

CELLULAR AND MOLECULAR BIOLOGY OF INSULIN ACTION, INSULIN RESISTANCE AND DIABETES MELLITUS*

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INTRODUCTION

Investigations of the cellular and molecular mechanisms of insulin action facilitate a better understanding of the regulation of intermediary metabolism and of basic cell biology. In turn, the knowledge gained in these areas contributes towards elucidation of the mechanisms of insulin resistance which is a characteristic feature of the most prevalent form of diabetes mellitus. Accordingly, this article will highlight some major aspects of the cellular and molecular biology of insulin action and discuss these topics in the context of the increasing prevalence of diabetes mellitus in developing countries including Ethiopia.

Insulin is a polypeptide hormone that plays a central role in the control of a number of cellular metabolic and growth promoting processes. It stimulates the synthesis of glycogen, lipid and protein (anabolic effects) while it inhibits the degradation of these substances (anticatabolic effects). Its growth promoting effects include stimulation of DNA and RNA synthesis as well as stimulation of cell growth and differentiation. Thus, a deficiency of insulin and/or resistance to its biological effects result in profound alterations of cellular function, the most important manifestation of which is the syndrome of diabetes mellitus [1].

Diabetes is the most common endocrine-metabolic disorder that affects an estimated 100-200 million people world-wide and the incidence of new cases is rapidly increasing [2]. It is a complex disorder characterized by abnormalities of carbohydrate, lipid and protein metabolism resulting from a deficiency of insulin or of its cellular metabolic effects. The metabolic derangements are manifested by hyperglycaemia and the time-dependent development of chronic degenerative changes in the eyes, kidneys, nerves, and blood vessels. These changes result in the well-known chronic clinical complications of the disease and lead to considerable morbidity and mortality world-wide.

Diabetes mellitus is currently classified into two major types. Approximately 5-10% of all diabetics have type I or insulin-dependent diabetes mellitus (IDDM) which is characterized by absolute insulin deficiency resulting from pancreatic β -cell damage. Such patients are susceptible to developing ketoacidosis and are dependent on exogenous insulin administration to sustain life.

Type II or non-insulin-dependent diabetes mellitus (NIDDM) comprises over 90% of the diabetic population. Subjects with NIDDM in whom the disease is usually

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diagnosed in adulthood are not prone to ketoacidosis. They are not dependent on exogenous insulin to sustain life, although insulin may be used to treat persistent hyperglycaemia that does not respond to other therapeutic measures. A characteristic feature of NIDDM is resistance to the biologic actions of insulin at the level of target tissues. Therefore, delineation of the pathophysiology of NIDDM will first require characterization of the molecular mechanisms of insulin action.

CELLULAR AND MOLECULAR BIOLOGY OF INSULIN ACTION

The diverse cellular actions of insulin are all initiated by binding of the hormone to specific high-affinity receptors on the surface of target cells [3]. Figure 1 depicts the insulin receptor (IR) which is a transmembrane glycoprotein macromolecule composed of subunits of molecular mass 125,000 daltons (α -subunit) and molecular mass of 90,000 daltons (β -subunit). Both subunits are derived from a single-chain polypeptide precursor (proreceptor) that is the product of the 22 exons of the IR gene located on the short arm of chromosome 19. The proreceptor contains the entire amino acid sequence of the α - and β -subunits. Intracellularly, the proreceptor undergoes terminal glycosylation and proteolytic cleavage into the individual subunits that are covalently disulfide-linked into the mature β - α - α - β heterotetrameric IR that is inserted into the plasma membrane where it mediates insulin binding and signal transduction.

