The Role of Insulin in Regulating Ca2+-ATPase and Intracellular Calcium Homeostasis

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ABSTRACT

Insulin, in addition to being a pivotal metabolic hormone, plays a significant role in intracellular calcium homeostasis. Ca^{2+}-ATPase, an integral membrane protein found in the plasmalemma or organellar membrane of all cells, responsible for the maintenance of intracellular calcium homeostasis. It has been extensively studied with respect to its structure and mechanism of action, and more recently to gene expression. Though the concept of insulin mediated calcium transport is not new, an involvement of insulin in calcium homeostasis is still under discussion. Therefore, the analysis of the transporter responses to insulin would offer mechanistic understanding of calcium specific transporters to insulin and how cellular processes respond to insulin signaling in the intracellular milieu.

KEYWORDS: Ca^{2+}-ATPase, Insulin, Calcium homeostasis, PMCA, SERCA.

I. INTRODUCTION

A variety of cellular functions are regulated by intracellular free calcium concentration mainly depends on the functional status of the cell. Under physiological resting conditions, the cytosolic calcium concentration is maintained at or below a micromolar value, while in the extracellular fluids it is in the millimolar range [36], by Ca^{2+} homeostasis systems involved in extrusion and compartmentalization activities [5]and thus responsible for the generation and maintenance of steep ion gradients [38].To balance any inward leakage, the cell needs to extrude calcium and this function is accomplished by transport mechanisms such as the sodium-calcium exchanger or the outwardly directed active calcium pump which is the calcium-stimulated ATPase) localised in the endoplasmic reticulum (ER) membrane and in the plasma membrane (PM). These ion pumps built up and kept the concentration gradients at the cost of the energy from ATP [40].

Although the physiological and biochemical importance of activation of calcium influx has been well documented and repeatedly confirmed for many types of cells [30],the accurate signaling mechanism coupling ER to PM calcium channels has remained unknown so far [40]. It is evident from the previous studies that the depletion of intracellular calcium stores leads to the opening of calcium channels in PM and massive calcium entry into the cell [35]. As the activation of calcium influx depends on the filling state of ER calcium stores, these channels are called store-operated channels (SOC). This regulatory phenomenon was formerly known as the capacitative regulation of calcium entry [35]. On the other hand, several studies demonstrated that mitochondria play an important role in the regulation of SOC [14], [19], [13], [25]. In various cell lines deenergisation of mitochondria greatly impaired the store-operated calcium influx [39].

The activation of cell by extracellular stimuli like hormones or growth factors results in the elevation of low basal level of calcium concentration [40]. Among hormones, insulin has a central role in regulating cell metabolism, gene expression, growth, and differentiation. The mechanism by which insulin regulates cellular metabolism remains unknown although indirect evidence suggests that alterations in intracellular calcium are important. The role of intracellular calcium as a mediator of insulin action was originally proposed by Clausen *et al*. (1974) and by Kissebah *et al*. (1975), proposed that insulin triggers an increase in intracellular calcium which is responsible for the subsequent modification of metabolic activities [10]. Since then, considerable evidence favoring this hypothesis has been accumulated. Although some investigators failed to observe a relationship between calcium and insulin action, diverse aspects of insulin action have been demonstrated to be dependent upon extracellular and cytoplasmic Ca^{2+} [10]. However, it is interesting to highlight that calcium may be the signal molecule responsible for mediating the intracellular actions of insulin, and that the interaction of insulin with its receptor triggers an increase in intracellular calcium which is responsible for the subsequent changes in metabolism. Keeping in this view, the present review briefly explains the interaction of insulin with calcium transporters, responsible for maintaining a proper intracellular calcium homeostasis.

II. DIRECT ADDITION OF INSULIN INHIBITS A HIGH AFFINITY PLASMA MEMBRANE Ca^{2+}-ATPase

The cell maintains a large electrochemical gradient for ionised calcium between the cytoplasm and the extracellular environment. The plasma membrane may, therefore, be important in the regulation of calcium homeostasis, as a slight alteration in the processes maintaining this gradient could result in marked changes

in cytoplasmic calcium. One such process is the active extrusion of calcium from the cell by a high affinity calcium-stimulated ATPase (Ca^{2+}-ATPase) [7].

Among calcium transporters, plasma membrane Ca^{2+}-ATPase (PMCA), members of the large family of P-type ion pumps, found in the plasmalemma or organellar membrane of all cells, characterized by the formation of a phosphorylated intermediate (hence their name) during the reaction cycle [31]. Pershadsingh and McDonald (1979) identified a high affinity Ca^{2+}- ATPase in a plasma membrane-enriched subcellular fraction isolated from rat adipocytes which may provide the enzymatic basis for a calcium extrusion pump [32]. Similarly it has been well documented in the subsequent studies that these integral membrane proteins plays an important role in the regulation of cytosolic free calcium concentration, and it is assumed that in most cell types, it represents the major high-affinity mechanism for Ca^{2+} extrusion to the extracellular fluid [6], [37] and thus maintain a steep intracellular ion gradients [38]. It has been demonstrated that the Ca^{2+}-ATPase is specifically inhibited by the direct addition of physiological concentrations of insulin to the isolated plasma membranes. This effect suggests that direct regulation of calcium homeostasis may indeed represent an important event in the mechanism of action of insulin [32].

