Positive and negative regulation of glucose uptake by hyperosmotic stress

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SUMMARY
This review will provide insight on the current understanding of the intracellular signaling mechanisms by which hyperosmolarity mimics insulin responses such as Glut 4 translocation and glucose transport but also antagonizes insulin effects. Glucose uptake induced by insulin is largely dependent on the PI 3-kinase/PKB pathway. In both adipocyte and muscle cells, hyperosmolarity promotes glucose uptake by multiple mechanisms which do not require PI 3-kinase/PKB pathway but are dependent on the cell type. In muscle, osmotic stress induces glucose uptake by stimulation of AMP-Kinase and/or inhibition of Glut 4 endocytosis. In adipocytes, activation of Gab1-dependent signaling pathway plays an important role in osmotic stress-mediated glucose uptake. Apart of its insulin-like effects, hyperosmolarity can lead to cellular insulin resistance mediated by both prevention of PKB activation and inhibition of the Insulin Receptor Substrate-1 (IRS1) function. Serine phosphorylation and degradation of IRS1 negatively regulate its functions. Understanding how osmotic stress induces glucose transport or mediates insulin resistance may provide novel targets for strategies to enhance glucose transport or to prevent insulin resistance.

Key-words: Stress · Signaling · Glut 4 · Glucose uptake · Insulin resistance.

RéSUMÉ
Régulation positive et négative du transport du glucose par le stress hyperosmolaire
Le stress hyperosmolaire a un double effet : il mime l’effet de l’insuline sur la translocation des transporteurs de glucose Glut 4 et sur le transport du glucose, mais il inhibe aussi les effets de l’insuline sur ces mêmes paramètres. Cette revue décrit les mécanismes intracellulaires par lesquels ces effets s’exercent. La captation de glucose induite par l’insuline est largement dépendante de la stimulation de la voie PI 3-kinase/PKB dans le muscle et l’adipocyte. Si l’hyperosmolalité induit également la captation de glucose, cet effet s’exerce par de multiples mécanismes qui sont indépendants de cette voie PI 3-kinase/PKB et qui diffèrent dans le muscle squelettique et dans l’adipocyte. Dans les lignées musculaires, l’augmentation du transport de glucose en réponse à un stress hyperosmolaire implique l’activation de l’AMP-Kinase et également une inhibition de l’endocytose des transporteurs de glucose Glut 4. Dans les lignées adipocytaires, c’est l’activation d’une voie dépendante de Gab-1 qui explique l’effet de l’hyperosmolalité. Indépendamment de ses effets insulinomimétiques, l’hyperosmolalité provoque également une insulinorésistance au niveau cellulaire qui s’explique d’une part par une désactivation de la PKB et d’autre part par une inhibition de la fonction d’IRS1. Cette rétro-régulation du signal insulinique est due à court terme à une phosphorylation d’IRS1 sur des résidus sérine et à long terme à une dégradation d’IRS1, deux processus que l’on retrouve dans l’insulinorésistance. Une meilleure connaissance des voies stimulées par le stress hyperosmolaire peut augmenter le transport de glucose ainsi que des mécanismes mis en jeu dans l’apparition de l’insulinorésistance peut fournir de nouvelles pistes et cibles thérapeutiques du diabète et de l’insulinorésistance.

