PHYSIOLOGICAL AND DYSFUNCTIONAL SECRETION OF INSULIN

BOGDANA VIRGOLICI[1*], PETRUTA ALEXANDRU[2], DANIELA LIXANDRU[1]

[1] *UMF „Carol Davila" Bucharest, Romania*
[2.] *Institute of Biochemistry of the Romanian Academy, Splaiul Independentei 296, 060031Bucharest, Romania*

Glucose triggers insulin secretion trough a pathway that involves the cytoskeleton of the pancreas beta cell. Transient F-actin reorganization allows the insulin granules access to the plasma membrane for subsequent docking, fusion (mediated by SNARE complex) and insulin release. Small Rab-, Ras- and Rho-family GTPases are involved in insulin secretion. Physiologically, a balance is maintained between the load of insulin translation into the endoplasmic reticulum (ER) and the folding capacity of the ER. An increased demand for insulin negatively affects the homeostasis of β cells and leads to ER stress. The PERK (protein kinase RNA-like endoplasmic reticulum kinase) pathway keeps the capacity of the pancreas beta cell to respond adequately to the ER stress. Chronically elevated glucose creates a huge stress on the *β*-cell which cannot be relieved and this lead to dysfunction. The best example is insulin resistance which leads to type 2 diabetes mellitus development. Fasting intact proinsulin and the ratio proinsulin/C peptide are biomarkers for β-cell dysfunction, insulin resistance and cardiovascular risk in type2 diabetes mellitus patients. Understanding better their significance is important for diabetes mellitus management and for finding new therapies aiming at preventing diabetes.

Key words: proinsulin, exocytosis, SNARE complex, C peptide , diabetes mellitus

INTRODUCTION

Almost 100 years ago, the romanian physiologist Nicolae Paulescu, using pancreatic extracts from dogs reported the discovery of a substance called pancrein, which lowered glycaemia in diabetic dogs. Nowadays, it is known that insulin, the product of the β-cells of the Langerhans islets, is initially synthesized as a precursor (preproinsulin), from which the mature hormone is excised by enzymes: prohormon convertase 2 (PC 2) and 3 (PC3) and carboxypeptidase E. Studies on cell culture demonstrated that high demand of insulin, triggered by chronic glucose exposure, is followed by co-ordinate increased transcription for both preproinsulin and PC2 and PC3 enzymes (1). Inherited defects in preproinsulin convertases action were described

[*] Corresponding author (E-mail :hvirgolici@yahoo.com)

in human and experimental animals but their importance in diabetes mellitus type 2 is weak (2, 3).

Intragranular medium and chaperone-like proteins influence preproinsulin cleavage process (4). Proinsulin is folded in the endoplasmic reticulum (ER) lumen, whereby three disulfide bonds are formed. Properly folded proinsulin is then delivered to the Golgi apparatus and packaged into secretory granules. Acidifying clathrin-coated secretory vesicles represent the proper environment for proinsulin cleavage (5,6**).**

The β cell is electrically excitable and couples changes in extracellular glucose levels to insulin exocytosis. As plasma glucose rises, GLUT-2 receptors on the cell surface transport glucose across the plasma membrane and into the cytosol. As glucose is metabolized and ATP levels increase, the rise in the ATP/ADP ratio closes K^+ATP channels, resulting in cell depolarization. In response, voltage-dependent calcium channels open and the Ca^{2+} influx initiates the beginning of biphasic insulin exocytosis. As insulin promotes glucose uptake by the target organs, plasma glucose falls, reversing this pathway to end the secretory process (7, 8).

EXOCYTOSIS IN BETA CELLS

Insulin secretion is biphasic. Beneath the plasma membrane of the beta cells, insulin granules are located and form the readily-releasable pool (RRP). KCl and glucose trigger the first phase of insulin release which develops rapidly and peaks within 5-10 minutes after stimulation. Releasing of prepacked vesicles from RRP and initiation of RRP replenishment represent the first phase of insulin secretion. But, the vast majority of insulin granules are located in the cytosol and the second phase release of insulin constitutes the recruitment of these vesicles to refill the RRP. The second phase of insulin secretion occurs exclusively following stimulation by nutrients. Also in contrast to the first phase, the second phase release develops slowly, but can be sustained over several hours during elevated glucose conditions (8,9,10).

