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PATHOPHYSIOLOGY OF DIABETES MELLITUS

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Metabolic disorders of diabetes mellitus are caused mainly by the insufficiency of insulin action on the insulin target cells which contain an insulin receptor on the surface of their cell membrane. The insufficiency of insulin action in diabetes can be classified into three forms: firstly, insufficiency of insulin secretion from B-cells of pancreas; secondly, insufficiency of the action of secreted insulin on the target cells; and thirdly, insufficiencies of both insulin secretion and insulin action in peripheral tissues.

1. Secretion of insulin

Insulin is synthesized in B-cells of pancreas; in the ribosome of B-cells, the newly synthesized preproinsulin is converted to proinsulin, and in the Golgi apparatus proinsulin is further converted into insulin and is stored in β -granules. A small amount of insulin is continuously secreted from the pancreas without any specific stimuli; this is called "basal secretion". Various substances are known to be stimulatory for the augmented secretion of insulin. Several tests in vivo have revealed an intimate negative correlation between the basal level of plasma IRI and the insulin sensitivity. In some conditions, for example, in obesity, lipotrophic diabetes, the Cushing syndrome, and in the insulin-resistant hyperglycemic syndrome (type A, type B), basal IRI levels are elevated.

2. Stimulants to insulin secretion

Glucose is physiologically the most important substance for the secretion of insulin. Therefore, when one wants to investigate clinically the insulin secretory capacity of patients and normal subjects, blood glucose levels should be taken into consideration. As we discussed in earlier sessions of this meeting, the delayed and low insulin secretory response after oral glucose load may be adopted as an index for the diagnosis of primary diabetes.

There are two phases in insulin secretion after glucose stimulation: rapid and sharp secretion of the first phase and, appearing more slowly, the continuous secretion of the second phase. Glucose also enhances the insulin secretion which is evoked by other substances. In addition, glucose stimulates the synthesis of insulin in B-cells. Moreover, B-cells can discriminate α -anomers and β -anomers of D-glucose in the early phase of insulin secretion (Niki et al). This is considered one of the most important pieces of evidence supporting the theory of a glucoreceptor for insulin secretion. In vitro experiments show insulin secretion to be relatively small when blood glucose level is normal, it reaches its maximum at the glucose level of ~ 300 mg/dl. Mannose is metabolized in B-cells and stimulates insulin secretion also. Non-metabolizable sugar cannot stimulate the insulin secretion. In addition, amino acids are stimulants of insulin secretion. Among them, arginine is most potent. Moreover, gastrointestinal hormones, such as secretin, gastrin, and pancreaticozym, stimulate

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insulin secretion. The effects of these peptide hormones on insulin secretion are rapid; therefore, the first phase of insulin secretion appears to be stimulated by these hormones through the effect on cell membrane receptor(s). Recently, GIP (gastric inhibitory polypeptide) has been recognized as an important stimulant to insulin secretion. The physiological role of these gastrointestinal polypeptide hormones may be very important, because insulin secretion is more pronounced when glucose is administered via jejunum as compared with the intravenous administration of glucose, while the rise in blood glucose is more prominent in the intravenous administration of glucose. Moreover, in the Langerhans' islet cells other than B-cells are identified with a specific anatomical arrangement. The A-cell produces glucagon, while somatostatin is synthesized in the D-cell. Insulin secretion is stimulated by glucagon, while it is inhibited by somatostatin.

3. Autonomic nervous system and insulin secretion

The Langerhans' islets have a complex system of nerve fibres. Augmented insulin secretion has been reported by the electrical stimulation of the efferent portion of the vagal nerve. In addition, a number of cholinergic drugs stimulate insulin secretion, while atropin inhibits the secretion. Thus, the stimulation of the parasympathetic system contributes to insulin secretion. As for the sympathetic system, insulin secretion is inhibited by the injection of epinephrine, even though hyperglycemia is produced by it. Moreover, epinephrine inhibits the increased insulin secretory response induced by other substances. The effects of epinephrine on the B-cells are suppressed by blocking agents acting against α -adrenergic receptors. Recently Ui et al. have purified islet-activating protein (IAP) from the supernatant of culture media of *Haemophilus pertussis*. This protein has a potent effect on B-cells. The insulin secretion during feeding is significantly increased by the previous intravenous administration of 1 μ g of this protein to a rat. Although the true mechanism of the role of bioactive protein in insulin secretion from the B-cell remains to be determined, the activation of β -adrenergic action via a β -adrenergic receptor has been suggested; therefore, the Ca^{++} transport through the β -cell membrane may be modified by this IAP. At this moment, the very important question of whether or not insulin secretion is controlled by insulin itself remains to be determined.