Mots-clés : Stress · Signalisation · Glut 4 · Transport du glucose · Insulinorésistance.
Insulin regulates blood glucose levels through multiple regulatory mechanisms such as suppression of endogenous glucose production by the liver and stimulation of glucose uptake into muscle and adipocytes. Glucose transport in muscle and adipose tissues is due to the translocation of the glucose transporter Glut 4 from an intracellular pool to the plasma membrane [1]. These biological responses require tyrosine phosphorylation of IRS1, which leads to, in turn, binding and activation of PI 3-kinase. Downstream effectors of PI 3-kinase such as Protein Kinase B (PKB) or atypical PKC are involved in Glut 4 translocation. Furthermore, it has been recently shown that insulin-induced Glut 4 translocation also requires the activation of a second pathway which is completely independent of PI 3-kinase activity. In adipocytes, the Cbl proto-oncogene, associated with CAP (Cbl-associated protein) and APS (adapter protein containing PH and SH2 domain) is phosphorylated in response to insulin and regulates glucose uptake. Once phosphorylated, Cbl recruits the adapter protein Crk-II in a complex with C3G, a GDP to GTP exchange factor for TC10, a Rho-family GTPase, allowing for its activation which regulates the traffic of Glut 4-containing vesicles. GTP-bound TC10 could participate in Glut 4 translocation through a modification of cortical actin or a stimulation of actin polymerization at the level of Glut 4 compartments [2-4] (Fig. 1).

Various other stimuli can promote Glut 4 translocation through insulin—and PI 3-kinase—dependent mechanisms. In skeletal muscle, contraction and hypoxia can stimulate glucose uptake [5-7]. Insulin and contraction have fully or partially additive effects on glucose transport indicating that both stimuli induce glucose uptake via distinct mechanisms [5, 8]. In L6 myocytes and 3T3-L1 adipocytes, inhibition of oxidative phosphorylation by 2,4-dinitrophenol or rotenone stimulates glucose transport [9-11]. Metformin, which is widely used for the therapy of type 2 diabetes mellitus, and adiponectin, a hormone secreted by the adipocytes, stimulate the uptake of glucose in muscle [12-15]. Finally, hyperosmolarity can also mimic, at least in part, insulin effects on Glut 4 translocation and glucose uptake in muscle and 3T3-L1 adipocytes [16-20]. Hyperosmotic stress-induced glucose uptake is largely independent of IRS1 phosphorylation and activation of the PI 3-kinase/PKB pathway [16, 17, 20-24]. Interestingly, hyperosmolarity induces this response via distinct mechanisms depending on the cell type. Furthermore, like several other insulinomimetic agents, hyperosmolarity does not only partially activate several insulin-specific biological responses but also induces a state of insulin resistance [22, 25]. We first review, in the present article, the molecular mechanisms of Glut 4 translocation and glucose uptake induced by hyperosmotic stress. We then examine by which mechanisms(s) hyperosmotic stress antagonizes insulin responses. It should be noted that hyperosmotic stress (usually induced by high extracellular concentrations of sorbitol) is not a physiological stimulus but is used as a tool that could lead to discovery of novel molecular mechanisms of glucose transport and also to better understand the molecular mechanisms of cellular insulin resistance.

Osmotic stress stimulates glucose transport

In muscle and adipose cells, hyperosmolarity triggers the cell surface accumulation of Glut 4 leading to an increase in glucose transport. The mechanisms controlling this osmotic stress-mediated response have not yet been fully elucidated. Different molecular mechanisms have been reported and seem to be different in adipose tissues and skeletal muscle.

