-RT-PCR, quantitative RT-PCR; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; TM, total membrane.

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

which is considered to be insulin dependent in peripheral tissues, is still unclear, and its possible involvement in the neuronal glucosensing is a reasonable hypothesis.

Recent reports have colocalized Glut4 and insulin receptor in glucose-excited neurons of the hypothalamic ventromedial nucleus, which are considered glucosensing neurons (26). As in peripheral tissue, insulin activates insulin receptor substrate (IRS)/phosphatidylinositol 3-kinase (PI 3-kinase) signaling pathway in the hypothalamus. The inhibition of this signaling cascade abolished the anorexic effect of insulin (27). In insulin-sensitive tissues such as adipose tissue or skeletal muscles, Glut4 responds to insulin signaling by translocation to the PM allowing increased glucose uptake (28, 29). Although Glut4 is colocalized with the insulin receptor in the hypothalamus and other areas of brain, Glut4 association with hypothalamic action of insulin is still unknown.

We have recently shown that both insulin and leptin receptors are expressed in a human neuronal cell line, SH-SY5Y (30–32), and that leptin and insulin both activate Janus kinase (JAK) 2/signal transducer and activator of transcription (STAT)-3 and IRS/PI 3 kinase signaling pathways (33). To investigate whether insulin and leptin affect Glut4 translocation and glucose transport, we have used SH-SY5Y human neuronal cells. Here we show that both insulin and leptin activate the translocation of Glut4 to the PM but are without effect on translocation of Glut3. Furthermore, we show that Glut4 translocation and glucose uptake are PI 3 kinase-dependent in this cellular model. To our knowledge, this is the first study in a neuronal cell system demonstrating that Glut4 is translocated to the PM in response to two anorexic hormones: insulin and leptin. This finding might contribute to the understanding of the role of neuronal Glut4 in *in vivo* glucoregulatory reflex involving a neuronal network and the anorectic hormones: insulin and leptin.

Materials and Methods

Chemicals

DMEM, fetal bovine serum, L-glutamine, penicillin, streptomycin, and other cell culture reagents were obtained from Life Technologies, Inc. (Invitrogen, Cergy Pontoise, France). BSA (fraction V radioimmunoassay grade), leupeptin, aprotinin, wortmannin, all *trans*-retinoic acid, and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Premade polyacrylamide solution Protogel was from National Diagnostics (Prolabo, Paris, France). Antibodies raised against Glut-4 and Glut-3 from human origin were from Santa Cruz Biotechnology (Tebu, France). 2-Deoxy-D-[1, 2-³H]glucose was purchased from ICN (Vannes, France). Nitrocellulose membranes were from Euromedex (Mundolsheim, France). Human leptin was produced as previously described (34). Briefly, a plasmid encoding human leptin was prepared and over-expressed in *Escherichia coli* as insoluble inclusion bodies. Those were isolated and refolded, and the human leptin was subsequently purified to homogeneity by ion-exchange chromatography yielding electrophoretically pure, over 95% monomeric biologically active protein.

Cell culture and stimulation

SH-SY5Y human neuroblastoma cells (kindly provided by Dr. B. Dufy, Centre National de la Recherche Scientifique, Bordeaux II, France) were grown in DMEM supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin in 5% CO₂ atmosphere at 37°C; differentiation of SH-SY5Y cells was achieved by treatment with retinoic acid. Differentiated cells

were used after 15 d of retinoic acid treatment to obtain a high percentage of cells that showed a clear morphological differentiation. Chronic insulin and leptin treatment of differentiated SH-SY5Y was performed as previously described previously (33) with minor modifications. Briefly, serum-starved cells were incubated for 16 h at 37°C in serum-free DMEM in presence or absence of leptin (15 nM) or insulin (100 nM). After washing, cells were stimulated for 15 min at 37°C with or without insulin (100 nM), leptin (15 nM), or the combination of both hormones. Where indicated, wortmannin (1 µM) was added to the medium during 30 min and 15 min before the beginning of hormonal stimulation.

2-Deoxyglucose uptake measurement

The SH-SY5Y cells were seeded in six-well dishes and differentiated as described previously (33). Differentiated SH-SY5Y cells were serum starved for 16 h, and then incubated in presence or absence of insulin (100 nM) or leptin (15 nM) for 16 h. Cells were then rinsed twice with modified DMEM lacking glucose, pyruvate, and serum and incubated in the same medium for 30 min at 37°C with or without insulin (100 nM), leptin (15 nM) or the combination of both hormones. Glucose transport was initiated by the addition of 100 µM 2-deoxy-D-[³H]glucose (0.33 µCi/dish) for the last 10 min; and wortmannin (1 µM) was added 15 min before the addition of 2-deoxy [³H]glucose. Finally, cells were washed three times with ice-cold PBS, lysed in 1 N NaOH, and the cell-associated radioactivity was counted using liquid scintillation counter. Aliquots of cell lysates were saved for the determination of proteins content.

