# Editorial
# The Endoplasmic Reticulum – the possible site of the primary beta cell defect in diabetes mellitus

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The endoplasmic reticulum (ER) is a membranous cell structure that is connected with the pores of the nucleus membrane and forms a network of canals around the nucleus, network that is already connected to the Golgi apparatus. Part of the ER has ribosomes attached to its walls and is known as the Rough Endoplasmic Reticulum (RER), the site of protein synthesis. The other part of the ER is known as the Smooth Endoplasmic Reticulum (SER) and represents the site for the synthesis of complex lipid molecules. The ER and the Golgi apparatus can represent up to one third of the cell volume.

Since the translation of protein molecules takes place in the ribosomes attached to the walls of the RER, the newly formed protein molecules pass quickly (tens of seconds – minutes) inside the ER.

The pancreatic beta cells, with a very intense secretory activity, have also a very well developed ER. Even if the main synthesis product of the beta cell are the molecule of proinsulin and proamylin (and finally insulin and amylin), the production of insulin and amylin does not represent more than 50% of the total protein synthesis (in a state of relative repose of the insulin secretion, this percentage decreases below 50%). The rest of the proteins secreted by the pancreatic beta cell are represented by different structural pieces included in the structure of the various cell organelles (mitochondria, lysosomes, etc.), or other functional proteins (enzymes, ion channels, receptors, etc.) [1-3]. A number of these proteins contribute to the continuous generation of the insulin secretory vesicles. Not only the protein component of the vesicle membrane is secreted in the RER but also their proteic content (proinsulin, insulin, C peptide, proamylin, amylin, PC2 and PC3 convertases, carboxipeptidase E, a number of chaperon molecules and other small but numerous peptides with uncertain functional significance).

The main activity that takes place inside the pancreatic beta cell is that of proinsulin processing (folding and proper packaging of these molecules), a phenomenon very important for allowing the access to the molecule of the enzymes for proinsulin cleavage in insulin and C peptide, cleavage that takes places during the process of vesicle generation inside Golgi apparatus [4].

A wrong folding and packaging of proinsulin inside the ER will have three main consequences:

(**a**) the molecules will occupy a much larger space (physically) producing the so called crowding stress [5], which, if not controlled, can lead to the blocking of the traffic of proinsulin and proamylin molecules towards the Golgi apparatus and the secretory vesicles;

(**b**) a defect in the sorting process of the miss-folded proinsulin molecules, which normally are directed towards the un-regulated secretory pathway (the constitutive pathway), will result in their inclusion in the secretory vesicles where they will remain up to the final stages of exocytosis [6-8]. Also here takes place the sorting of the rest of synthesised proteins that will be conducted towards the corresponding cell organelles. As a consequence, the flux of molecules inside the ER is very high (tens of thousands of molecules per second). Without a good functioning of this system, the traffic of the protein molecules inside the ER can be hardened or even blocked in any moment;

(**c**) if the crowding stress surpasses a critical level, the pressure sensors from the ER membrane represented by some complex proteins, will trigger the reaction known as *unfolding protein response* (UPR) [9-13].

Any defect in the ER will have repercussion on the successive insulin secretory stages that take place initially in the Golgi apparatus and then in the secretory vesicles. Our hypothesis, based on the arguments we shall present later in this paper, suggests that an important percentage of the general population inherits a bigger or smaller defect in one ore more of the ER proteins involved in the good functioning of the molecular traffic inside this cell structure, including also the folding and packaging of proinsulin. This leads to the persistence of an increased percentage of proinsulin inside the secretory vesicles, which explains the high percentage of plasma proinsulin. [6-8]

In order for this phenomenon to occur, all the mechanisms for the regulation of the ER function have to be surpassed. If these regulation mechanisms can restore the traffic of proinsulin on the pathway ER – Golgi apparatus insulin-secretory vesicles – exocytosis, a minor beta cell defect can be compensated and diabetes will remain only a potential phenomenon, the clinical apparent form of the disease occurring only in the case of an increased beta cell demand. In the rare cases for which the defect in the processing of proinsulin is very high, the agglomeration of the molecules and the complete blocking of the ER will lead to the extreme outcome, i.e. the trigger of cell apoptosis. [14-17].

**Unfolding protein response (UPR)** is the physiologic reaction includes several ER membrane proteins: protein kinase RNA – dependent – like ER kinase activating transcription factor 6 (ATF6) and inositol – requiring ER to nucleus kinase 1 (IRE 1). This reaction aims to decrease ER protein levels by: (1) attenuating translation of pre-proinsulin mRNA at the level of ribosomes (by activating the protein kinase PERK); (2) up-regulating the expression of genes encoding ER chaperons (by activating ATF6) in order to increase the folding capacity of ER; (3) degrading misfolding protein via ER associated degradation pathway [14]; (4) degradation of mRNAs encoding ER targeted proteins by IRE1 (inositol requiring ER to nucleus kinase 1) decreasing the production of new proteins in ER [13,15,16].

These reactions have as aim the decrease of the molecule agglomeration inside the ER, de-blocking the pathways for the transport of proinsulin, but also of other molecules that follow to be included beside proinsulin in the secretory vesicles.