Effect of hyperosmotic stress in muscle cells

Two distinct intracellular pools of Glut 4 have been characterized in muscle. One is recruited by insulin and the other by exercise. As previously described for exercise-induced glucose uptake, hyperosmolarity does not recruit Glut 4 from the insulin-sensitive pool since the time-courses of insulin—and hyperosmotic stress-induced Glut 4 translocation are different. Moreover, the v-SNARE VAMP2, a protein which is associated to the insulin sensitive Glut 4 pool and required for the fusion of the vesicles with the membrane, is not involved in the sorbitol-induced Glut 4 translocation [26]. Exercise and the adenosine analogue, AICAR, increase the activity of the AMP-activated protein kinase (AMPK) that correlates with glucose uptake in muscle cells [8, 13, 27]. Treatment of muscle cells with sorbitol also promotes the activation of AMPK [19, 27] and over-expression of a dominant-negative form of AMPK blocks the stimulation of Glut 4 translocation by hyperosmotic stress [28]. Altogether, these observations are in favor of an important role of AMPK activation in hyperosmolarity-induced glucose uptake. Following AMPK activation, different downstream pathways have been proposed. Fryer et al. have reported that AMPK phosphorylates and activates...
nitric oxide synthase (NOS) and that NOS inhibitors prevent sorbitol-induced glucose transport. Moreover, guanylate cyclase activity, which is increased following NO production, plays an important role in sorbitol-mediated glucose uptake [19] (Fig 2). In contrast, the group of Farese has reported that activated AMPK promotes the activation of the non-receptor proline-rich tyrosine kinase-2, PYK2, leading to the activation of the GRB2/SOS/RAS/RAF/MEK1/ERK pathway [29]. ERKs activate phospholipase D (PLD) which generates phosphatidic acid (PA). Accumulation of PA stimulates atypical PKCs, which are apparently required for the increase in Glut 4 translocation and glucose transport in response to osmotic stress [30, 31] (Fig 2).

Glut 4 accumulation at the cell surface in response to sorbitol in muscle cells seems also to be due to the inhibition of Glut 4 endocytosis, a tyrosine kinase independent process (Fig 2). This could be due to the alteration of the formation of clathrin-coated pits that are required for Glut 4 endocytosis [26].

Effect of hyperosmotic stress in adipose cells

While activation of AMPK is essential for sorbitol-induced glucose uptake in skeletal muscle, activation of AMPK by AICAR is without effect on glucose uptake in 3T3 L1 adipocytes [32]. In contrast, AMPK activation antagonizes insulin-stimulated glucose transport [33]. Furthermore, inhibition of NOS by L-NMMA or guanylate cyclase by LY83583 did not prevent hyperosmolarity-stimulated glucose uptake (personal communication). In 3T3-L1 adipocytes, glucose transport activity induced by osmotic stress is fully dependent on a tyrosine kinase activity and requires the t-SNARE protein syntaxin-4 for docking/fusion of Glut 4-containing vesicles [16, 18]. Among the proteins phosphorylated on tyrosine following sorbitol treatment, Gab-1 (Grb2-associated binder-1) seems to play a key role. Indeed, microinjection of anti-Gab-1 antibodies strongly inhibits osmotic stress-induced Glut 4 translocation without altering the insulin effect [18]. Gab-1 is an adapter
protein, which possesses an N-terminal pleckstrin homology (PH) domain and 16 potential tyrosine phosphorylation sites [34, 35]. In response to osmotic stress, Gab-1 acts as a docking protein for PLCγ1, the p85 subunit of PI3 kinase and Crk-II. Both Gab1-dependent PI 3-kinase and PLC-γ activities appear to play a role in osmotic stress-induced membrane ruffling [18] and gene expression [36], respectively, but are not required for glucose transport [16, 17, 20-24]. In contrast, the formation of Gab1-Crk-II complex correlates with Glut 4 translocation and glucose uptake [20]. Upon sorbitol stimulation, the phosphorylated Gab1 recruits Crk-II via its SH2 domain. The Crk-II SH3 domains are constitutively associated with C3G, a GDP to GTP exchange factor for several small GTP-binding proteins including TC10. In favor of this pathway, we have shown that osmotic stress-mediated Glut 4 translocation and glucose uptake are inhibited by overexpression of the dominant-interfering TC10/T31N mutant or by inactivation of TC10 by *Clostridium difficile* toxin B [20]. The full activation of CrkII/TC10 pathway is mediated via Gab1 but could be also dependent on Cbl, which is phosphorylated on tyrosine residue in response to sorbitol [16]. However, signaling pathways downstream of Gab1, and specifically the Gab1/CrkII/C3G/TC10 signaling, play a crucial role in osmotic-stress-induced glucose transport [18, 20] (Fig 3). Since Gab1, CrkII/C3G, TC10 are expressed in muscle [34, 37-38], it is possible that osmotic stress-stimulated glucose uptake could also be mediated by this signaling pathway.