Immunocytochemistry

Differentiated SH-SY5Y cells were starved in serum-free DMEM for 16 h and stimulated for 15 min at 37°C with or without insulin (100 nM), leptin (15 nM), or the combination of both hormones. After washing in PBS, the cells were fixed for 30 min in 2% paraformaldehyde-PBS, washed three times in PBS, and blocked in 5% normal rabbit serum and 0.2% gelatin fish. Cells were then incubated with goat polyclonal antibodies raised against the extracellular domain of human Glut-4 or Glut-3. The negative control was prepared by omitting the primary antibody. After extensive washing with PBS, cells were incubated with fluorescein thiocyanate rabbit anti-goat IgG (1:500; Vector Laboratories, Burlingame, CA). All samples were briefly counterstained with 4,6-diamine-2-phenylindole at 1:300 dilution to count the number of nuclei per field of view. Cells were mounted and cover slipped with anti-fade medium (Vector Laboratories). Cell analysis was carried out using a DMRB microscope (Leica Microsystems, Wetzlar, Germany) equipped with a mercury light source and filter system to visualize the green fluorescence. The microscope settings were consistently maintained when comparisons were made.

PM preparation and immunoblotting

After hormonal treatment, differentiated SH-SY5Y cells were washed twice in ice-cold PBS and membrane preparation was performed as previously described (35). Briefly, cells were scraped in buffer A containing 10 mM NaHCO₃ (pH 7.0), 250 mM sucrose, 5 mM Na₂S₂O₈, and 100 µM phenylmethylsulfonyl fluoride. The resulting homogenates were clarified at 1300 × g for 10 min. The supernatant was centrifuged at 9000 × g for 10 min and then at 190,000 × g for 1 h. The resulting pellet (total membrane or TM) was homogenized in buffer A, one aliquot of TM was saved (to estimate total Glut3 and Glut4) and remaining applied on discontinuous sucrose gradients (25, 32, and 35%, wt/wt), and ultracentrifuged at 150,000 × g for 16 h at 4°C. PMs at the 25/32% interphases were recovered, diluted with sucrose-free buffer A and ultracentrifuged at 190,000 × g for 1 h. Pellets of fractions were resuspended in buffer A, and protein content was determined by bicinchoninic acid (BCA)-based method (BCA protein assay; Pierce, Rockford, IL), with BSA as standard. Proteins from the different fractions (50–100 µg) were solubilized for 1 h at room temperature in buffer B (3% sodium dodecyl sulfate, 2 M urea, 1 mM EDTA), treated with Laemmli buffer and separated by SDS-PAGE with 12% resolving gel. The same treatment was applied on TM. Proteins were transferred to nitrocellulose membranes, and immunoblots were blocked with 3% BSA for 1 h at room temperature. After incubation with the appropriate primary and secondary antibodies, nitrocellulose membranes were washed, and targeted pro-

teins were detected using enhanced chemiluminescence reagents (ECL; Amersham Biosciences, Amersham, Buckinghamshire, UK).

Quantification of suppressor of cytokine signaling (SOCS)-3 and Glut4 mRNA expression by quantitative RT-PCR (Q-RT-PCR)

Total RNA from differentiated SH-SY5Y cells was extracted using RNA Insta pure kit (Eurogentec, Seraing, Belgium) according to manufacturer's recommendations. A 1- μ g portion of total denatured RNA was reverse transcribed with 50 U of Moloney murine leukemia virus reverse transcriptase (Ozzyes, Saint Quentin en Yvelines, France) as previously described (33), and the resulting cDNAs were submitted to quantitative PCR analysis. The PCR primers were as follows: SOCS3 sense, 5'AGGAATGTAGCAGCGATGGAA3'; SOCS3 antisense, 5'GC-CCTGTCCAGCCCAATAC3'; Glut4 sense, 5'GGAGCTGGTGTGGT-CAACACA3'; Glut4 antisense, 5'GGAGCAGAGCCACAGTCATCA3'; rpL19 sense, 5'CAATGCCAACTCCCGTCA3'; rpL19 antisense, 5'GCT-GTACCTTCGGTTACCTAT3'. Real-time PCR was carried out using the Roche LightCycler apparatus and the Fast Start DNA Master SYBER Green I kit (Roche Diagnostics, Mannheim, Germany). PCR amplification was performed in triplicates using the following conditions: initial activation of the hot start DNA polymerase for 15 min at 94 C followed by denaturation for 10 sec at 94 C, annealing for 10 sec at 60 C and extension for 10 sec at 72 C. Forty cycles of PCR were programmed to ensure that the threshold crossing point (cycle number) was attained. Fluorescence emission was monitored continuously during cycling. At the completion of cycling, melting curve analysis was carried out to establish the specificity of the amplified product. The level of expression of each mRNA and their estimated crossing point in each samples were determined relative to the standard preparation using the LightCycler computer software (Roche). A ratio of specific mRNA/rpL19 amplification was then calculated, to correct for any differences in efficiency at RT.

