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Review

Insulin Signalling: The Inside Story

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ABSTRACT

Insulin signalling begins with binding to its cell surface insulin receptor (IR), which is a tyrosine kinase. The insulin receptor kinase (IRK) is subsequently autophosphorylated and activated to tyrosine phosphorylate key cellular substrates that are essential for entraining the insulin response. Although IRK activation begins at the cell surface, it is maintained and augmented following internalization into the endosomal system (ENS). The peroxovanadium compounds (pVs) were discovered to activate the IRK in the absence of insulin and lead to a full insulin response. Thus, IRK activation is both necessary and sufficient for insulin signalling. Furthermore, this could be shown to occur with activation of only the endosomal IRK. The mechanism of pV action was shown to be the inhibition of IRK-associated phosphotyrosine phosphatases (PTPs). Our studies showed that the duration and intensity of insulin signalling are modulated within ENS by the recruitment of cellular substrates to ENS; intra-endosomal acidification, which promotes dissociation of insulin from the IRK; an endosomal acidic insulinase, which degrades intra-endosomal insulin; and IRK-associated PTPs, which dephosphorylate and, hence, deactivate the IRK. Therefore, the internalization of IRKs is central to insulin signalling and its regulation.

Mots clés :
insulinase endosomique acide
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tyrosine kinase

RÉSUMÉ

La signalisation de l'insuline commence par la liaison de l'insuline à son récepteur (IR) situé à la surface des cellules, soit la tyrosine kinase. La kinase du récepteur de l'insuline (KRI) s'est subséquemment autophosphorylée et activée vers les principaux substrats cellulaires de la tyrosine phosphorylée qui sont essentiels au déclenchement de la réponse à l'insuline. Bien que l'activation de la KRI commence à la surface des cellules, elle est maintenue et augmentée à la suite de l'internalisation du système endosomal (SEN). Il a été découvert que les composés de peroxovanadium (pV) activent la KRI en l'absence d'insuline et mènent à une réponse insulinique complète. Par conséquent, l'activation de la KRI est nécessaire et suffisante à la signalisation de l'insuline. De plus, il pourrait être démontré qu'elle apparaît avec l'activation de la KRI endosomale seulement. Il a été démontré que le mécanisme de l'activité du pV est l'inhibition des phosphotyrosines phosphatases (PTP) associées à la KRI. Nos études ont montré que la durée et l'intensité de la signalisation de l'insuline sont modulées au sein du SEN par le recrutement de substrats cellulaires du SEN; l'acidification intra-endosomale, qui favorise la dissociation de l'insuline de la KRI; une insulinase endosomique acide, qui dégrade l'insuline dans les endosomes; les PTP associées à la KRI, qui déphosphorylent et, donc, désactivent la KRI. Par conséquent, l'internalisation des KRI est essentielle au déclenchement de la signalisation de l'insuline et à sa régulation.

Introduction

Type 2 diabetes mellitus is characterized by both resistance to the action of insulin and defects in insulin secretion. The former has been an important motivating factor in the exploration of insulin action on its target tissues. By 1970, the notion that insulin and other peptide hormones interacted with specific cell surface receptors was established. Thus, incubating cells with ^{125}I-insulin and increasing quantities of unlabelled insulin defined receptors for insulin as cell surface binding sites of high affinity (~10^{-9} M) and specificity (1). Shortly thereafter, my colleagues and I recognized that the demonstration of specific binding sites (i.e. receptors) in any tissue was a new way of defining hormone target tissues. We demonstrated insulin receptors in the classic target tissues (liver, fat and muscle) but also in a range of other tissues not previously regarded as insulin targets (e.g. placenta and brain) (2).

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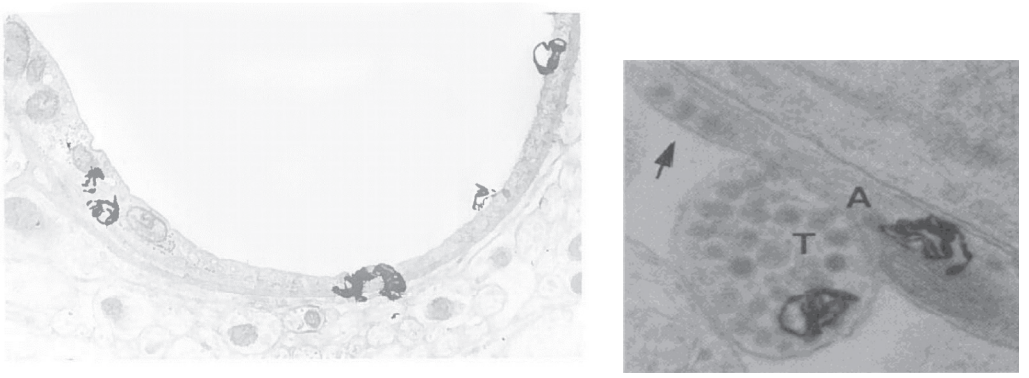

Figure 1. Electron microscope radioautographs illustrating the localization of ^{125}I-insulin-specific binding sites (silver grains). *Left,* insulin binding sites on the endothelium of rat brain microvessels. *Right,* insulin binding sites over a free nerve terminal (T) and a preterminal axon (A) containing dense core vesicles *(arrow)* in the external median eminence of the rat hypothalamus.

