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THE INSULIN RECEPTOR

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The plasma membrane of the cell serves to provide anatomical limits for the functions of the cell while providing ready access into the cell of metabolites required by the cell for normal activity. It has been known for many years that passage into the cell, and from the cell, of nutritive and breakdown metabolites is governed by highly complex systems of diffusion and energy dependent transport that may be specific for individual or related groups of molecules. In recent years the plasma membrane has also been found to be endowed with highly developed cognitive powers in terms of recognizing signals from the extracellular milieu that regulate the metabolic functions of the cell. This capability to receive signals is now known to be accomplished by complex proteins or glycoproteins that are integral units of the cell membrane. Among the most important regulatory signals received by cells are those transmitted through the endocrine system.

Among the most extensively studied receptors for protein hormones is the insulin receptor. It has been found in a variety of cells that are known targets of insulin action including hepatocytes, adipocytes, cells of skeletal and cardiac muscle and placenta, erythrocyte precursors and erythrocytes, monocytes and vascular endothelial cells. A variety of tumor cells also bear insulin receptors in their plasma membranes including lymphoid tumors and erythroleukemic cells. Fibroblasts growing in tissue culture bind insulin and this binding is enhanced if they assume the phenotypic functions of adipocytes (3T3 cells).

It is abundantly clear that the insulin receptors are in a state of continuous dynamic flux. Following exposure to high concentrations of insulin, a sharp diminution in the number of receptors occurs. This phenomenon of mediation of receptor binding by insulin itself is referred to as "down regulation" of insulin receptors. It has been shown to occur in vitro in lymphoblastic cells and fibroblasts. In vivo, a diminution of binding occurs in subjects with hyperinsulinism such as is found with insulinoma. Conversely, when insulin deficiency is induced in animals with agents such as streptozotocin, increased receptor binding is found in cells such as hepatocytes.

The mechanisms underlying receptor loss are not clearly understood but they may involve internalization of the receptor-insulin complex followed by intracellular degradation of the insulin. It is not clear if the receptor can then return to the surface of the cell for further binding with insulin or if the receptor itself is destroyed. In that case all receptor activity of the cell would require new protein synthetic mechanisms. Protein synthesis inhibitors such as cycloheximide have been shown to inhibit the reappearance of receptor binding following down regulation but these experiments must be interpreted with caution. Cycloheximide exerts a general inhibitory action on protein synthetic activity of the cell and lack of reappearance of insulin binding activity may be a consequence of interference with synthesis of other cellular proteins.

Several attempts have been made to isolate the receptor and to determine its physical characteristics. Purification of the insulin receptor by solubilization and quantitative polyacrylamide gel electrophoresis has yielded a band with insulin binding activity. The active protein had an estimated molecular mean geometric radius of 68 A corresponding to a globular protein with an estimated weight of 10^6. The receptor appears to be composed of smaller subunits with molecular weights of 135,000 and 45,000. It has been estimated that the native receptor is composed of two each of such subunits. For the present, it is not possible to resolve the apparent discrepancies between these different methods of estimating the physical properties of the receptor.

Specific receptors for insulin like growth factors including IGF I and II, somatomedins and multiplication stimulating activity occur in many cells. These receptors have weak affinities for insulin and the insulin receptor has weak affinities for the growth factors. It has been suggested that acute metabolic effects of insulin and the growth factors, such as glucose oxidation, are mediated through the insulin receptor whereas the more prolonged growth effects of both classes of hormones are mediated through the growth factor receptors. This attractive hypothesis still requires adequate confirmation.