Statistical analysis

Statistical analysis was performed using ANOVA (Statview Software program, version 5) (ASAP Software, St. Ouen, France) to detect significant intergroup differences. Values are expressed as means \pm SEM, and $P < 0.05$ was considered statistically significant.

Results

Glut-4 but not Glut-3 translocation is insulin and leptin sensitive

The presence of Glut-4 and Glut-3 proteins was detected in SH SY5Y cells by Western blotting (Fig. 1, A and B) and immunohistochemical analyses (Fig. 2, A and B). In non-stimulated SH-SY5Y cells, Glut 3 was present in the PM and Glut 4 in the cytoplasm. Changes in Glut4 and Glut3 subcellular localization in response to acute stimulation with leptin (15 nM) and/or insulin (100 nM) was next assessed. The presence of GLUT 4 in the PM fraction was significantly increased in response to both hormones alone or combined (Fig. 1A), whereas Glut 3 was unaffected (Fig. 1B). These changes occurred with no alteration of total Glut 3 or Glut 4 proteins in SH-SY5Y cell extracts. Glut 4 translocation from cytoplasm to the PM in response to insulin and/or leptin was confirmed by immunohistochemical analysis (Fig. 2A). By contrast, Glut 3 localization at the PM was similar in unstimulated and stimulated cells (Fig. 2B).

Long-term exposure of SH-SY5Y cells to insulin or leptin down-regulates Glut 3 and Glut 4 expression and abolishes insulin- and leptin-dependent Glut4 translocation

To study the chronic effect of insulin and leptin on Glut3 and Glut4 expression, SH-SY5Y cells were treated with in-