Insulin exocytosis is regulated by SNARE complexes which are a superfamily of proteins that function in membrane fusion. The majority of SNARE proteins are anchored to the membrane at the C-terminal. The SNARE core complex is composed of three protein in a heterotrimeric 1:1:1 ratio: SNAP (soluble N-ethylmaleimide-sensitive factor attachement protein), VAMP (vesicle-associated membrane protein) and Syntaxin. The SNARE complex is extremely stable and its dissociation is mediated by an ATPase chaperone, that requires αSNAP to bind the core complex. Dissociation of this complex allows the components to recycle for another round of membrane fusion. Because all these proteins: VAMP, syntaxin, and SNAP-25 bind to αSNAP, they have been referred to as αSNAP receptors or SNAREs (8,11).

Syntaxins are transmembrane anchored proteins and are localized on cell compartments such as the ER, Golgi and endosomes. Pancreatic β-cells contain syntaxin (Syn) isoforms 1, 2, 3 and 4. Syntaxin proteins have “open” or “closed” conformation (10,12). Into the SNARE complex, syntaxin protein, in the open conformation, stops current activity trough K+ATP channel inhibition. The consequence of cell depolarisation is increased calcium influx which end with

exocitosis (9). While Syn-1 is involved in the first phase of glucose stimulated insulin secretion, Syn-3 and -4 participate at both phases of insulin secretion. Syn-2 is not involved in insulin secretion (13,14). Syn-4 is present on plasmatic and on vesicules membranes in pancreatic β cell beeing important in insulin exocytosis, while in adipose tissue, muscle and liver helps for GLUT4 translocations. It acts in association with MUNC18c protein. Munc18 (SM) proteins are a family of cytosolic proteins which have been isolated in various species of mammals and yeast lines. Munc18-c appears to closely mediate Syn-4 accessibility to VAMP-2, which has implications for granule doking at the plasma membrane after glucose stimulation (8,12,15). Several additional binding partners on SM-syntaxin interactions were described. As an example, in islet β cells, Doc2β protein (the Munc accessory factor double C2-like domain-containing protein β) overexpression increases glucose stimulated insulin secretion by 40% and the siRNA-mediated depletion of Doc2β attenuates insulin release (8).

Cytoskeleton-remodeling Rho-family GTPases mediate insulin secretion. Cdc42 and Rac1, the F-actin-remodeling small Rho-family GTPases, participate to the second phase of insulin release. Several Rab- and Ras-family GTPases are also known to be involved in granule doking and/or priming but their roles in beta cells will become more clear in the future (9,12) (see Fig. 1).

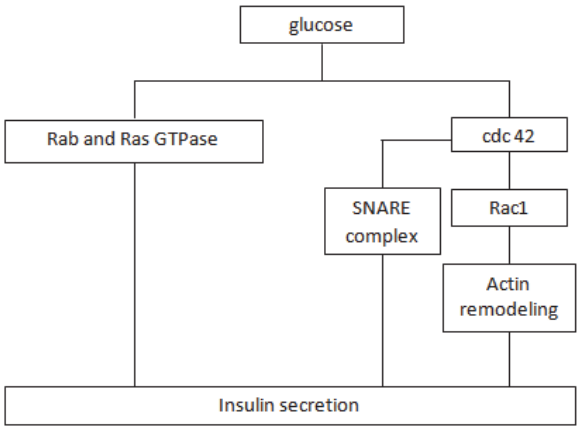


Fig. 1. Mediators of insulin secretion triggered by glucose.

DEGRADATION OF BETA-GRANULES

Aged β-granules are those that remained unused in the storage pool for approximately 5 days. Then, they follow an intracellular degradation mechanisms, either via crinophagy and/or autophagy. When an imbalance between insulin production and secretion arises, as may occur in type 2 diabetes, autophagy further increases to maintain β-granule numbers. If autophagy becomes chronic, it could contribute to β-cell loss (16).

ENDOPLASMIC RETICULUM STRESS AND INSULIN SECRETION

A great demand for insulin is followed by an imbalance between the physiological load of insulin translation and the folding capacity of the ER and ER stress is generated. The unfolded protein response (UPR) is an intracellular system that combates the ER stress. The cellular response and susceptibility to ER stress is likely to be cell-type specific, but three pathways are generally described: ATF-6, IRE1 and PERRK (17). If the overload of unfolded proteins in the ER is not resolved by the UPR, apoptosis can occur via JNK kinase (6).

In the pancreatic *β*-cell, the PERK (protein kinase RNA-like endoplasmic reticulum kinase) mediates the cell response to ER stress (17,18). Prolonged ER stress, via PERK, activates the transcripton factor ATF4, which leads to CHOP gene expression and finally the cell enters apoptosis (see Fig. 2).