Figure 2
Signaling pathways activated by hyperosmotic stress to induce Glut 4 translocation in muscle cells. Osmotic stress increases the activity of AMPK, which, in turn, leads to the stimulation of two distinct pathways. AMPK phosphorylates and activates the eNOS leading to the production of NO and activation of guanylate cyclase. AMPK could also activate the PYK2 tyrosine kinase activity leading to the activation of ERK through the Grb2/SOS/Ras/Raf/MEK1/ERK pathway. ERK activate PLD, which generates phosphatidic acid (PA). Accumulation of PA stimulates aPKC, which plays a role in hyperosmotic stress-induced glucose uptake. Both pathways promote the translocation of Glut 4 to the cell membrane from VAMP2-negative vesicles. In addition, Glut 4 accumulation at the cell surface in response to osmotic stress also results from the inhibition of its endocytosis, a tyrosine kinase independent process. This could be due to the alteration in formation of clathrin-coated pits that are required for Glut 4 endocytosis.
As described above for muscle cells, it has been reported similarly that hyperosmotic stress activates the PYK2/ERK/PLD/aPKC pathway in 3T3 L1 adipocytes and adipose cells, leading to Glut 4 translocation and glucose uptake [30] (Fig 3). However, several reports are not in favor of a role of ERK activation in osmotic stress-induced glucose uptake in 3T3 L1-adipocytes. Indeed, inhibition of ERK activity by cell treatment with a MEK1 inhibitor PD98059 or expression of a dominant-interfering mutant ∆SOS, did not change the effect of osmotic stress on glucose transport [17, 20].

In adipose cells from type 2 diabetic subjects, insulin-signaling pathways are severely impaired, including IRS1 phosphorylation, PKB activity, and glucose transport [39]. In these cells, activation of Gab1-signaling pathway is still responsive to growth factor [40]. Since human insulin resistance correlates with inhibition of IRS1 function, the activation by pharmacological agents of pathways dependent on Gab1 could overcome the inhibition of IRS1 function and partially or totally rescue glucose transport.

While a unifying mechanism of hyperosmotic stress induced glucose uptake has not yet been identified, the new emerging pathways involved in this response give a novel viewpoint of potential insulin-independent mechanisms for glucose uptake.

Hyperosmotic stress leads to cellular insulin resistance

The earliest abnormality observed in insulin resistance is a decrease in the insulin-induced glucose uptake in skeletal muscle and adipose tissue and a reduced ability of the hormone to suppress hepatic glucose production [41, 42]. The inability of insulin to promote normal cellular glucose uptake could result from both a decrease in the cellular Glut 4 gene expression and the alteration in insulin signaling pathways controlling Glut 4 translocation and glucose uptake [3]. In rat epididymal adipose cells, hyperosmotic stress markedly reduces insulin-induced glucose transport [43]. Similarly, pretreatment of 3T3-L1 adipocytes with sorbitol strongly decreases the ability of insulin to stimulate glucose uptake, lipogenesis and glycogen synthesis [22]. Concomitant molecular mechanisms by which hyperosmotic stress antagonizes insulin-mediated responses have been reported, that we will review below.

Inactivation of PKB

Chen et al. have reported that hyperosmolarity prevents insulin-induced PKB activation. This suggests that stimulation of a calyculin A—or okadaic acid-sensitive protein phos-
phosphatase leads to the deactivation of PKB [22] (Fig 4). A strong inhibition of insulin-mediated PKB activation has also been detected in adipocytes from diabetic patients and their non-diabetic relatives, which could be attributed to 70% reduction in IRS1 protein expression and PI 3-kinase activity. In contrast, the activity of PKB appears to be normal or only impaired in vitro in skeletal muscle from the same subjects [39]. Since the impairment of insulin signaling at the level of PKB is specific for adipose tissue from diabetic patients, identification of a unifying mechanism leading to inhibition of glucose uptake could provide attractive targets for strategies to prevent insulin resistance.