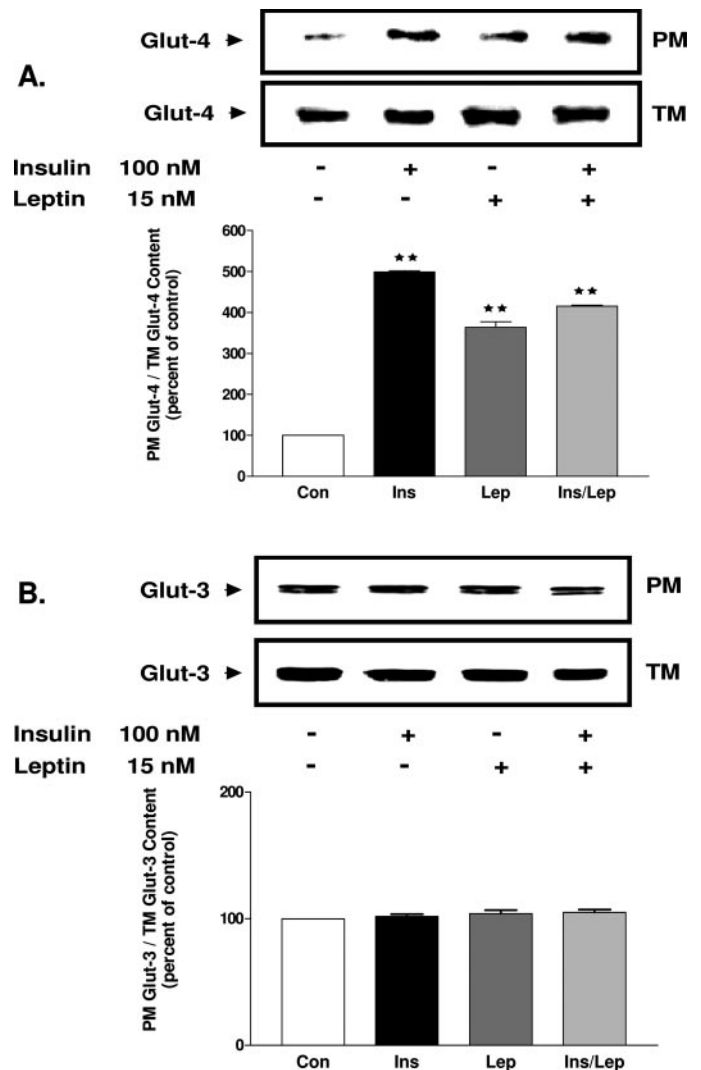


FIG. 1. Glut4 and Glut3 characterization and translocation to PM of SH-SY5Y neuronal cells. Serum-deprived cells were incubated in the absence (Con) or presence of 100 nM insulin (Ins), 15 nM leptin (Lep), or 100 nM insulin plus 15 nM leptin (Ins/Lep) for 15 min. Cells were lysed, and then TM and PM were prepared. Western blots were performed using specific Glut4 (A) or Glut3 (B) antibodies. The blotted proteins were revealed by ECL, the results were expressed as PM/TM ratio for Glut4 and Glut3, and presented as means \pm SEM ($n = 3$). **, $P < 0.001$.

ulin (100 nM) or leptin (15 nM) for 16 h before measuring Glut4 and Glut3 contents in total cell lysates by Western blot. Both insulin and leptin treatment significantly down-regulates Glut4 and Glut3 protein expression (Fig. 3, A and B), by roughly 2-fold whatever the isoform. The impact of the chronic exposure to insulin or leptin on Glut4 PM translocation in response to acute stimulation was investigated. Insulin- and leptin-dependent Glut4 appearance on the PM was abolished, in cells pretreated by leptin or insulin pretreatment (Fig. 4). This indicates that, in addition to a profound down-regulation of the total Glut4 cellular content, chronic exposure to insulin or leptin deactivates the mechanisms involved in Glut4 translocation to the PM.

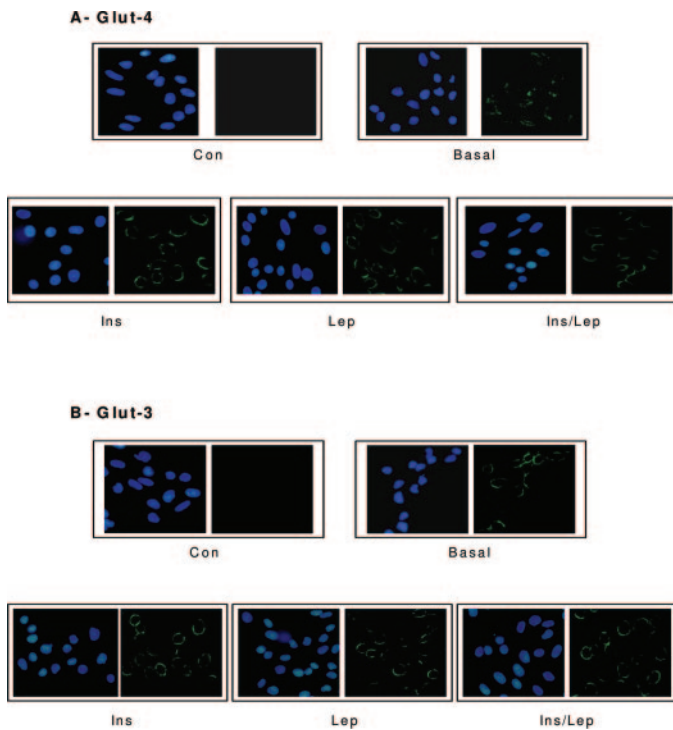


FIG. 2. Localization of Glut4 and Glut3 in SH-SY5Y cells in response to insulin or leptin using immunocytochemistry. Starved SH-SY5Y cells were incubated in the absence (basal) or presence of 100 nM insulin (Ins), 15 nM leptin (Lep) or 100 nM insulin plus 15 nM leptin (Ins/Lep) for 15 min, and then incubated in the presence of specific antibodies directed toward Glut4 (A) or Glut3 (B) shown at the *right* part of each box. 4,6-Diamidino-2-phenylindole staining was used to reveal cell nuclei (*left* part of each box). The negative control was prepared by omitting the primary antibody (Con). The microscope settings were consistently maintained.

Glut 4 translocation to PM and glucose transport in response to insulin or leptin are PI 3 kinase-dependent in SH-SY5Y neuronal cells

To determine whether Glut 4 translocation in response to insulin or leptin in SH-SY5Y neuronal cells is dependent upon the activation of PI 3-kinase signaling pathway, Glut4 translocation was measured in absence or presence of wortmannin, a specific PI 3-kinase inhibitor. The presence of wortmannin precluded the appearance of Glut 4 in the PM fraction in response to either insulin, leptin, or both (Fig. 5).