Properly folding of proinsulin in ER is made according to the further disulfide linkage between the A and B chain of insulin insuring their correct alignment. This process is followed by a packing the molecule in a more small volume, making it accessible to the enzymatic splitting which start already in ER and is completed in Golgi apparatus and in clathrin coated nascent secretory vesicles. The last process takes place in the *cis* and *trans* Golgi network. The assembly of the various molecules given the shape of the secretory vesicles is an ATP – dependent process, needing $Ca^{+2}$, guanosine triphosphate and some chaperon proteins.

Our hypothesis regarding a bigger or smaller defect in the ER as the primary cause of diabetes mellitus is based on the finding that in all the phenotypes of diabetes, plasma proinsulin is more or less increased in the majority of cases. Placing the insulin-secretory defect inside the ER is logical since any defect at this level will present in all further steps of the insulin secretory pathway, affecting the maturation of the secretory vesicles with the persistence of an increased amount of intact proinsulin inside the secretory vesicles. That explains the increased proinsulin levels also in the peripheral circulation. A β cell defect located only the secretory vesicles is less probable since the faith of the insulin-secretory vesicles inside the ER and the Golgi apparatus. The secretory vesicles have no obvious mechanisms for the correction of some defects appeared in the previous steps of their formation.

The defect in the processing of proinsulin appear early during disease progression and can aggravate or not according to the intervention of various environmental factors. The case of type 1 diabetes, which needs the presence of a second genetic defect (in the function of the immune system), requires a separate discussion. In the case of type 2 diabetes, the problems that arise are the following: in what degree the defect in the processing of proinsulin affects all the pancreatic beta cells or just part of them? The answer to this question is very important since the involvement of just a part of the beta cells, with a decreased lifespan for these cells, would imply that the physiological beta cell regeneration process could potentially match the apoptosis process maintaining the β cell mass for several decades. Other several decades will be necessary for the beta cell number to decrease below threshold required for the onset of overt hyperglycaemia. The balance between beta cell apoptosis / regeneration can explain the large spectrum of age for the onset of the glycemic decompensation, which can be anywhere between 5 and 80 years. Finally, if our hypothesis is correct, then the genetic predisposition for diabetes can be much more frequent than currently estimated. Even in the families with a diabetic heredity, an insulin-secretion defect can remain silent and latent for lifelong if some other external diabetogenic factor (from those recognized in the modern lifestyle) would not intervene. Analysing a much larger population it is not excluded to find out that the cases with a predisposition for diabetes, attested by the increased level of proinsulin, are much more frequent than currently believed.

Recently, an Australian group [13] published several papers regarding the relationship between type 2 diabetes and the ER stress. The experimental models used by

the authors are inspired by the hypothesis of lipotoxicity/glucotoxicity as the potential diabetogenic factor. Indeed, the prolonged incubation of the MIN6 insulin secreting cells with saturated fatty acids leads to an extensive ER response, explaining the apoptosis which can occur in this condition. A quite similar ER stress activation has been found in the pancreatic islets in diabetic db/db mice and also in archival formalin-fixed paraffin – embedded pancreatic tissue obtained from 11 diabetic patients and 12 non-diabetic patients. As they expected, type 2 diabetes was accompanied by reduced immunostaining for insulin and enhanced production of the pro-apoptotic BAX (bcl2- associated x protein). No data has been given about the age or duration of disease or their treatment and metabolic control. The main intensity of the immunostaining was significantly higher in diabetic patients vs. non-diabetic subject for HSPA5 (heat shock 70kDa protein 5), DDIT3 (DNA- damage inducible transcript 3, known as Chop) and DNAJC3 (DnaJ – Hsp 4 – homologue C3, known as p58).

The histological images presented by the authors are illustrative for the hyperexpression of these molecules in the Langerhans islets from the pancreas of diabetic patients, without being possible to make a clear distinction between the cells for which these alterations were identified. The authors suggest these alterations could be the expression of the endoplasmic reticulum reaction to stress induced by the plasma FFA and glucose increase. Evidently, the explanation is simplistic since the data obtained in a single experiment performed on isolated cells cannot automatically transfer to the interpretation of human diabetes. In the same time, the hypothesis of lipotoxicity / glucotoxicity cannot be used for the explanation of diabetes pathogenesis (as the authors suggest), since the increase in blood glucose, and in great proportion of the FFA, appear late in the evolution of diabetes, when the beta cell mass has already decreased below half of the initial value. However, the immunohistochemical documentation of the ER stress reaction, both in diabetic mice and humans, represent an objective element that could benefit from a more proper interpretation than that provided by the authors. Even if for this reaction the authors mention also the expression of HSPA5, a component of the UPR belonging to the chaperones family with a protective role against the ER stress, it is evident that their attention was excessively focused on the pro-apoptotic effect of this reaction, reaction in fact usually present in the beta cells in T2DM and having moreover a character of protective and corrective reaction and not of pro-apoptotic reaction.

More strange is the absence of a referral to the consequences of ER stress on the defects in the processing of proinsulin at this level, with a great impact both on the exocytosis itself and on the increased levels of proinsulin.

Apart the main insulin secretor line (pre-proinsulin/proinsulin/insulin), an important pathogenic role for diabetes may have the β cell secretory line, pre-proamylin/proamylin/ amylin, which has been put in light by Westermark and his school (Hull, Paulsson). About the crucial role of these two secretory lines for the pathogenesis of diabetes, we will come back in the next issue of this journal.

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