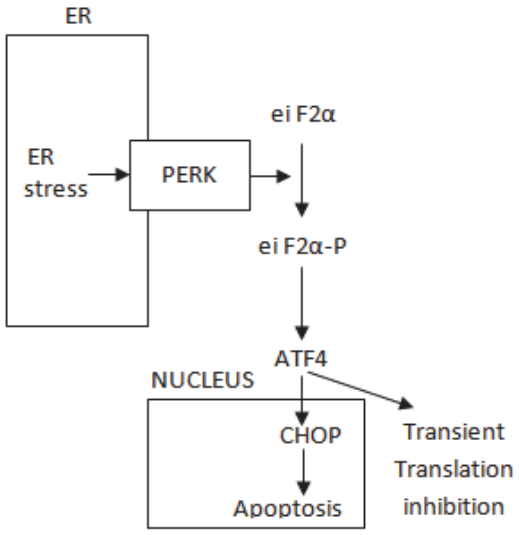


Fig. 2. The cell response to the ER stress mediated by PERK and eiF2α proteins**.**

The second role for PERK, in the form of PERK-eIF2*α* system, is to modulate insulin translation under physiological situations. Under normal fasting conditions when glycaemia is low and insulin translation is not stimulated, the levels of phosphorylated eIF2*α* are actually high (17). In postprandial state, the phosphatase PP1 is activated and decreases the level of phosphorylated eIF2*α*. *The effect is an increased overall translation* including translation for insulin, which will continue as long as glycaemia is high. Increased insulin translation will generate ER stress and via PERK pathway an increased eIF2*α* phosphorylation is initiated and finally, translation is attenuated. Therefore, the physiological balance between increased and inhibited transcription of the insulin gene, according to glycaemia, is maintained by tight control of PERK and eIF2*α* protein levels (17,19).

FACTORS THAT INFLUENCE INSULIN SECRETION

Chronically elevated glucose creates stresses on the *β*-cell which can lead to dysfunction. Increased oxidative stress and low antioxidant defence are important pathogenic culprits (17).

Long-chain fatty acids metabolism in peroxisomes generate hydrogen peroxide which is not mitigated because β-cells almost completely lack catalase. The increased oxidative stress will lead to β-cell dysfunction and ultimately β-cell death (20). Oleate and palmitate trigger ER stress via Ca(2+) depletion in ER and elicit UPR response, resulting in either survival or apoptosis outcomes (20).

Chemical chaperones and incretin hormons have been used to protect β-cells from lipotoxic ER stress (21). Glucagon like peptide-1 stimulates insulin secretion via increased cAMP and stimulates beta cell differentiation and proliferation via PI3K (phosphatidil-inositol 3 kinase). For example, in patients with type 2 diabetes mellitus, GLP-1 rescues the first-phase defect in insulin secretion and this is mediated in part through cAMP-Epac2 signaling (22).

CELL CULTURE STUDIES FOR INSULIN SECRETION

Even the cell lines used in pancreas research work are not similar to physiological β cells, they are important tools for studying the molecular events underlying secretion of insulin. Insulinoma-derived cell lines contain mainly insulin and small amounts of glucagon and somatostatin. They have the advantage of unlimited growth in tissue culture, but they are different in their insulin-secretory responsiveness to glucose. As an example, βTC mouse cell lines manifested hypersensitivity to glucose and their dose-response curve is markedly shifted to the left, while INS-1 rat cells retain normal glucose regulation of insulin release. PANC-1 and MIA-PaCa human cell lines have properties of poorly diferentiated adenocarcinomas, they respond to intestinal hormons and are used mainly for evaluation of new pancreas anticancer agents.(23,24)

In 2011, Ravassard *et* al. obtained a human pancreatic cell line, *EndoC*-β*H1* cells, that secreted insulin in response to glucose (25). Nowadays, there is an urgent need to do research work on human cell lines which mimic the physiological secretion of insulin instead of the cancerogenic rat or mouse β cell lines (24,26).

CLINICAL STUDIES FOR INSULIN SECRETION

Physiologically, inside the beta pancreas cells, proinsulin is cut by carboxypeptidases into insulin and C peptide. Aging is associated with a loss of beta cells function which is reflected by a higher proinsulin and proinsulin/insulin ratio values (27,28). In a healthy subject, only a minor quantity of uncleaved intact proinsulin is postprandially released into the circulation. Intact proinsulin can bind to the insulin receptor. However, it has only 10–20% of the glucose-lowering effect of insulin, but almost the same adipogenic activity (29,30). Lifestyle factors can influence beta cell function. Brhyni *et* al. demonstrated that proinsulin/insulin ratio was positively correlated with smoking and negatively with heart rate (27).