To examine the impact of Glut4 translocation on glucose uptake, glucose transport was measured in SH-SY5Y cells by determining the incorporation labeled 2-deoxyglucose (^3H]-2-DOG). Insulin and leptin significantly increased ^3H]-2-DOG incorporation in SH-SY5Y cells, and this augmentation was totally abolished in the presence of the PI 3-kinase inhibitor (Fig. 6A).

To determine whether ^3H]-2-DOG transport is intimately associated to Glut4 translocation, SH-SY5Y cells were pre-treated with insulin or leptin, conditions where Glut4 is down-regulated and not translocated to PM (as shown in Fig. 4). Both insulin (Fig. 6B) and leptin pretreatment (Fig. 6C) completely inhibited ^3H]-2-DOG transport in response to acute insulin or leptin stimulation compared with untreated cells (Fig. 6A). In this case, wortmannin has no additional

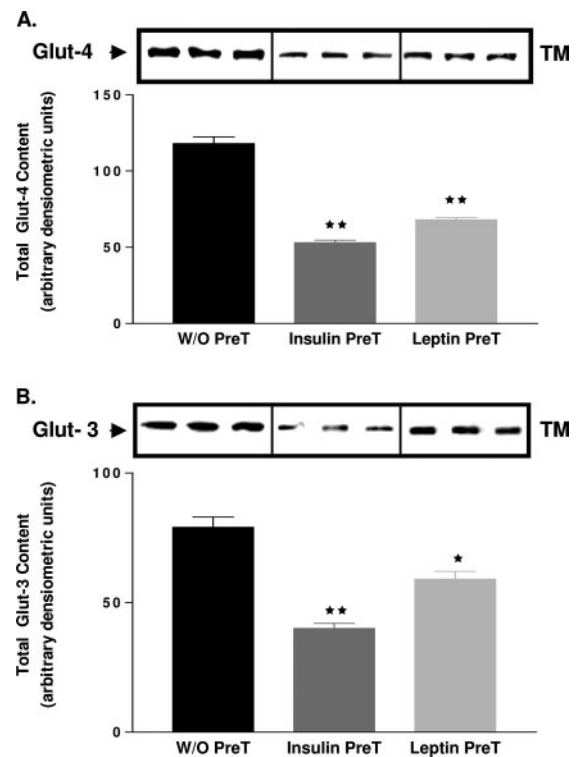


FIG. 3. Insulin and leptin down-regulate Glut4 and Glut3 expression in SH-SY5Y cells. Serum-deprived cells were incubated in the absence (W/O PreT) or presence of 100 nM insulin (Ins PreT) or 15 nM leptin (Lep PreT) for 16 h. After TM preparation and solubilization, cell lysates were subjected to SDS-PAGE and Western blot using antibodies directed toward Glut4 (A) or Glut3 (B). The experiment was conducted in triplicates. The blotted proteins were revealed by ECL, the results were expressed as total content of Glut4 and Glut3 and presented as means \pm SEM ($n = 3$). *, $P < 0.01$; **, $P < 0.001$.

effect, indicating that the residual glucose transport is not dependent upon PI 3-kinase activation (Fig. 6, A and B).

Chronic insulin or leptin treatment increased SOCS-3 and decreased Glut 4 expression at the mRNA level in SH-SY5Y neuronal cells

The inhibition of Glut4 translocation and stimulated glucose transport by chronic exposure to insulin or leptin may involve alterations of insulin and leptin signaling pathways. Indeed, we have previously shown that chronic exposure of SH-SY5Y cells to insulin or leptin alters JAK2/STAT-3 and IRS/PI-3kinase pathways (33). Here, we hypothesized that chronic insulin or leptin treatment induced inhibitors of these pathways. To corroborate this hypothesis, the expression of SOCS-3 was measured using Q-RT-PCR. Chronic insulin and leptin treatment significantly increased SOCS-3 expression in SH-SY5Y cells by more than 2-fold (Fig. 7B). The treatment significantly reduced the expression of Glut4 mRNA in the same cells (Fig. 7A), confirming result obtained previously at the level of Glut4 protein (Fig. 3).