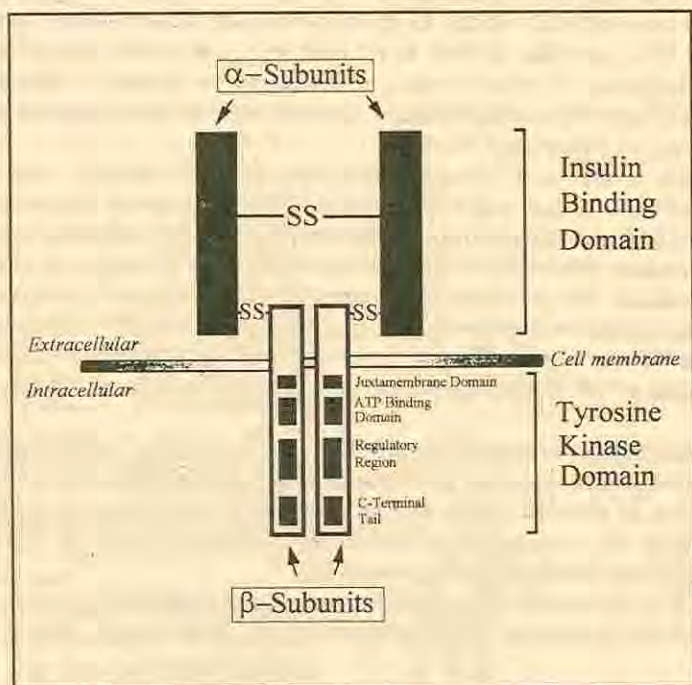


Figure 1. Insulin receptor subunit structure and functional domains. See text for details.

The α -subunit of IR contains the insulin binding site and is on the external surface of the cell membrane. The β -subunit consists of a small extracellular region, a single transmembrane segment that anchors the IR to the cell membrane, and a larger intracytoplasmic portion that contains the insulin-stimulable tyrosine-specific protein kinase (TPK) domain. Insulin binding to the α -subunit triggers ATP-dependent enhanced phosphorylation of specific tyrosine residues within the β -subunit TPK domain [3]. This autophosphorylation reaction activates the β -subunit as a kinase towards other intracellular protein substrates and is a key early postbinding step in insulin signal transmission to post receptor effector molecules (Figure 2). One of these is the insulin receptor substrate-1 (IRS-1) which undergoes rapid phosphorylation on specific tyrosine residues in response to insulin binding and activation of the IR kinase. The activated IRS-1 then interacts with several other downstream protein molecules that are involved in insulin signal transmission. Some of these proteins contain src homology 2 (SH 2) domains and include the p85 subunit of phosphatidylinositol 3-kinase (PI 3-kinase), Syp, Grb2, Shc, SOS and GTPase-activating protein (GAP). The linking of the activated IR kinase with these signaling molecules is further coupled to a sequential series of intracellular protein phosphorylation-dephosphorylation reactions (protein kinase cascades) that finally culminate in the well-known biological actions of insulin. These include: stimulation of glucose transport (via translocation of intercellular glucose transporter molecules to the cell surface); stimulation of glycogen, lipid and protein synthesis (through modulation of the amounts and/or activities of the relevant enzymatic processes); and regulation of gene expression and cell growth (via modulation of specific gene transcriptional and translational processes). It should be noted that although much has been learned about the insulin signaling cascade, our knowledge of how each component of the cascade is linked to specific insulin bioresponse is far from complete.