III. PLASMA MEMBRANE CA^{2+}-ATPASE OVER EXPRESSION REDUCES CA^{2+} OSCILLATIONS AND INCREASES INSULIN RELEASE

Glucose, the major physiological stimulator of insulin release, induces the release of the hormone from the pancreatic β-cell by generating both triggering and amplifying signals through distinct pathways [17]. In a study by Kamagate *et al*. (2002) examined the role played by cytosolic-free Ca^{2+} concentration oscillations and the over expression on glucose-induced Ca^{2+} oscillations in the process of insulin release [22]. Similarly, it has been evident from the previous studies that glucose generates large amplitude β-cell cytosolic-free Ca^{2+} concentration oscillations are thought to result from the opening of voltage-sensitive Ca^{2+} channels and that are synchronous to insulin release oscillations. Therefore, it has been suggested that oscillations of insulin secretion could be driven by such cytosolic-free Ca^{2+} concentration oscillations [15], [16], [22].

IV. INSULIN RECEPTOR SIGNALING AND SARCO/ENDOPLASMIC RETICULUM Ca^{2+}-ATPase IN β - CELLS:

Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is an intracellular membrane-bound enzyme that utilizes the free energy of ATP to transport Ca^{2+} against a concentration gradient and thus to maintain intracellular free Ca^{2+}levels within the physiological range. Ca^{2+} uptake into sarco/endoplasmic reticulum (SR) via SERCA is responsible for the removal of 90% of Ca^{2+} from the cytoplasm [2]. In particular, it has been evident that cellular Ca^{2+} is a critical element in β cell function and an increase in intracellular calcium is important for insulin exocytosis. β-Cell metabolism of glucose results in an increase in the ATP-to-ADP ratio, leading to closure of the ATP-sensitive K^+ channel, depolarization of the β-cell, and influx of extracellular Ca^{2+} through voltage-dependent Ca^{2+} channels [12]. The subsequent increase in intracellular Ca^{2+} then activates insulin exocytosis Likewise, altered Ca^{2+}metabolism has also been reported to affect β cell function, including insulin biosynthesis [27] and it has become apparent that the β cell also has many of the elements of the insulin receptor signal transduction pathway, including the insulin receptor and insulin receptor substrate (IRS) proteins 1 and 2 [3].

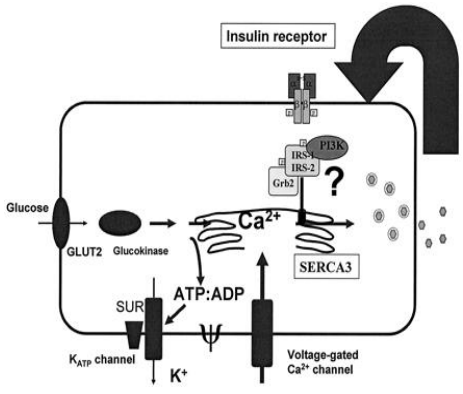

FIG. 1. Insulin receptor signaling in the β-cell (adapted from Borge et al., 2002). In this scheme, insulin has a positive autocrine feedback loop on its own secretion in the presence of glucose. Secreted insulin binds to the insulin receptor present on the plasma membrane of the β-cells, which initiates a signal transduction cascade mediated by the IRS proteins. Tyrosine phosphorylation of IRS-1 results in inhibition of the activity of SERCA located in the ER, which in turn causes an increase in cytosolic calcium and insulin secretion. Grb2, growth factor receptor-bound protein 2; K_{ATP}, ATP-sensitive K^+ channel; PI3K, phophatidylinositol 3-kinase; SUR, sulfonylurea receptor.

Studies with transgenic models have shown that the β cell–selective insulin receptor knockout and the IRS-1 knockout lead to reduced glucose-induced insulin secretion [3]. Similarly, over expression of the insulin receptor and IRS-1 in β cells results in increased insulin secretion and increased cytosolic Ca^{2+} [3]. The study also reported that this glucose-dependent interaction occurs at the level of IRS-1 and the sarco (endo) plasmic reticulum calcium ATPase, the calcium pump of the endoplasmic reticulum. However, it has been postulated the existence of an autocrine-positive feedback loop of insulin on its own secretion involving interaction with the insulin receptor signal transduction pathway (Fig. 1) and regulation of intracellular calcium homeostasis [3].