Discussion

To assess the role of Glut4 in neuronal cell glucose transport and the impact of insulin and leptin on its translocation to the PM, we used the SH-SY5Y cell line that naturally

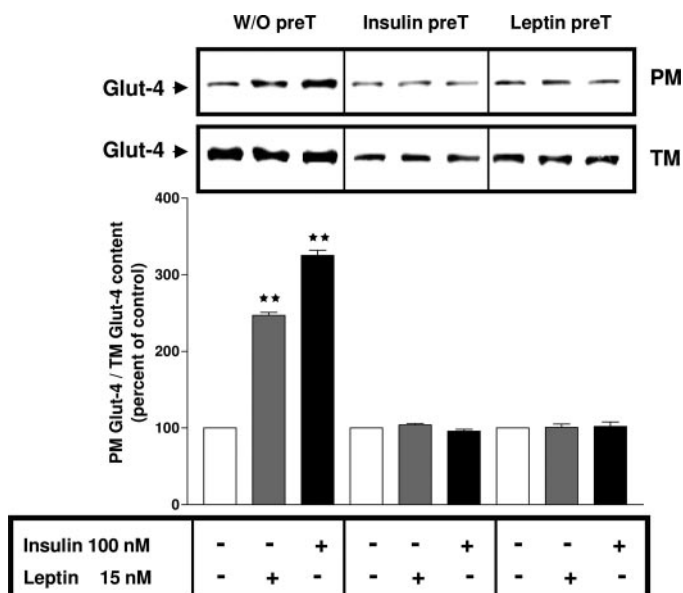


FIG. 4. Insulin or leptin pretreatment abolished Glut4 PM translocation in response to insulin or leptin in SH-SY5Y cells. Serum-deprived cells were incubated in the absence (W/O PreT) or presence of 100 nM insulin (Ins PreT) or 15 nM leptin (Lep PreT) for 16 h. After washing, cells were incubated in the absence or presence of insulin or leptin for 15 min, and then TM and PM were prepared and solubilized. Cell lysates were subjected to SDS-PAGE and Western blot using antibodies directed toward Glut4. The blotted proteins were revealed by ECL, the results were expressed as PM/TM ratio of Glut4 (% to the control) and presented as means \pm SEM ($n = 3$). **, $P < 0.001$.

expresses insulin and leptin receptors (33). In the present paper, we demonstrate that both insulin and leptin induced Glut4 translocation to PM in these neuronal cells, whereas Glut3 translocation is not sensitive to such stimulation. Glut3 was showed to be mostly located at the PM, in good agreement with previous reports on mouse neurons (6, 36).

The insulin-responsive Glut4 has been previously localized in neurons in different areas of the brain (8, 22), but to our knowledge this is the first study reporting Glut4 translocation to neuronal PM in response to insulin or leptin. To further investigate the mechanisms involved in Glut4 translocation in SH-SY5Y cells, the PI 3-kinase signaling pathway was inhibited by a specific inhibitor. Because inhibition of PI 3-kinase abolished insulin- and leptin-dependent Glut4 translocation to PM, this indicates that, as in peripheral tissues such as adipose tissue or muscles, Glut4 translocation is insulin sensitive in SH-SY5Y neuronal cells. Interestingly, we also show that Glut4 translocation is leptin sensitive, through a PI 3-kinase-dependent pathway. The translocation of Glut4 in response to insulin or leptin is corroborated with 2-DG uptake, which is increased after insulin or leptin stimulation and completely abolished in the presence of PI 3-kinase inhibitor. We have previously shown that leptin and insulin receptors share the IRS/PI 3-kinase signaling pathway in SH-SY5Y neuronal cells (33). Taken together, these data indicate that PI 3-kinase plays a key role for the integration of leptin and insulin action at the neuronal level as described by others in hypothalamic neurons (37). The role of PI 3-kinase that has been so far described concerns its involvement in insulin and leptin signaling through the ac-

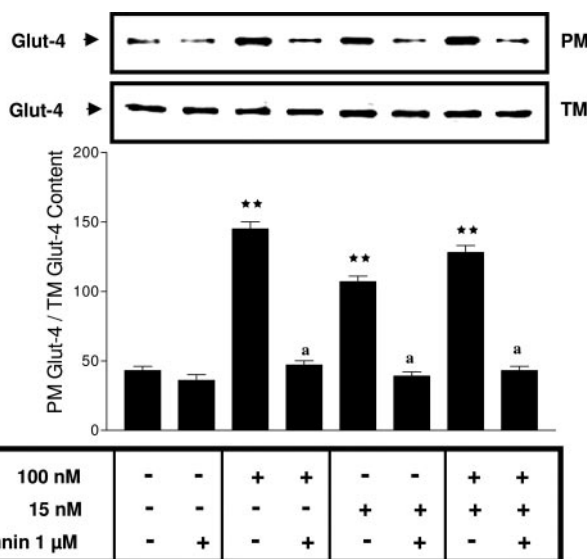


FIG. 5. Glut4 translocation to PM is wortmannin sensitive in SH-SY5Y cells. Serum-deprived cells were incubated in the absence or presence of wortmannin for 15 min then 100 nM insulin, 15 nM leptin or 100 nM insulin plus 15 nM leptin was added for an additional 15 min. Cells were lysed, and then TM and PM were prepared. Western blots were performed using specific antibodies directed toward Glut4. The blotted proteins were revealed by ECL, the results were expressed as PM/TM ratio for Glut4 ($\times 100$) and presented as means \pm SEM ($n = 3$). **, $P < 0.001$ when comparing the effect of leptin, insulin or leptin + insulin to untreated cells; a, $P < 0.001$ when comparing the effect of leptin, insulin, or leptin + insulin in the absence or presence of wortmannin.