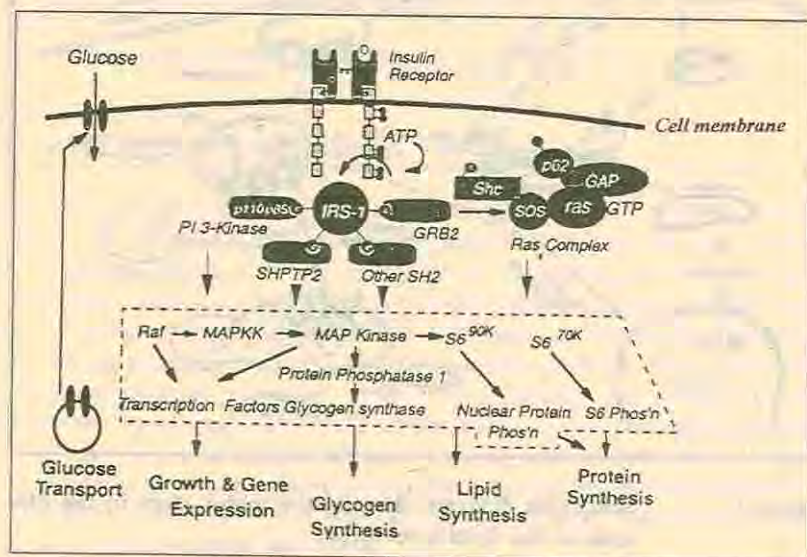


Figure 2. Schematic representation of the mechanism of insulin action. See text for details.

Insulin binding also triggers clustering of the bound insulin-receptor complexes along the plane of the plasma membrane. This is then rapidly followed by internalization of the complexes and their specific intracellular trafficking and processing (Figure 3). This process, termed receptor-mediated endocytosis, is a fundamental property in all insulin target cells and is thought to mediate certain biological functions of insulin [4]. These include termination of insulin action via internalization and degradation of the hormone, delivery of circulating insulin to cells in target tissues via transcytosis in endothelial cells, regulation of the level and signaling functions of the IR kinase, and mediation of the intracellular actions of insulin. In view of these factors, there has been considerable interest in attempting to better characterize the cellular and molecular mechanisms and the specific functional roles of the endocytosis, intracellular trafficking and processing of insulin-receptor complexes. To identify the IR structural domain(s) mediating insulin-induced endocytosis, various IR mutants have been constructed and functionally analyzed by means of *in-vitro* mutagenesis and stable transfection of full-length IR cDNA. The general consensus emerging from such studies is that the immediate submembranous domain of the β -subunit is important for mediating endocytic function [4]. However the identities of the specific amino acid residues within this domain that play a necessary role in endocytosis have not been conclusively established. It is significant to note that in addition to its role in endocytic function, the juxtamembrane domain of the IR β -subunit also participates in the coupling of the activated IR kinase to intracellular signal transduction molecules.

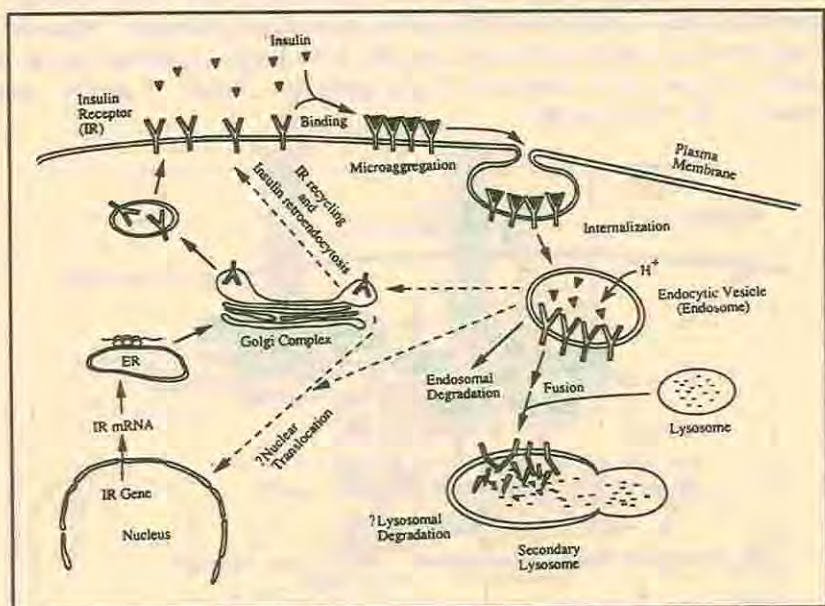


Figure 3. Schematic diagram showing the major steps in the life-cycle of the insulin receptor.

The rapidly advancing knowledge regarding the cellular and molecular biology of insulin action has in turn led to a better understanding of the mechanisms of cellular insulin resistance as discussed below.

