Insulin Action in Peripheral Glucose Uptake -
The Molecular Perspective
Acção Periférica da Insulina na Captação de Glucose – a Perspectiva Molecular

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RESUMO
Nos últimos anos, a insulinorresistência tem sido objecto de inúmeros estudos e um dos principais alvos da pesquisa e intervenção farmacológicas. Como consequência, importantes passos têm sido dados a um ritmo acelerado, com vista à compreensão dos mecanismos associados à acção da insulina, bem como às suas alterações. Deste modo, a tomada de conhecimento dos mais recentes avanços nesta área e de como eles se encaixam na panorâmica global da acção da insulina parece ser útil, tanto do ponto de vista da pesquisa como do ponto de vista clínico.

O presente é o primeiro de dois mini-artigos de revisão acerca da acção da insulina no aporte periférico de glucose. Esta primeira revisão tem como objectivo dar uma visão geral dos eventos intracelulares conducentes à captação de glucose insulino-dependente, enquanto que na segunda revisão será efectuada uma abordagem da acção da insulina numa perspectiva fisiológica, e, integrativa, dando particular ênfase às diferenças na acção da insulina de acordo com o estado prandial.

Assim, na presente publicação será dada uma visão sumária e geral das principais vias de transdução de sinal da insulina, envolvidas no aporte de glucose por tecidos periféricos (extra-hepáticos). Apesar de neste artigo não se fazer uma abordagem farmacológica, espera-se que constitua uma boa base para compreender os mecanismos associados à fisiopatologia e farmacologia das alterações na acção da insulina.

PALAVRAS-CHAVE
Insulina; Acção da insulina; Receptor de insulina; Transdução de sinal da insulina; Aporte de glucose.

ABSTRACT
In the recent years, insulin resistance has become the aim of numerous studies and one of the major focuses for pharmacological research and intervention. As a logical consequence, important steps towards the knowledge of insulin action and its alterations have been added at a high rate. Therefore, the awareness about the recent breakthroughs in this field and about how they fit within the whole picture of insulin action seems to be very useful, both in clinical and research practice.

The present article is the first of two mini-reviews concerning peripheral insulin action in glucose uptake. This first review article aims at the intracellular events leading to insulin-dependent glu-
cose uptake, whereas in the second review insulin action will be approached in a whole-body perspective, giving particular emphasis to differences in insulin action according to the prandial state. Thus, in the present review, we will provide a brief overview of the major insulin signaling pathways involved in peripheral (extra-hepatic) glucose uptake. Although this article does not aim pharmacological therapeutic, we hope that it may launch some minimum comprehensive basis to better understand the mechanism behind the pathophysiology and pharmacology of insulin action.

KEYWORDS
Insulin; Insulin action; Insulin receptor; Insulin signaling pathway; Glucose uptake.

INTRODUCTION

Insulin is probably the most important anabolic hormone in the human organism\(^1\). At the cellular level, its action is characterized by several effects, which suggests the involvement of multiple signaling pathways initiated by the binding of insulin to the receptor.

The present review aims to provide a brief overview of the major insulin signaling pathways involved in glucose uptake via GLUT4 translocation, in particular in adipose tissue and skeletal muscle, last of which is responsible for about 75% of the insulin-dependent glucose uptake\(^2\). Transposition from the cellular to the physiological level (i.e., whole-body) will be essayed in a second review, resulting in a broad outline of insulin action on glucose metabolism and on glucose uptake in particular.

INSULIN RECEPTOR

The insulin receptor is ubiquitous in vertebrate tissues, although it may be expressed in different concentrations in different tissues\(^3\). A general schematic representation of the insulin receptor is provided in figure 1.

Structurally, insulin receptor is an heterotetrameric glicoprotein, composed of two \(\alpha\)-subunits and by two \(\beta\)-subunits, with N-terminal complex carbohydrates capped by terminal sialic acid residues\(^4,5\). Insulin receptor structure is stabilized by 3 disulphide bonds that link the two \(\alpha\)-subunits to each other and to the \(\beta\)-subunits, presenting a \((\alpha\beta)_2\) organization\(^6,7\). The \(\alpha\)-subunits are entirely located in the outside the cell, whereas \(\beta\)-subunits contain one extracellular portion, one transmembrane region and an intracellular region, last of which includes a juxtamembrane domain, a regulatory domain (activation domain) and a C-terminal domain\(^6,8\), with different functional roles.

