Disruption of disulfide bonds of insulin receptor as a cause of insulin resistance.

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Abstract:-

Background

Many theories have been put forward to explain insulin resistance in DM2. The cause of insulin resistance still remained an enigma till date. Defect in insulin signaling pathway is one such possibility considered. For insulin signal transduction to occur downstream, the insulin should be in tetrameric, holo-enzyme form for the conformational changes and auto-phosphorylation steps to take place. A prerequisite to this is, linkage of the two α-sub-units and α,β -sub-units by disulfide bonds. Without this, the receptor is in the α,β dimer half-enzyme form, devoid of any binding affinity to the ligand or auto-phosphorylating activity.

Aim

To explore, disruption of the disulfide bond formation, as a possible cause of insulin resistance in DM2.

Conclusions

Under physiological conditions of carbohydrate metabolism as a source of energy, disulfide bond formation occurs normally. But when energy metabolism switches to β-oxidation of fats, disruption of disulfide bond formation of the insulin receptor occurs, leading to insulin resistance in DM2.

Key words:- Tetramer, ligand, auto- phosphorylation, disulfide bond, β oxidation.

Introduction:-

Hyperglycemia, in the presence of high levels of insulin in blood, is the hall mark of insulin resistance in DM2. Despite many mechanisms suggested, the issue of insulin resistance in DM2 is yet to be resolved. While resistance observed is to the endogenous insulin, the exogenously administered insulin is still effective. This suggests that the insulin resistance in DM2 is, relative and reversible. The defect in Signal transduction of insulin is believed to hold the key to insulin resistance. The role played by the disruption of disulfide bond formation of the insulin receptor as a cause of Insulin resistance in DM2 is explored.

A brief review of relevant literature:-

The insulin receptor:-

The readers are directed to the invited review article, "The insulin receptor structure, function and signaling" by LEE Jongson and PaulF,Pilch (1)for extensive and comprehensive review .

The insulin receptor (IR) is a trans-membrane protein having intrinsic tyrosinase activity. It's two dimensional structure has been elucidated since long. The IR has two α-and two β- subunits. The α- sub unit is extra-cellular and has the ligand (insulin) binding site/sites as detected by affinity labeling protocols (2,3,4). Besides it has a cysteine rich region. The β -sub unit has three compartmental regions - the extracellular, the transmembrane and cytosolic domains. The cytosolic Tyrosine Kinase domain has ATP binding consensus sequence and three clusters of Tyrosine residues that can be phosphorylated in response to insulin action. The three clusters are found in juxta-membrane domain residues, the "tri-tyrosine" residues in the tyrosine kinase domain and the carboxy-terminal residues. The insulin ligand binds to one α- sub unit in such a way that it precludes attachment of a second ligand to the other α-sub unit. The second α- sub unit shows negative co operability. The physiologically active form of the receptor is the tetramer which is a functional dimeric protein complex. This is the holo-enzyme which has high affinity to the insulin ligand and has the full phosphorylating activity.(5,6) α,β heteromer is devoid of full ligand affinity or has any phosphorylating activity. The potential receptor- hormone contact sites are –residues 20-120 (7) the

disulfide rich region (8,9) and around the residue- 390 (10) region just to the carboxy side of the disulfide -rich region.

The disulfide bond formation :-

The role of protein disulfide isomerase (PDI) in disulfide bond exchange:-

The protein disulfide isomerase is an enzyme in the endoplasmic reticulum (ER). It catalyzes the formation of disulfide bonds between cysteine residues within a protein, in this case, the insulin receptor.(11). It also acts as a chaperon catalyzing proper protein folding.(12). PDI has 4 thioredoxine-like domains, two of which have the canonical C X C C motif. In the oxidized form it catalyzes the formation of the disulfide bonds, of the general structure R-S-S-R. It is also called S-S bond or Disulfide Bridge.

The thiol- disulfide exchange:-

In the reduced state the PDI is a dithiol. The thiol – disulfide exchange is depicted by the following equation.

R'SH+ R'SSR" ---------- RSSR' + R"SH

The reaction proceeds through a Nucleophile substitution type2 (SN_2)mechanism. The nucleophile is the deprotinated thiol anion, which attacks the reacting sulfur of the disulfide bond making a S-S-S like transition state, with negative charge being delocalized but more abundant on attacking and leaving sulfurs.

The role of Endoplasmic oxido-reductin 4:-

Endoplasmic oxido-reductin (ERO) is a protein tightly bound to the inner membrane of ER. It transfers the preformed disulfide bonds in the endoplasmic reticulum (ER) to the insulin receptor(IR) through the PDI by way of thiol- disulfide exchange as seen above. It catalyzes the oxidation of Protein Disulfide Isomerase (PDI), by coupling the de novo disulfide bond formation to the reduction of oxygen to H_2O_2. It oxidizes the PDI by accepting electrons from the sulfur and it self gets reduced. Thus the SH HS thiol bond is acquired in exchange of the S-S disulfide bond to PDI. The ERO can oxidize next PDI molecule only if it loses its electrons to the Electron Transport Chain (ETC).