MECHANISMS OF INSULIN RESISTANCE

Insulin resistance is broadly defined as a state in which a normal concentration of insulin produces a subnormal biological response [5]. As such, the manifestations of insulin resistance could represent a wide spectrum of conditions ranging from subclinical insulin resistant states that can only be identified with sophisticated *in-vivo* testing (e.g. using the euglycemic glucose clamp technique), to impaired glucose tolerance, and finally to overt insulin resistant diabetic states.

Insulin resistance can involve any of the multiple metabolic and mitogenic effects of insulin and can potentially result from defect(s) at any point in the integrated sequence of steps from insulin synthesis and secretion to its transport and action at target cell level. Table 1 lists the mechanisms of insulin resistance in which the different abnormalities have been grouped as defects at prereceptor, receptor, and postreceptor phases of the insulin action scheme.

Table 1. Mechanisms of Insulin Resistance

1. Pre-Receptor Phase

- a) Increased insulin degradation.
- b) Binding of insulin by anti-insulin antibodies.
- c) Secretion of a mutant insulin molecule.
- d) Increased levels of counterregulatory hormones (e.g. growth hormone, cortisol, glucagon, catecholamines).
- e) Anti-insulin receptor antibodies.

2. Receptor Phase

- a) Alterations in insulin binding (acquired or genetic insulin receptor defects).
- b) Defects in insulin signal transduction (acquired or genetic).

3. Post-Receptor Phase

Any defect(s) in intracellular pathways of insulin action.

In general, most of the conditions listed as pre-receptor causes of insulin resistance are rare and do not produce significant clinical problems. Of these the study of the syndrome of insulin resistance due to production of circulating autoantibodies has provided important insights on the role of the insulin receptor in insulin action and resistance. The patients with this syndrome generally have autoimmune disorders and produce circulating antibodies directed against the insulin receptor. Such antibodies cause insulin resistance by acting as competitive antagonists for endogenous or exogenous insulin. Accordingly, depending on the severity of the syndrome, patients can have varying degrees of insulin resistance ranging from manifestation of mild glucose tolerance test to severe hyperglycaemia requiring large amounts of exogenous insulin administration in order to overcome the competitive antagonism by the endogenous anti-insulin receptor antibodies.

Receptor and post-receptor defects in insulin action constitute target tissue insulin resistance which is a well-documented characteristic finding in NIDDM [5]. Acquired defects in insulin binding (typically manifested by decreased numbers of cell surface insulin receptors) are generally thought to result from endocytosis and down-regulation

of the receptors by the prevailing chronic hyperinsulinemia in obese NIDDM subjects. Such alterations in receptor numbers are acquired and reversible since the lowering of ambient insulin levels by appropriate therapeutic interventions is generally associated with return of the cell surface insulin receptor numbers towards normal.

Various mutations in the insulin receptor gene have been identified as the basis for the insulin resistance and hyperglycaemia in certain rare states of extreme insulin resistance (e.g. type A syndrome of extreme insulin resistance). In general, depending on the location of the mutation, the insulin resistance arises due to decreased insulin binding affinity (α -subunit mutations), impaired proreceptor processing and generation of mature functional receptor (mutations in the proreceptor α - β cleavage site), or impaired receptor autophosphorylation / kinase function (mutations in ATP binding or TPK catalytic sites). However, the insulin receptor coding sequence is normal in ordinary cases of NIDDM and receptor mutations do not explain the insulin resistance in this condition [3].

Insulin resistance due to post-receptor defect(s) can be due to any abnormality distal to the initial binding of insulin to its receptor. Although the exact nature of the post-receptor abnormalities remains unclear, the results of several studies indicate that defects in insulin receptor kinase function are associated with insulin resistance of NIDDM and obesity. However, such defects are acquired and largely reversible upon vigorous treatment of the patients. Finally, although insulin-stimulated glucose transport is impaired in NIDDM, the coding sequence for the insulin-sensitive glucose transporter (GLUT-4) is normal. Thus, this functional defect is also acquired and reversible.