Presently, there are two types of insulin receptor described: types A and B. The difference between these two isoforms is the presence of a 12 aminoacid sequence between
positions 716 and 717 of the α-subunits of type A insulin receptor. Type B insulin receptor is highly specific for insulin and prominent in the major target-tissues for insulin action, such as liver, skeletal muscle and adipose tissue. Type A insulin receptor promotes binding of IGF-2 (insulin-like growth factor 2) instead of insulin and is present in many fetal tissues, central nervous system and haematopoietic cells. Patients with accumulation of type A receptor in skeletal muscle seem to be more prompt to the development of insulin resistance.

IN SU LIN  BIN D IN G  AN D  ACTIVAT I O N  O F  T H E  R ECEPTOR

Insulin binds to one of the α-subunits of the insulin receptor, bringing the two α-subunits closer upon disruption of the α₂-dimer. Although there are two major binding sites (in the two α-subunits – figure 1), only one insulin molecule binds to the insulin receptor with high affinity, presenting a negative cooperativity for insulin concentrations lower than 0.1 μmol/dm³.

Insulin binding to the α-subunit induces tyrosine kinase activity in the regulatory domain of the intracellular portion of the β-subunit, promoting phosphorylation of tyrosine residues of this domain and concomitant activation of the insulin receptor - autophosphorylation.

Autophosphorylation of the insulin receptor is the key step in the initiation of the intracellular signalling and it may occur at seven different tyrosine residues, located in the three regions of the β-subunits with tyrosine kinase activity (juxtamembrane, regulatory or activation and C-terminal). However, the process seems to be initiated by phosphorylation of the tyrosine₁₁₆₂ residue of the regulatory domain (figure 1).

Insulin binding induces conformational changes in the regulatory (or activation) domain that allow binding to ATP, favoring the initial phosphorylation of the tyrosine₁₁₆₂ residue (regulatory domain) and, subsequently, the remaining tyrosine residues of the regulatory domain of the insulin receptor. Phosphorylation of tyrosine residues in the insulin receptor allow the recruitment, docking and activation of the effector proteins involved in the signaling cascade that present SH2 (Src-2 homology) domains. Many of these effector proteins are small adaptive molecules, such as p85, which is the regulatory subunit of the enzyme phosphatidylinositol-3-kinase (PI3K) and of CrkII, a small protein G activation molecule.

After insulin binding and activation of the insulin receptor, the complex insulin-insulin receptor is internalized and incorporated into endosomes, still in an active form, which facilitates the binding of the cytoplasmatic substrates.

Interestingly, in the absence of insulin, α-subunits seem to exert a negative effect upon the regulatory domains, thus blocking the signal transduction cascade.

This unusual form of activation seems to allow small molecules to interact with the insulin receptor in distinct sites from the activation domains of insulin.

INSULIN SIGNALING PATHWAYS INVOLVED IN GLUCOSE UPTAKE

Both insulin receptor and the majority of the proteins involved in insulin signalling are activated by tyrosine residues phosphorylation.

There are several intracellular substrates of the insulin receptor that can be phosphorylated at tyrosine residues by the receptor itself: Gab1, p60δ, APS, Shc isoforms, Cbl and the proteins of the IRS family (insulin receptor substrate). Many of these proteins are common substrates of the insulin receptor and of the IGF-1 receptor; however, the different specificity of both recruitment and phosphorylation ensure an adequate regulation of the signalling cascades of insulin.
and IGF-1 receptors. Additionally, APS, Cbl and IRS proteins, in particular, have been associated with the process of glucose uptake through stimulation of glucose transporters-4 (GLUT4) translocation.

Figure 2 summarizes the major insulin signaling pathways that involve these substrates, leading to GLUT4 translocation and glucose uptake.

The most relevant mediators of insulin action in glucose uptake by skeletal muscle and adipocytes are the IRS proteins, in particular IRS-1 and IRS-2. In mammals, four major proteins of the IRS family were described: IRS-1, expressed in skeletal muscle and adipose tissue; IRS-2, present in the brain, ovary, liver and adipose tissue; IRS-3, expressed in adipose tissue, presumably in rodents only; and IRS-4, present in the thymus and kidney. IRS proteins present an amine terminal, with binding domains for the insulin receptor and a carboxyl terminal, with tyrosine phosphorylation sites.

Following tyrosine phosphorylation, IRS protein activates PI3K, which plays a central role in GLUT4 translocation. IRS activates PI3K by binding to p85 regulatory subunit, which presents two SH2 domains that bind to phosphorylated residues in IRS proteins. Besides p85 subunit, PI3K presents a p110 catalytic subunit, responsible for phosphoinositides phosphorylation at position 3, producing phospholipidic compounds of the phosphatidylinositol-3-phosphate (PI3P) family, namely phosphatidylinositol-3,4,5-triphosphate (PtdInsP3).