We subsequently developed an in vivo radioautographic method that permitted more precise cellular localization of peptide hormone receptors in a given tissue (3). Thus, it was possible to demonstrate insulin receptors on the endothelium of capillaries (4) and on nerve terminals in the median eminence of the hypothalamus (5) (Figure 1) as well as on other circumventricular organs of the brain where fenestrated capillaries permit access of circulating molecules directly to neuronal elements (6). The latter finding was extended to a range of peptide hormones, each with a specific pattern of neuronal interaction in the circumventricular organs (7). These findings provided a structural basis by which circulating insulin and other peptides could directly influence functions of the central nervous system.

Intracellular Receptors and Internalization

The localization of peptide hormone receptors was pursued at the subcellular level. Insulin binding sites had been observed in isolated hepatic Golgi fractions (GFs). However, it was unclear whether these sites existed on GF vesicles or on possible contaminating plasma membrane (PM). Insulin binding was examined in GFs and PM both before and after subjecting the cell fractions to freeze-thawing, a technique that would fracture vesicular structures (GFs) but not PM. As seen in Figure 2 (top), freeze-thawing markedly augmented insulin binding to GFs but not PM-indicating receptors in GF vesicles as well as on the cell surface. This was consolidated by demonstrating the different pattern of human growth hormone (hGH) binding to GFs and PM. As with other primate growth hormones, hGH binds to both lactogen and GH receptors. In the case of female rat liver, lactogen receptors predominate and are especially concentrated in GFs rather than in PM (Figure 2, bottom). In addition, we used electron microscope radioautography to demonstrate ^{125}I-insulin binding to GF vesicular elements (8).

The question then arose as to whether these were newly synthesized receptors transiting the Golgi apparatus on their way to the cell surface or were receptors internalized from the cell surface. This was first explored by injecting ^{125}I-insulin in vivo and following its association with different cell fractions with time. As seen in Figure 3 (left), ^{125}I-insulin was most highly concentrated in the GF fraction and, as demonstrated by electron microscope-radioautography, especially in lipoprotein-filled vesicles of this fraction (Figure 3, right). Coinjection of unlabelled insulin competitively inhibited the binding of ^{125}I-insulin to these elements (9). It was subsequently shown that the entities involved in insulin uptake could be resolved from true Golgi elements (10) and, hence, constituted a new organelle, which came to be known as the endosomal system or endosomes (ENS).

The cloning of the insulin receptor in the mid-1980s revealed it to be a tyrosine kinase capable of autophosphorylation and activation against external substrates. Furthermore, insulin receptor kinase (IRK) activity was shown to be necessary for insulin action to occur (11). Indeed, activation of the IRK in the absence of insulin was sufficient to realize the full range of insulin's effects (12). The development of antibodies to both the IR and phosphotyrosine enabled direct analysis of IRK fate and activation state. As seen in Figure 4 (left), the injection of insulin in vivo resulted in a large increase in the receptor content of ENS and a corresponding decrease in IR content of PM, as expected for insulin-dependent internalization of IRKs. As seen in Figure 4 (right), the internalized IRK was highly activated, both in effecting autophosphorylation and the tyrosine phosphorylation of downstream substrates (13). The much greater specific activity of the IRK in ENS vs. PM is consistent with an important role for the internalized receptor in determining the insulin response.

Insulin Degradation: An Endosomal Process

For some years prior, the degradation of insulin in tissues was attributed to an insulin-degrading enzyme, which was shown to have a pH optimum for insulin degradation of ~7.0, to be absent from ENS, present in the cytosol and especially enriched in peroxisomes, where it was found to be involved in the processing of peroxisomal proteins (14).

By 1988, it had become clear that degradation of insulin occurred subsequent to its internalization into ENS (15). Degradation of internalized insulin could be demonstrated in purified intact ENS (16) and by this means, characterized in some detail (Figure 5). It is noteworthy that there is a pH optimum of ~5.0. The requirement for adenosine triphosphate demonstrates the role of proton translocation into ENS to achieve the optimal intra-endosomal acidic pH. Thus, neutralizing the pH in ENS with either the proton ionophore, monensin, or the weak base, chloroquine, substantially inhibited insulin degradation. Also N-ethylmaleimide, an endosomal proton pump inhibitor, but not oligomycin, a mitochondrial proton pump inhibitor, inhibited insulin degradation, again emphasizing the importance of an acidic endosomal pH for this process (Figure 5).

The endosomal degrading activity showed relative specificity for insulin compared to epidermal growth factor (EGF) or prolactin (16). Subsequent studies have identified the aspartic acid protease cathepsin D as the insulin protease catalyzing the inactivating cleavage of endosomal insulin (17).