Serine phosphorylation of IRS1

In addition to dephosphorylation of PKB, another mechanism is involved in hyperosmolarity-induced insulin resistance. Indeed, in 3T3-L1 adipocytes, hyperosmotic stress inhibits both tyrosine phosphorylation of IRS1 and IRS1-associated PI 3-kinase activity in response to physiological insulin concentrations [25]. One attractive explanation is that hyperosmotic stress negatively regulates the function of IRS1 by increasing its serine phosphorylation. These post-translational modifications could be a general mechanism to regulate the functions of docking proteins (IRS1, Gab1,Gab2) [44-47].

The concept that IRS1 Ser phosphorylation impairs insulin action has emerged ten years ago when Tanti et al. showed that a Ser phosphatase inhibitor, okadaic acid, severely altered the effect of insulin on glucose transport and Glut 4 translocation in adipocytes and skeletal muscles [44, 48]. This effect was linked to a decrease in IRS1 tyrosine phosphorylation and PI 3-kinase activation. We found that okadaic acid markedly increases the Ser phosphorylation of IRS1 and that hyperphosphorylated IRS1 is a poor substrate for insulin receptor [44]. Emerging data demonstrate that several factors such as PDGF, chronic insulin stimulation or TNF-α, which are implicated in insulin resistance, use this process to down-regulate insulin signaling [49-53]. The mechanism by which serine phosphorylation of IRS1 inhibits its function has not been fully elucidated yet [54]. However, it has been shown that phosphorylation of serine 307 could play an important role. Ser307 is located close to the phosphotyrosine binding (PTB) domain of IRS1, which is involved in its interaction with activated insulin receptor. Phosphorylation of Ser307 could then induce a conformational change, which prevents the association between IRS1 and insulin receptor leading to a decrease in IRS1 tyrosine phosphorylation [55].

In 3T3-L1 adipocytes, hyperosmotic stress inhibits the IRS1 functions in response to insulin. As a consequence,
insulin-induced membrane ruffling, which is dependent on PI 3-kinase activation, is markedly reduced. Interestingly, these inhibitory effects are associated with an increase in the phosphorylation of IRS1 on Ser\textsuperscript{307} residue. This effect is completely dependent on mammalian target of rapamycin (mTOR). Indeed, rapamycin, the mTOR inhibitor, prevents the osmotic stress-induced phosphorylation of IRS1 on Ser\textsuperscript{307} and reverses the inhibitory effect of hyperosmotic stress on insulin-induced IRS1 tyrosine phosphorylation and PI 3-kinase activity. However, the phosphorylation of Ser\textsuperscript{307} was correlated with an increase in the activity of PKC-θ [56, 64].

The importance of the Ser\textsuperscript{307} in the negative regulation of IRS1 function seems to be a general mechanism. Several stimuli such as fatty acids [56], chronic administration of insulin or IGF-1 [52, 57-58] and TNF-α [57, 59] induce the phosphorylation of Ser\textsuperscript{307} leading to the reduction in both IRS1 tyrosine phosphorylation and IRS1-associated PI 3-kinase activity. However, the phosphorylation of Ser\textsuperscript{307} could be catalyzed by multiple kinases. In response to TNF-α, both the c-Jun amino terminal kinase (JNK) and the inhibitor κ B kinase-β (IKK-β) are involved [60-63]. In response to insulin, we and others have found the mTOR signaling pathway as predominantly involved in insulin-induced phosphorylation of Ser\textsuperscript{307} in adipocytes, muscles and hepatocytes [52, 58], although JNK could also be involved [62]. In response to fatty acids, the phosphorylation of Ser\textsuperscript{307} was correlated with an increase in the activity of PKC-θ [56, 64], but JNK could also be involved [61].