PI3P (and PtdInsP3 in particular) activates phosphoinositide-dependent kinase 1 (PDK1), which in turn activates protein kinase B (Akt/PKB) and the atypical protein kinase C (PKCζ and PKCλ). It has also been described that PtdInsP3 can bind directly to PKC (PKCζ and PKCλ) and to Akt/PKB, activating them, therefore not requiring PDK1 as an intermediate.

Active Akt/PKB then promotes phosphorylation of Akt Substract of 160 kDa protein (AS160), which is constitutively associated to GLUT4 vesicles and in particular to Rab proteins, small G proteins involved in the processes of transport and fusion of GLUT4 vesicles to plasma membrane. Thus, AS160 phosphorylation by Akt/PKB promotes activation of the Rab proteins, leading to a higher rate of GLUT4 translocation - this topic will be further explored in the next section. On the other hand, PI3K can activate phospholipase C (PLC), resulting in the production of the second messengers DAG and inositol triphosphate (IP3), which activate PKCζ, thus stimulating glucose uptake (figure 2).

An additional insulin signalling pathway contributing to GLUT4 translocation and somehow independent of IRS phosphorylation and PI3K activation is described, and also presented in figure 2.

Such pathway involves phosphorylation of both APS (adaptive protein with SH2 and PH domains, last of which is present in Akt/PKB, allowing this enzyme to bind
PtdInsP₃ and Cbl protooncogene (Casitas b-lineage lymphoma, c-Cbl)²⁻²⁵ directly by the IR¹⁻²⁵. APS is involved in Cbl recruitment for the insulin receptor²⁵. In the majority of insulin-sensitive cells Cbl is associated with the adaptive protein CAP (Cbl-associated protein)². Following phosphorylation, the Cbl-CAP complex is transported into lipid rafts in the plasma membrane, where it binds to flotillin and recruits CrkII protein². CrkII then forms a complex with the guanylnucleotide exchange protein C3G²⁷, which activates TC10²²,²⁸. TC10 is a GTP-binding protein present in the lipid rafts that contributes to GLUT4 translocation and their docking at the plasma membrane²⁶,²⁸, possibly though regulation of actin microfilaments dynamics²²,²⁹,³⁰.

Although the TC10 pathway can be seen as an separate pathway from the PI3K-dependent one, some studies have suggested that TC10 activates PI3P³¹ and others have described that atypical PKC (PKCζ and PKCλ) are also able to promote TC10 activation²⁶,³². Thus, atypical PKC may represent a point of convergence for the PI3K and TC10 signaling pathways¹⁹, both of which contributing synergistically to GLUT4 translocation (figure 2).

**GLUT4 TRANSLOCATION**

GLUT4, present mostly in skeletal muscle and adipocyte, are located within vesicles that move in a cyclic manner between the intracellular storing sites and plasma membrane. Insulin promotes the presence of GLUT4 at the plasma membrane in two distinct, but synergistic ways: by increasing the rate of GLUT4 exocytosis and by reducing their internalization rate²⁻²³. In basal conditions, AS160 associates with GLUT4 vesicles, maintaining Rab proteins in their inactive form (Rab-GDP)³³,³⁴. Insulin-stimulated phosphorylation of S160 (PI3K pathway) inhibits AS160 negative effect on Rab proteins, causing a shift towards Rab-GTP complex formation and allowing for Rab-dependent GLUT4 translocation to occur³³,³⁴,³⁷.

As mentioned earlier, TC10 can also stimulate the Rab proteins mechanism through activation of PI3P (figure 2). Additionally, TC10 seems to activate actin-related protein 3 (Arp3), actin-regulatory neural Wiskott-Aldrich syndrome protein (N-WASP)³⁰ and exocyst protein complex³⁸, which are involved in the regulation of actin polymerization (N-WASP and Arp3), as well as docking and anchoring of GLUT4 vesicles to the plasma membrane (exocyst protein complex)²²,³⁰,³⁸. This TC10-mediated process is required not only for translocation of the GLUT4 vesicles, but also to their fusion with the plasma membrane².

As stated in the previous section and presented in figure 2, the remodeling or reorganization of the actin filaments in response to insulin binding to the receptor appears to be modulated by both the TC10 and IRS/PI3K pathways, through activation of the Rab proteins²².