V. SARCO/ENDOPLASMIC RETICULUM Ca^{2+}-ATPase MODULATE INSULIN SECRETION:

To function as an intracellular Ca^{2+} store, the sarco/endoplasmic reticulum needs to express at least three different types of proteins [34]. (1) Ca^{2+} pumps for uphill transport of Ca^{2+} from the cytosol to the lumen; (2) luminal Ca^{2+}-binding proteins for storing Ca^{2+}; and (3) Ca^{2+} channels for the controlled release of Ca^{2+} to the cytosol along its electrochemical gradient. Although the ER is generally assumed to form a continuous compartment, it can be heterogeneous at the level of its Ca^{2+}-handling proteins [26]. A heterogeneous distribution allows on the one hand localized Ca^{2+} pumping and release, and on the other hand, the setting up of Ca^{2+} signals without disturbing Ca^{2+}-dependent processes within the ER lumen [33], [1], [26], [29]. It has been reported that the exocytosis of insulin-containing granules from pancreatic beta-cells is tightly regulated by changes in cytosolic Ca^{2+} concentration [20]. Similarly, previous studies documented the involvement of leucin, which induces insulin secretion in the absence of glucose, suppressed pancreatic islet Ca^{2+}ATPase activity [24]. Kulkarni *et al*. (2004) [23] reported that deletion of IRS-1 (insulin receptor substrate) in knockout mice islets dramatically reduced expression of SERCA2b and 3 genes. Furthermore, some studies demonstrated that IRS-1 and SERCA were localized in ER vesicles from beta-cells and could interact directly with one another [3]. These authors found that pharmacological inhibition of SERCA in beta-cells resulted in enhanced secretion of insulin [3] and chronic activation of insulin receptor signaling by IRS-1 over expression in beta-cells inhibited gene expression of SERCA [40]. Similarly, in insulin-dependent diabetes of acute and chronic streptozotocin rat models a 30% decrease of the SERCA mRNA level was also described [11]. These findings support the hypothesis that Ca^{2+} - ATPases are involved in the specificity of islet response and in insulin secretion. [18].

Insulin-dependent Ca^{2+} mobilization in skeletal muscle cells and cardiomyocytes were reported by Contreras-Ferrat *et al*. (2014) [8]. Specifically, insulin activates the sarco-endoplasmic reticulum (SER) channels that release Ca^{2+} into the cytosol and the study signifies a possible involvement of the Ryanodine Receptor (RyR) and the inositol 1, 4, 5-triphosphate receptor (IP3R). In skeletal muscle cells, a rapid, insulin-triggered Ca^{2+} release occurs through RyR, that is brought about upon S-glutathionylation of cysteine residues in the channel by reactive oxygen species (ROS) produced by the early activation of the NADPH oxidase (NOX2), which further promotes the mitochondrial Ca^{2+} uptake. However, in cardiomyocytes insulin induces a fast and transient increase in cytoplasmic free calcium concentration through L-type Ca^{2+}channels activation. In both cell types, a relatively slower Ca^{2+} release also occurs through IP3R activation, and is required for GLUT4 translocation and glucose uptake [8].

VI. CYTOSOLIC FREE CALCIUM CONCENTRATION IN OBESITY AND INSULIN RESISTANCE

Insulin resistance may be caused by a reduced number of insulin receptors, by mutation of insulin receptors leading to reduced affinity of insulin binding or by the reduced activity of the IRTK. The plasma insulin concentration is compensatively increased in insulin resistance. The increased insulin concentration can cause insulin resistance by down-regulating insulin receptors and desensitizing post receptor pathways [21]. The most important risk factor in insulin resistance is obesity and obesity-induced insulin resistance may involves an alteration in Ca^{2+} metabolism plus other mechanisms. It has been hypothesized that abnormal cellular calcium handling, particularly elevations in cytosolic free calcium concentrations, may represent a common intracellular abnormality (a missing link) that is responsible for the frequent co-existence of insulin resistance and hypertension [10]. The data shown that sustained elevations of cytosolic free calcium in insulin target cells, such as are observed in patients with obesity and non-insulin-dependent diabetes mellitus and in some patients with hypertension, may lead to the development of insulin resistance. Although the mechanisms that lead to such increases are not yet well understood, they appear to include an enhanced influx of calcium via calcium channels [10]. Similarly, there are several reports suggesting the possible involvement of obesity-associated high levels of intracellular free Ca^{2+} in cellular resistance to insulin [42], [28]

VII. CONCLUSION

It is concluded that Ca^{2+}-ATPase, assumed to participate in active transport of Ca between intracellular compartments and to constitute a Ca-accumulating system which returns the cytosolic free Ca concentration to the resting state plays a significant role in insulin action. Additionally, there are several novel mechanisms, including post-translational modifications and microRNAs (miRNAs) are emerging as integral regulators of Ca^{2+} transport activity and its interaction with insulin action (not included in the present review). Currently, several clinical trials are underway that utilize novel therapeutic approaches to overcome impaired cellular Ca^{2+}homeostasis in the pathogenesis of impaired insulin secretion and in the pathogenesis of impaired insulin action. Taken together, the literature indicates that regulation of cytosolic free calcium coupled with insulin action, may be an important target for the development of therapeutic strategies in the prevention and treatment of obesity.

VIII. REFERENCES

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