4. Insulin receptor and insulin action

The presence of an insulin receptor in the cell membrane is obligatory for the biological action of insulin on target cells. Evidences of the presence of insulin receptors are present in polymorphonuclear leukocytes, mononuclear leukocyte, uterine, bone, placenta, liver cell membrane, adipocytes, and muscle cells. Although insulin receptors are shown in lymphocytes, red cells, and kidney-cell membranes, the biological action of insulin has not been demonstrated in these cells. The insulin molecule has significantly changed during evolution from primitive vertebrate, such as frog and Atlantic hagfish to mammals. On the other hand, the insulin receptors have preserved functional similarities of binding characteristics in these various animals. Therefore, insulin receptors are more primitive proteins than insulin itself.

5. Muscle and adipose tissue metabolism and insulin action

Insulin facilitates the glucose transport through the cell membranes of muscle and adipose tissue and increases the intracellular glucose metabolism, especially the metabolism of glucose through the pentose cycle. In low concentrations of glucose, glucose transport through the plasma membranes of adipocytes and muscle cells acts as a rate-limiting step of the overall intracellular glucose metabolism. Glycogen synthesis is stimulated by insulin, but it is not solely due to the facilitated transport of glucose through cell membranes. Similarly, it is not due to a decrease in the cyclic AMP. By the action of insulin, the I form (active form) of glycogen synthetase is increased in muscle; furthermore, this increase in the enzyme activity is due to the increased activity of synthetase-D-phosphatase caused by the action of insulin. This effect of insulin is not related to the concentration of cyclic AMP. Pyruvate dehydrogenase (PDH) present in mitochondria is also activated by the action of insulin, without any relation to glucose transport or the cyclic AMP level. This effect of insulin is only demonstrated when intact cells are used; thus, the insulin action on the cell membrane triggers

the activation of mitochondrial PDH. Sakamoto et al. reported that insulin-dextran complexes with a large molecular weight activated adipocyte PDH when the complexes were added into incubation media containing rat epididymal adipocytes. As the macromolecular insulin-dextran complex cannot enter the cells, there must be some kind of mediator between the insulin receptor of the cell membrane and the mitochondria.

6. Liver and insulin action

Liver has a primary role in the regulation of blood glucose. Liver produces glucose during fasting, thus preventing hypoglycemia. In the fed state, liver takes up glucose from circulating plasma. Contrary to adipose tissue and muscle, glucose transport through the liver-cell membranes is not rate-limiting for subsequent intracellular glucose metabolism. The effects of insulin on the activities of glycolytic enzymes in liver cells, including hexokinase (type IV), phosphofructokinase and pyruvate kinase, have been demonstrated. Numa et al. reported that the diabetic condition induced in rats by streptozotocin injection influenced the activity of acetyl CoA carboxylase under two different conditions - that is, quick, allosteric control and chronic control. The latter is essentially due to the level of enzyme protein. The same group reported that the reduction of acetyl CoA carboxylase activity in the liver of diabetic mice was accompanied by the reduction of the synthesis of this enzyme protein.

The quick, allosteric control of this enzyme in the diabetic liver has been reported to be mainly due to the increased amount of long-chain fatty acyl CoA in this organ. The activity of citrate clearance enzyme is also reduced in the diabetic liver.