The human fetus synthesizes insulin as early as seven weeks of age and a rapid increase in the pancreatic content of the hormone follows between 11 and 20 weeks. In the fetus, insulin is an important anabolic agent. The inadequately controlled pregnant diabetic woman transfers excessive quantities of glucose to the fetus across the placenta, leading also to fetal hyperglycemia. As a consequence, the islets of the fetal pancreas are stimulated to produce insulin in excessive quantities and this fetal hyperinsulinism leads to fetal macrosomia. Induction of fetal hyperinsulinemia by infusion of insulin directly into the fetus of the rhesus monkey leads to marked macrosomia and organomegaly. On the other hand, human infants with "transient diabetes of the newborn" due to congenital insulin deficiency are undersized both in weight and length but grow to normal proportions with adequate insulin therapy. The potency of insulin as an anabolic hormone is difficult to demonstrate postnatally because of the associated hypoglycemia. Fetal hyperinsulinemia, however, is not attended by severe fetal hypoglycemia because of the continuing supply of glucose across the

placenta. The long-term anabolic effects of hyperinsulinemia on the fetus can be ascertained therefore without the complicating effects of hypoglycemia. There is ample evidence that insulin enhances transport of amino acids into cells and increases DNA-directed RNA and protein synthesis in a variety of cells and subcellular systems.

Studies of binding of insulin to plasma membranes of livers of human fetuses were carried out in our laboratories. We demonstrated that the liver of the human fetus has specific receptors for insulin at least as early as the fifteenth week of gestation. The capacity of the liver to bind insulin, however, is restricted at this time as compared to later in pregnancy. Between 15 and 18 weeks of fetal age, human liver membranes bound less than one-fourth as much insulin as "physiological" concentrations of insulin as did membranes from fetuses aged 26 to 31 weeks. The affinity of the receptor for insulin was substantially less between 15 and 18 weeks than it was in older fetuses. These studies on humans were restricted because the availability of the material was limited to fetuses between 15 and 31 weeks of gestation.

In studies on rat fetuses we found a similar increase in hepatic receptor binding of insulin with advancing age of the fetus. Of interest was our observation that receptor binding at term in the fetus exceeded the binding capacity of post natal animals by substantial amounts. In these studies it was not possible to determine whether the insulin receptors were on cells of hematopoietic origin or on hepatocytes. The increase in insulin binding occurred just before term at a time when the hematopoietic cells undergo a reduction in mass while the hepatocytes increase substantially. Hematopoietic cells constitute about 61% of the liver volume in the rat at day 15 of gestation decreasing to about 46% at term. On the other hand, hepatic parenchymal cells which constitute about 35% of the liver on day 15 of gestation increase to about 46% at term. It is unlikely, therefore, that increased insulin binding at term in the rat liver is a reflection of increased binding to hematopoietic cells. Increased binding of insulin to fetal tissues as compared to adult tissues is also found in other fetal tissues both human and subhuman, as we shall see presently.

Circulating monocytes possess insulin receptors with high affinity for insulin. While monocytes are not typical target cells for insulin action, insulin has been shown to exert effects on the metabolism and function of these cells. Considerable evidence also exists that changes in insulin receptors in major target cell systems such as adipocytes and hepatocytes are reflected by parallel changes in monocyte insulin receptors--a fortunate circumstance because of the ready availability of monocytes for study in experimental subjects.

In agreement with the studies of rat liver referred to above monocyte insulin receptors of human umbilical cord blood are much more numerous than those of monocytes of children and adults. Normal newborn infants have about 38,000 insulin binding sites per monocyte as compared to about 25,000 for adults. The fetus therefore appears to have an enhanced capacity for binding insulin. We have calculated that the monocytes of normal infants bind nearly four times as much insulin as do the monocytes of

normal adults. The fetus, however, manifests an extraordinary property of the insulin receptor. Unlike the cells of the post natal individual that undergo "down regulation" of receptor number in the face of hyperinsulinemia, insulin receptors of the fetus are actually increased or "up regulated" by hyperinsulinemia. The fetus of the poorly controlled diabetic mother is macrosomic because of fetal hyperinsulinemia. In the face of this hyperinsulinemia, insulin receptor binding is substantially increased. In a series of 8 hyperinsulinemic infants of diabetic mothers, average monocyte receptor sites per cell were increased from 38,000 (in the normal infant) to about 105,000. At an insulin concentration of 1 ng/ml, 10 times as many sites bound insulin as would be expected in the adult and about 3 times as many as compared to the normal infant.