Rab proteins have been shown to be necessary effectors in vesicle trafficking, docking and fusion. In particular, Rabs 2A, 8A, 10, and 14 are expressed in insulin-sensitive tissues and appear to be substrates of the AS160 GAP domain (IRS/PI3K pathway) and are associated with insulin-responsive GLUT4-containing vesicles³⁴-³⁶. AS160 thus may represent a convergence between insulin signaling and vesicle trafficking². AS160 is a negative regulator of basal GLUT4 exocytosis, ie, in basal conditions, AS160 associates with GLUT4 vesicles, maintaining Rab proteins in their inactive form (Rab-GDP)³¹,³⁴. Insulin-stimulated phosphorylation of S160 (PI3K pathway) inhibits AS160 negative effect on Rab proteins, causing a shift towards Rab-GTP complex formation and allowing for Rab-dependent GLUT4 translocation to occur³³,³⁴,³⁷.

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receptors (SNARE), namely SNAP-23, syntaxin-4, Synip, Munc18c and vesicle-associated membrane protein-2 (VAMP2) and and the plasma membrane proteins synaptoosome-associated 25-kDa protein and syntaxin-1A.

**INHIBITION OF THE INSULIN SIGNALING CASCADE**

Besides tyrosine phosphorylation (figure 2), both insulin receptor and IRS proteins have the potential to be phosphorylated at serine or threonine residues, which blocks or impairs the insulin signaling pathway. Such inhibitory effect of serine/threonine phosphorylation is achieved by reducing the number of phosphorylated tyrosine residues, by dissociating IRS proteins from their receptor, hindering tyrosine residues phosphorylation, by releasing IRS from the intracellular complexes that maintain them in close proximity to the receptor, by promoting IRS degradation, or by inducing IRS interaction with other proteins rather than with the tyrosine kinase catalytic site of PI3K.

These inhibitory (serine/threonine) phosphorylations constitute a physiological feedback mechanism in insulin signaling and allow the establishment of cross-talk mechanisms with different pathophysiological pathways that promote insulin resistance. Indeed, most of the stress and/or inflammation pathways studied so far stimulate serine/threonine phosphorylation of either IRS or insulin receptor (or both) as a way to induce insulin resistance.

Several kinases are known to be involved in the process of serine/threonine phosphorylation-dependent regulation, namely PI3K, Akt/PKB, glycogen synthase kinase-3 (GSK3) and mammalian target of rapamycin (mTOR), as well as PKC and the inhibitor of nuclear factor κ (IkB) kinase; these last two (PKC and IkB) have been suggested to be involved in the obesity-induced insulin resistance.

Insulin action is also attenuated by protein tyrosine phosphatases (PTPases) that promote tyrosine dephosphorylation of the insulin receptor and its substrates, a mechanism that seems to be augmented in many insulin resistant conditions, particularly in those associated with inflammation. Indeed, in studies using transgenic knockout of PTP1B models was observed an increase in the number of phosphorylated tyrosine residues, in both the receptor and IRS proteins, as well as an amelioration of insulin sensitivity in muscle and liver, improving or avoiding the diabetic condition.

**CONCLUSION**

Insulin plays a central role in carbohydrate metabolism. Although insulin presents different effects in different target-organs, one can consider that its major role in extrahepatic tissues, such as skeletal muscle and adipose tissue, is to promote glucose uptake. The knowledge of the molecular aspects of insulin action is important to understand the mechanism underlying pathophysiology and pharmacology of insulin resistance. In the present mini-article, we provided a brief review of the main signaling pathways that ensure insulin-stimulated glucose uptake.

The insulin receptor is an obvious target molecule to pharmacologically potentiate insulin action. However, other molecules can be key players for this purpose. Akt/PKB is also a pivotal molecule for insulin signaling pathways. However, in those tissues that are dependent on insulin to acquire glucose, GLUT4 is the main glucose transporter available. Indeed, most insulin signaling pathways will ultimately lead to GLUT4 expression and/or translocation. Furthermore, even insulin-independent pathways promote glucose uptake via GLUT4 translocation. Therefore, GLUT4 can be considered as an essential key player and...
target molecule for the study and/or modulation of different insulin signaling pathways involved in glucose uptake, since GLUT4 compliance should always be ensured in order to allow insulin-dependent glucose uptake.

The molecular aspects summarized herein constitute the basis for a second review, in which insulin action will be approached from a whole-body physiological perspective, more directed to the clinic.

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