The reverse electron transport (RET) :-

The mitochondrial ETC consist of 4 multi sub -unit complexes (complex I-IV) which along with F0-F1-ATP synthase, (complex V),are encoded by either mitochondria or DNA .The complexes normally transfer electrons to the final accepter, the molecular oxygen which is reduced by 4 electrons, to water at complex IV. Premature single electron reduction of molecular oxygen, earlier in the chain forms the super oxide radical. RET is a set of reactions that allow electrons to be transported against the gradient of redox potential of electron carriers from reduced coenzyme Q to NAD+ instead of oxygen. The reduction of Q enzyme requires FADH2 -linked oxidizable substrates like glycerophosphate or succinate.

The redox carriers and the centres of ETC can potentially leak single electrons to oxygen and convert it to superoxide anion, a progenitor ROS. Given a moderate redox potential of the superoxide oxygen couple,(E1/2= 0.16 V, the reaction of one electron reduction of oxygen is thermodynamically favorable to many oxido-reductases.

Reactive oxygen species production (ROS):-

To maintain the oxidative state of ERO I activity, the transfer of disulfide bond by ERO to PDI is coupled to reduction of molecular oxygen in the ETC by the electrons acquired from PDI. The reduction of oxygen is not complete since the end product is not water but H_2O_2. Thus ERO 1 activity constitutes an important source of ER derived oxidative stress, due to production of ROS. ROS includes super oxide(O_2^{-}-),Hydroxyl radical, (OH^{-}) and hydrogen peroxide (H_2O_2) (13) MnSOD catalyzes the dis mutatation O_2^{-}- to H_2O_2. (14). ROS production implies that there is reverse electron transport. Among the ETC complexes complex1 and complex 3 are the major sites of super oxide production, the former generating super oxide within the mitochondrial matrix only, whereas the later generate them in the inter-membrane space also.. Complex 1 oxidizes NADH with enzyme Q as electron acceptor coupled with proton pump generating trans-membrane potential. Complex I can generate super oxides in presence of NADH. Since the electrons derived from sulfur are at a higher redox potential than complex I, RET occurs to reduce

the NAD Both NAD reduction and Reactive Oxygen Species (ROS) production require high membrane potential provided by ATP hydrolysis.

Role of Peroxy-Redoxin (PROX) 4:-

Hydrogen Peroxide (H_2O_2) formed in ERO I reaction is harmful, so it is removed by another protein, peroxy-redoxin (PROX 4) . These are group of enzymes present in ER which remove H_2O_2also form the disulfide bond. The peroxidatic cysteines in the PROX 4 , take an oxygen from $H_2O_2$and form water and a –SOH group which reacts with the adjacent SH group to form a disulfide bond. The disulfide bond so formed is transferred to PDI by ERO1 which by thiol-disulfide exchange forms disulfide bond between the two α and α,B sub units of the insulin receptor.

The proposed Hypothesis;-

Under the conditions of normal carbohydrate metabolism, the intermediate products of glycolysis and TCA enter ETC through the ComplexI through NADH and complex 11 through $FADH_2$. The electrons passed by ERO 1 to complex1 of the ECT, result in RET and which generates ROS from which the disulfide bonds are generated in the ER for ERO I to initiate oxidation of the reduced PDI, When the metabolism shifts to beta oxidation, the intermediate products of TCA cycle enter through complex -II, no RET occurs from succinate to complex I. Hence no ROS production and hence no disulfide bonds are formed which could be transferred to the insulin receptor. The tetramer or holo -enzyme which can initiate conformational change and auto phosphorylation of the two sub units as well as consequent insulin receptor substrate(IRS) and cascade of auto phosphorylation down stream, is not formed. The free fatty acids (FFA) especially the long chain free fatty acids (LCFA), inhibit RET from succinate to complex I, thus decreasing the succinate-dependent ROS production, in spite of increased FADH2 production due to β- oxidation of FFA (15)This situation continues until the - β - oxidation pathway of energy metabolism prevailing over the normal carbohydrate based energy metabolism is overcome. On the other hand if carbohydrate based energy metabolism is restored, normal disulfide formation is resumed and the insulin sensitivity is restored.

Conclusion:-

The insulin receptor can effect conformational, auto- phosphorylation and signal transduction functions only when it is in tetrameric , holo-enzyme form. For this, the disulfide bond / bridge formation is an essential pre-requisite. These disulfide bonds are formed normally under physiological conditions of carbohydrate based energy metabolism but not under conditions of β- oxidation of fats, prevailing in DM2.Thus whether the Insulin receptor is functional or not is determined by presence or absence of disulfide bond formation which in turn determines the sensitivity or resistance of insulin in DM2.

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