tivation of pro-opiomelanocortin hypothalamic neurons leading to the inhibition of food intake (36). Furthermore, intracerebroventricular administration of PI 3-kinase inhibitors blocks the ability of leptin and insulin to inhibit food intake (27, 38). It has been also shown that both hormones induced neurons hyperpolarization through a PI 3-kinase-dependent mechanism contributing to neuropeptide release and rapid changes in energy intake (39, 40).

In the present paper, we suggest another mechanism by which leptin and insulin may exert their role as peripheral indicators of energy balance and inhibitors of food intake through an IRS/PI 3-kinase/Glut4 signaling pathway. The insulin and leptin-dependent translocation of Glut4 and subsequent increase in glucose uptake may affect the neuronal glucokinase (GK).

It has been described that the neuronal GK is expressed in glucose-excited neurons in the hypothalamus and is considered as a potential glucosensing gatekeeper with similar properties to pancreatic β -cells GK (26). GK is sensitive to changes in intracellular glucose concentrations and plays a key role in the glycolytic flux inducing an increase in ATP-to-ADP ratio leading to the inactivation of K_{ATP} (ATP-sensitive potassium) channel and consequently to membrane depolarization. GK is also able to use glucose transported through Glut4 in neurons and this hypothesis is reinforced by the colocalization of insulin receptor and Glut4 in glucose-excited neurons in the hypothalamus (26). Another glucose transporter, Glut 2, was suggested to play a role in neuronal glucose sensing; however, its glial or/and neuronal cells localization is still matter of debate (15).

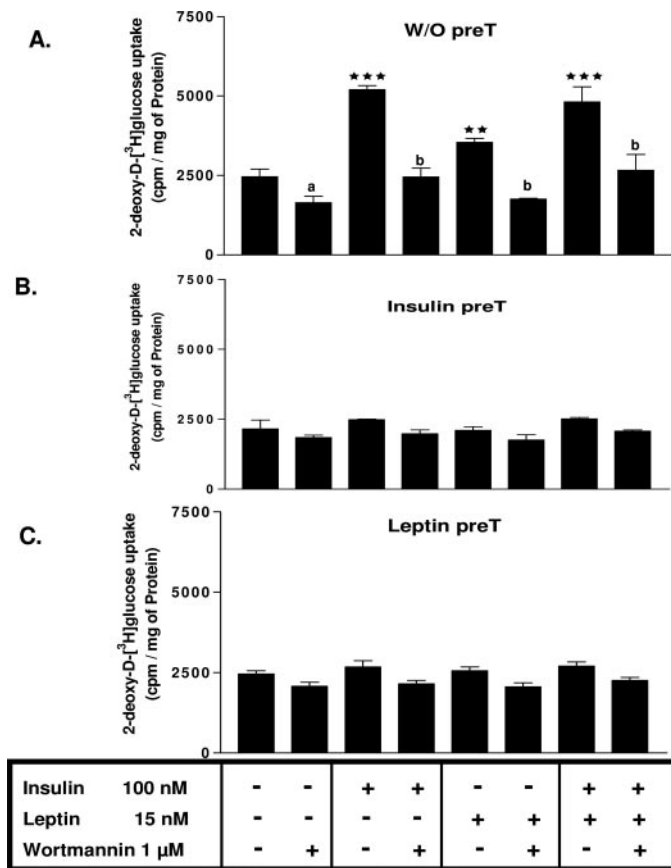


FIG. 6. Glucose transport is insulin and leptin sensitive in SH-SY5Y and is PI 3-kinase dependent. Serum-deprived cells were incubated in the absence (W/O PreT) or presence of 100 nM insulin (Ins PreT) or 15 nM leptin (Lep PreT) for 16 h. Cells were then incubated for 30 min in a glucose-free medium in the absence or presence of insulin, leptin, or insulin + leptin. In the last 15 min of incubation, radiolabeled 2DOG was added in the absence or presence of wortmannin. After washing, cells were lysed in 1 N NaOH, and cell-associated radioactivity measured. Results were expressed as incorporated radioactivity per milligram of protein and presented as means \pm SEM (n = 3). **, $P < 0.01$; ***, $P < 0.001$; a and b, $P < 0.05$ and $P < 0.01$, respectively, where treatment in absence or presence of wortmannin were compared.