Since IRS1 contains nearly 100 potential serine phosphorylation sites, other serine phosphorylation sites and kinases are likely involved in the inhibition of IRS1 functions. We recently determined that hyperosmotic stress also promotes the phosphorylation of Ser\textsuperscript{632} (personal communication). Furthermore, several groups identified that Ser\textsuperscript{612} and Ser\textsuperscript{632} are also phosphorylated in response to insulin and TNF-α. Both serine residues are located close to tyrosine residues which are major phosphorylation sites involved in the binding of PI 3-kinase and required for insulin-stimulated glucose uptake. The role of the phosphorylation of these two serine residues is not firmly established but several studies suggest that it could modulate the interaction between IRS1 and PI 3-kinase and/or its activation [65, 66]. The phosphorylation of these sites is mediated by MAPK and/or mTOR signaling pathways in response to both insulin [52] or TNF α [63, 67]. We recently reported that the basal level of IRS1 phosphorylation on Ser\textsuperscript{632} (corresponding to Ser\textsuperscript{636} in human IRS1 sequence) and MAPK activity are abnormally high in primary cultures of skeletal muscle cells obtained from type 2 diabetic patients [68]. Concomitantly, insulin-induced IRS1 tyrosine phosphorylation was altered. Moreover, inhibition of MAPK normalized the level of IRS1 Ser\textsuperscript{632} phosphorylation [68]. These results favor a role of MAPK in the decrease in insulin sensitivity in type 2 diabetic patients.

Enhanced degradation of IRS proteins

While serine phosphorylation is usually considered as a short term inhibitory mechanism, regulated degradation of IRS proteins might also promote long term insulin resistance. Adipocytes from type 2 diabetic subjects, obese patients and relatives of diabetic subjects display a 70% decrease in IRS1 protein expression. In contrast, no significant decrease in IRS2 protein expression has been reported. Likewise, IRS1 protein expression appears to be unchanged in skeletal muscle or primary cultures of skeletal muscle cells from either type 2 diabetic subjects or relatives of diabetic patients. A small (30%) reduction in IRS1 protein expression was reported in morbidly obese subjects [39].

In 3T3-L1 adipocytes, prolonged osmotic stress promotes the degradation not only of IRS1 but also of IRS2 (Fig 4) [25]. Oxidative stress, a putative causative factor for cellular insulin resistance [69, 70], also leads to IRS1 degradation [71]. In contrast to recent data [72], we have shown that osmotic stress stimulates IRS2 degradation through a proteasome-independent process [25]. The decrease in IRS1 protein expression by oxidative stress is also insensitive to proteasome inhibitors [71]. This could indicate that both oxidative and osmotic stress induce these effects through a lysosomal process. In contrast, the prolonged treatment of adipocytes with insulin reduces the level of IRS1 through a proteasome-dependent process. Moreover, it has been proposed that the mTOR-dependent IRS1 phosphorylation on serine could allow for its degradation [51, 73-75]. In conclusion, although both insulin and stress (oxidative and osmotic) induce the serine phosphorylation of IRS1, other events activated only by insulin treatment could be required to trigger IRS1 degradation by the proteasome. Since the N-terminal region of IRS1 contains a structural element that is crucial for the specificity of ubiquitination and proteasome degradation in response to insulin [76], another region of this protein could be required for stress-induced degradation.

In conclusion, insulin resistance is a very complex phenomenon, which includes defects at the level of insulin receptor and also at the various steps of insulin action leading to glucose transport (IRS1 phosphorylation, PI 3-kinase activity, Glut 4 gene expression, IRS1 protein expression...). More studies are required to better understand the mechanism of insulin resistance in its entirety. This review provides hypothetical targets for novel strategies to enhance glucose transport or to prevent insulin resistance. Stimulation of Glut 4 translocation to cell membrane through insulin-independent mechanisms could enhance glucose uptake in...
insulin-resistant patients. Likewise, inhibition of specific kinases involved in impairment of IRS1 functions could increase the insulin sensitivity.

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