7. Insulin receptors under pathological conditions

As for insulin receptors under diabetic conditions, many investigators, including our group, have reported an increased binding of insulin to the insulin target cells in experimental as well as in clinical insulin-dependent diabetes. On the other hand, Olefsky et al. found about a 50% decrease in insulin receptors on circulating mononuclear leukocytes from a group of 20 thin, untreated, adult, nonketotic insulin-independent diabetic subjects. As these diabetics were also mildly hyperinsulinemic in the fasting state, the decrease in the number of insulin receptors may be explained by the "down regulation mechanism" mentioned earlier. We studied the insulin binding to circulating mononuclear leukocytes from normal controls, diabetics with FBS less than 140 mg/dl, and diabetics with 140 mg/dl or more. We could not demonstrate any significant differences in insulin binding among them. We have recently had the opportunity to study the sera obtained from two female patients with the Sjögren syndrome and a particular diabetic syndrome which is accompanied by extraordinary insulin resistance. One patient needed a maximal dose of 6600 U/day of insulin, while the other used 440 U/day to control blood glucose. The plasma insulin levels also reflected marked resistance, with basal values of 900 and 340 μ U/ml respectively. In addition, one patient had acanthosis nigricans. The patients were not obese, showed no signs of lipotrophic diabetes, and had none of the usual known causes of insulin resistance. Rat adipocytes pretreated with the sera obtained from the patients bound reduced amount of insulin as compared with control adipocytes. This inhibition of insulin binding was prevented completely by the addition of an anti-human IgG anti-serum to the patients' plasma during preincubation with rat adipocytes. Thus, the study supported the idea that the patients' plasma contained an anti-insulin receptor antibody. One patient was subsequently treated with prednisolone and cyclophosphamide, resulting in a remission of the insulin-resistant, hyperglycemic state, accompanied by a reduction of the plasma γ globulin level and the erythrocyte sedimentation rate and the disappearance of the anti-nuclear antibody. Serum obtained at the remission stage of this case revealed no difference from normal controls as far as the binding characteristics of insulin to rat adipocytes were concerned. Only nine cases of this peculiar type of autoimmune disease have been reported in all the world. The reported cases are all Negroes, or Japanese, with the exception of one Creole. The reduction of the binding of insulin to insulin receptors of the cells treated with patients' sera appears to mean a loss of affinity, while in some cases reported by others there appears to be a decrease in number of receptors. The circulating monocytes of these cases were normal, except for the defect of insulin binding, in number, morphology, histochemical staining, and

phagocytosis. Fasting, which returned with any improvement in insulin binding. Thus, in contrast to obesity, the insulin-receptor defect in this syndrome does not appear to be secondary to the hyperinsulinemia; it appears essentially to be due to the presence of a circulating antibody which blocks insulin binding to its receptor. When one uses human adipocytes as well as rat adipocytes for the in vitro experiments, the binding of the antibody to the insulin receptor is associated with dose-dependent biological activities, such as glucose transport, glucose oxidation, lipogenesis, the inhibition of lipolysis, and protein synthesis. Although this antibody revealed insulin-like activities in vitro, the presence of this antibody in vivo is associated with insulin resistance and glucose intolerance. Clarifying the discrepancy of the results between in vivo and in vitro experiments will require further study. One case of this syndrome reported by Kahn and Flier et al. died after intractable hypoglycemia. In the late stage of the clinical course, the patient's mononuclear leukocytes revealed an increase in the number of insulin receptors, especially of the low affinity sites. The reason why the proliferation of the insulin receptors in low affinity occurred waits further study.

The most common form of clinical and experimental insulin resistance is that associated with obesity. The insulin resistance of obesity is characterized by hyperinsulinemia, both basal and stimulated, variable degrees of glucose intolerance, and resistance to both endogenous and exogenous insulin. The major defect appears to be at the level of target cells. At least part of the insulin resistance observed in obesity appears to be due to a decrease in the number of receptors on target cells. In addition, post-receptor disturbances contribute significantly to the insulin insensitivity of these cells; perhaps post-glucose transport, intracellular defects may be important for the insulin insensitivity of obesity. The decreased insulin receptors are demonstrated in the genetic form of obesity and in the acquired obesity following the treatment of rats with ventromedial hypothalamic lesions. However, in the latter condition it took a week or more before there was a reduction in the number of insulin receptors, while the plasma insulin levels were increased two or three days after VMH lesion. Thus, we need more studies before clarifying the mechanism of the down regulation of insulin receptors by insulin.

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