Insulin resistance and beta cell dysfunction precedes type 2 diabetes mellitus. The gold standards to assess insulin sensitivity is the euglycemic hyperinsulinemic clamp and to estimate the beta cell function is the hyperglycemic clamp (31).

Nowadays, in clinical and epidemiologic studies, indices derivated from calculated parameters of the oral glucose tolarance test, HOMA-IR (homeostasis model assessment of insulin resistance index), C peptide**,** are more used as surrogat markers for assessing both insulin sensitivity or beta cell function (32,33).

High fasting proinsulin level is also a surrogate marker of insulin resistance or β-cell dysfunction and reflects pancreatic β-cell stress because of the increased demand for insulin or abnormalities of insulin processing and secretion, respectively (33,34). It seems that proinsulin provides a better prediction of cardiovascular diseases than insulin (34).

The conclusion of the Hoorn study was that "proinsulin concentration is an independent predictor of all-cause and cardiovascular mortality"(35). Lindahl B. *et* al. studies demonstrated that proinsulin predicts the first-ever stroke (36) and miocardial infarction (37). Insulin resistant subjects have high plasma proinsulin (38) levels and proinsulin stimulates PAI-1 secretion and inhibits fibrinolysis (39). Insulin resistance atherosclerosis study, a prospective, multicenter, epidemiological study found a relationship between low acute insulin response and high proinsulin levels with the risk of diabetes and cardiovascular disease (40).

Other studies showed that high plasma fibrinogen concentration (41) and microalbuminuria (42), known as cardiovascular risk markers, are correlated with higher levels of proinsulin. In diabetic subjects the positive correlations between proinsulin levels and dyslipidemia variables are also arguments for considering proinsulin as a cardiovascular risk marker (43).

However, recent research demonstrated that proinsulin is not directly involved in cardiac diseases pathogegenesis. The reasearchers found nine genetic loci that were associated with fasting proinsulin concentration (44).

It is estimated that in a healthy subject, the ratio proinsulin/insulin is less than 15-20% and a higher level than 20% is a predictor of future type 2 diabetes mellitus (39).

Zethelius B *et* al. demonstrated that proinsulin plasma level predicted type 2 diabetes over a 7-year period in eldery men (45). Random proinsulin levels and proinsulin/C peptide ratio were useful to identify relatives at high risk of type 1 diabetes and these results were added to immune and genetic tests (46).

In gestational diabetes, high plasma levels for proinsulin have the meaning of insulin resistance and do not reflect dysfunction of beta cells (47).

Although proinsulin secretion is not associated with diabetes duration and proinsulin secretion may precede the onset of clinically overt type 2 diabetes mellitus, fasting proinsulin concentration can be used for type 2 diabetes mellitus staging (30). Pfuziner A. emphasezis that in the earlier stages of diabetes mellitus type 2, drugs which stimulate insulin secretion can be used, but in beta cell dysfunction, in advanced stages of diabetes, these treatment „should safely not be recommended, because further proinsulin secretion may result in an increased cardiovascular risk".

In healthy subjects, fasting plasma insulin concentration ranges from 10 to 60-90 pmol/L, fasting C-peptide between 0.5-2 nmol/L (48) and proinsulin average is 6.7 +/- 1.7 pmol/l (49). Immunoassay methods are used for these measurements. In deficient C peptide states, its administration by infusion is beneficial but when it is in

excess, it produces undesirable effects, activating proinflammatory pathways. In diabetic patients high values for C peptide were associated with macrovascular complications. (50). In type 2 diabetic patient, serum C peptide values lower than 1 ng/ml in the fasting status or lower than 2ng/ml in the postprandial condition have the meaning of beta cell dysfunction and the diabetologist should recommand insulin administration (51).

In comparison with proinsulin/ insulin ratio, a better predictor for diabetes mellitus is the ratio proinsulin/ C-peptide because C-peptide is not affected by hepatic insulin clearance (52). It is known that after glucose stimulation, C peptide is cosecreted from the pancreas beta cell in the molar ratio 1:1 with insulin. The extensive and variable extraction of insulin by the liver makes improperly the estimation of beta pancreas cell secretion from peripheral insulin (53). Also, direct measurements of endogenous insulin by immunoassay interferre with the insulin analogs that the patient is taking. Moreover, these assays are affected by the presence of antiinsulin antibodies (54). So, the American Diabetes Association (ADA) recommands C-peptide measurement for estimation and monitoring beta pancreas cell function (55). Additionally, the use of C-peptide and insulin measurements together provides valuable information for the evaluation of hypoglycemia and the diagnosis of insulinoma (56).

Understanding molecular mechanisms of preproinsulin maturisation and β-cell failure is important for finding new therapies aiming at preventing diabetes.

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