In summary, much has been learned in recent years regarding the mechanism of insulin action and resistance. The basic knowledge gained in these areas is in turn increasingly applied to the elucidation of the pathogenesis of insulin resistant disease states such as NIDDM.

CONCLUDING REMARKS: FUTURE PERSPECTIVES ON THE RELEVANCE OF INSULIN RESISTANT STATES TO EVOLVING HEALTH CONDITIONS IN ETHIOPIA

Aside from its scientific and academic value, the consideration of the cellular and molecular mechanisms of insulin action and resistance also has important implications for the evolving health conditions in developing countries such as Ethiopia. Currently, in most of these countries, poverty, malnutrition and communicable diseases take such a heavy toll on human longevity and productivity that they tend to mask the importance of non-communicable diseases including diabetes mellitus as major health problems [6]. However, based on the experiences gained from other parts of the world as discussed below, it is expected that this situation will change drastically with economic development such that non-communicable diseases, especially NIDDM, will increasingly constitute major health problems.

Presently, diabetes mellitus (mostly NIDDM) and disorders of glucose tolerance are quite common world-wide and their prevalences are rapidly increasing as many societies attain economic development and urbanization with the accompanying nutritional change and adoption of sedentary life-style [2]. In the US, for example, diabetes and its complications constitute the third leading cause of morbidity and mortality resulting in over US \$90 billion of direct and indirect costs in 1992 [7]. To explain the unusually high prevalence of NIDDM in modernized societies, James Neel formulated the "thrifty genotype" hypothesis in 1962 [8]. It proposes that in traditional

populations subject to alternating periods of "feast and famine", a survival advantage was afforded to those with a metabolism which utilized and stored energy with maximum efficiency during periods of relative nutritional deficiency (in this context insulin plays a major role since it is considered a "thrifty hormone" that has both anabolic and anticatabolic actions). With modernization and the accompanying assured supply of highly refined calories, coupled with a sedentary life-style of urbanization, the thrifty genotype becomes disadvantageous and leads to obesity, insulin resistance, and the eventual development of NIDDM. One of the strongest supports for this hypothesis is derived from epidemiologic observations showing rapidly increasing prevalence of NIDDM among American Pima Indians and inhabitants of the South Pacific islands of Nauru as these two populations experienced relatively rapid changes from traditional to Westernized diet and life-styles [9 - 11]. Due to the limited information available on the prevalence and demographics of diabetes in Ethiopia and many developing countries such as in sub-Saharan Africa [12], the impact of demographic and nutritional changes on disease prevalence presently can not be ascertained in these countries. However, as a recent study from Cape Town in South Africa shows, urbanization of the African population is associated with a marked increase in the prevalence of NIDDM that could potentially approach or exceed the prevalence in many developed countries [13]. In view of the large-scale urbanization occurring in Ethiopia and throughout the developing world, such results have important implications for public health planning that the expected increase in NIDDM prevalence requires.

These considerations have important implications for the assessment of diabetes mellitus, especially NIDDM, as a growing health problem in Ethiopia. If the country successfully makes the hoped for transition to economic development and attains higher standards of living, the likely consequences of such changes will be increasing urbanization with its accompanying dietary and life-style changes. Concomitantly, the health impact of malnutrition and communicable diseases should decline while that of non-communicable diseases, especially insulin resistance, NIDDM, hypertension and cardiovascular disease will rise as has already happened in many economically developed countries. Accordingly, it will be prudent to set in motion appropriate educational and research endeavours that will address and plan for such predictable changes in the health sector of the country. Thus, a consideration of the cellular and molecular biology of insulin action and insulin resistance is of importance not only for the understanding of basic cell biology but also should contribute to the awareness of the growing importance of insulin resistance and NIDDM that is predicted to accompany socio-economic development.

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