We have also investigated the impact of chronic leptin or insulin exposure on Glut4 translocation and glucose uptake. Both hormones down-regulate Glut3 and Glut4 glucose transporters at the level of protein and significantly decreased Glut4 mRNA expression. The overexposure to leptin and insulin may mimic hyperleptinemia and hyperinsulinemia states that are observed during the onset of leptin and insulin resistance as previously reported (33). Chronic leptin or insulin treatment abolished Glut4 translocation and glucose transport in response to both hormones, and interestingly a cross-down-regulation between insulin and leptin signaling pathways was observed because the overexposure to insulin affects leptin action and *vice versa*. We have previously reported such events in SH-SY5Y neuronal cells concerning JAK2/STAT-3 and IRS/PI 3-kinase pathways (33). The cross-down-regulation may be, at least partly, attributed to the overexpression of SOCS-3 as we report here, where both leptin and insulin induced the expression of SOCS-3.

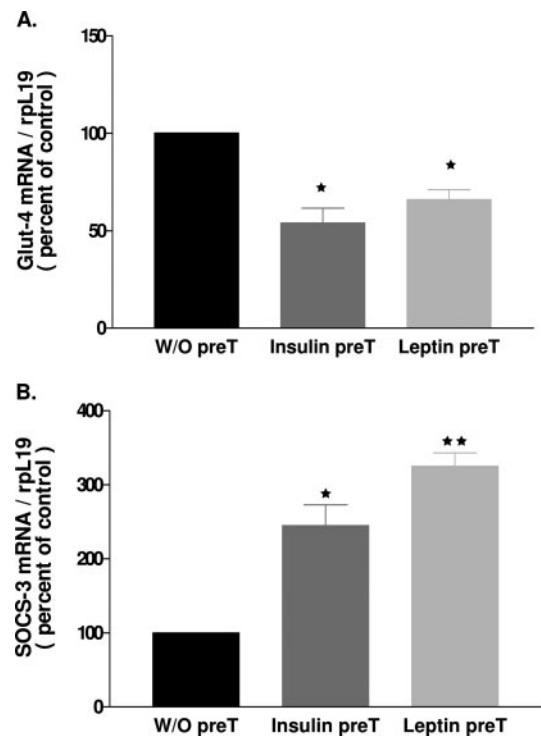


FIG. 7. Insulin or leptin pretreatment down-regulate Glut4 mRNA and up-regulate SOCS-3 mRNA in SH-SY5Y cells. Serum-deprived cells were incubated in the absence (Con) or presence of 100 nM insulin (Ins) or 15 nM leptin (Lep) for 16 h, then total RNAs were prepared and subjected to reverse transcriptase. The resulting cDNAs were used to perform quantitative PCR as described in *Materials and Methods* using specific primers for Glut4 (A) and SOCS-3 cDNA (B). RPL9 was used to normalize the expression results. Results were expressed as ratio of specific mRNA/rpl9 and presented as means \pm SEM (n = 3). *, $P < 0.01$; **, $P < 0.001$.

SOCS-3 affects leptin action as previously demonstrated in SOCS-3-deficient mouse where leptin sensitivity of the brain was clearly increased (41). In addition, by inducing the degradation of IRS proteins via a ubiquitin-dependent mechanism, SOCS-3 blocks insulin action (42). Thus, the chronic leptin or insulin action is mediated by the overexpression of SOCS-3 that reduces the responsiveness of SH-SY5Y cells to leptin and insulin by altering IRS/PI 3-kinase/Glut4 and JAK2/STAT-3 signaling pathways in addition to the alteration of Glut4 expression.

In conclusion, we report for the first time, in our knowledge, that both insulin and leptin are able to increase Glut4 translocation to neuronal PM and glucose transport in a PI 3-kinase-dependent manner. In addition, the chronic leptin or insulin treatment induced a cross-desensitization of Glut4 translocation and glucose transport in response to both hormones, which may contribute to the understanding of the complex relationship between leptin resistance and insulin resistance at the neuronal level.

Acknowledgments

We are grateful to Pr. C. Magnan for permitting the realization of Q-RT-PCR experiments in his laboratory.

Received November 17, 2005. Accepted February 10, 2006.

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We thank Conseil Régional de Ile de France for the financial support (SESAME GRANT A01947).

Disclosure statement: Yacir Benomar, Nadia Naour, Alain Aubourg, Virginie Bailleux, Arieh Gertler, Jean Djiane, Michèle Guerre-Millo, and Mohammed Taouis have nothing to declare.

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