This propensity of the fetus to enhance its binding capacity for insulin in the face of high ambient concentrations of insulin places the fetus of the diabetic mother in double jeopardy. Irrespective of the mechanism, down regulation of receptor binding in the presence of hyperinsulinemia in the adult is a protective mechanism that partially shields the cell from the effects of increased insulin concentrations. The infant of the diabetic mother affords itself no such protection. Hyperinsulinemia in this case enhances insulin binding and may aggravate the toxic effects of the hormone. No wonder the infant experiences the profound effects of marked increase in size, profound post natal hypoglycemia and respiratory distress. This "up regulation" of insulin binding is not an innate feature of the diabetic state because when the pregnant mother is adequately controlled and fetal hyperinsulinemia is mitigated, receptor binding in fetal monocytes returns to levels comparable to those of normal newborn infants.

Infants of poorly controlled diabetic mothers are at greater risk than normal infants for development of respiratory distress in the newborn period. We have developed evidence that demonstrates that hyperinsulinemia in the fetus is at least partially responsible. Lung slices from rabbit fetuses delivered prematurely were prepared and incorporation of palmitate and glucose into saturated phosphatidylcholine was studied in the presence and absence of added insulin in concentrations of 100 μU/ml. Incorporation of ^{14}C-palmitate into saturated phosphatidylcholine was significantly depressed by exposure to insulin as was the incorporation of ^{14}C-glucose. These experiments were confirmed in a series of in vivo studies. Diabetes was induced in pregnant rabbits by injection of alloxan (60 mg/kg) on the 14th day of pregnancy for study of insulin receptors from the lungs of the does and the fetuses at the 27th day of gestation. The diabetic does and their fetuses were hyperglycemic as compared to normal controls. In addition, the fetal diabetic offspring had plasma insulin concentrations that were significantly higher than control fetuses (84.8 ± 25 vs. 23.2 ± 3.7 μU/ml, mean $\pm$ S.D., $p<0.05$) confirming the presence of the fetal hyperinsulinemic state.

Lung membranes from fetuses of diabetic animals bound significantly more insulin than did those of the control fetuses. Scatchard analysis yielded curvilinear plots. Assuming two classes of receptors, one of high affinity and low capacity and another of low capacity and high affinity, we found that fetal

membranes had a fivefold increase in binding capacity of high affinity receptors as compared to adult lung membranes. Once again, in the offspring of the diabetic animals, the fetal lung, far from experiencing a down regulation of insulin receptor binding, showed a significant increase of binding. Thus we have produced evidence in two separate fetal tissues, lungs and monocytes, that hyperinsulinemia is associated with an increase in insulin receptor binding.

Insulin receptors with high specificity and affinity have also been described on circulating erythrocytes. This cell type is of particular interest to investigators studying infants and children because of the small quantities of blood necessary for analysis. Erythrocytes have only one-fifth to one-tenth of the number of receptors of monocytes but because they are so much more abundant, complete analyses can be carried out on as little as 10 ml whole blood. Erythrocytes from cord blood bind considerably more insulin than do those of older children and adults. This is in agreement with studies of insulin receptors and monocytes. We have not yet been able to study the effects of hyperinsulinemia on insulin binding to erythrocytes.

Diabetes mellitus type I, the insulin dependent type that occurs in children, does not appear to be associated with significant disturbance of insulin binding. Type II diabetes in which insulin resistance is a feature may be associated with diminished receptor binding in certain instances, for example "chemical diabetes" and diabetes with fasting hyperglycemia. Obesity is characteristically associated with diminished insulin receptor binding both in adults and children and with hyperinsulinemia and insulin resistance. When obese subjects undergo loss of weight, insulin binding returns to normal. It appears that carbohydrate intolerance, hyperinsulinemia and impaired insulin receptor binding are all consequences rather than the cause of the disorder. Conversely, insulin binding is increased in anorexia nervosa, a state in which there is enhanced sensitivity to administered insulin. Increased sensitivity to insulin accompanied by increased insulin receptor binding also follows muscular exercise. In growth hormone deficiency increased sensitivity to insulin does not appear to be mediated through increased insulin binding, according to studies recently completed in our laboratory. Nor does increased resistance to insulin in states of growth hormone excess, or following injections of growth hormone, appear to be related to impairment of insulin receptor binding.

Disease states may be associated with receptor antibodies. For example, myasthenia gravis is associated with presence of antibodies to the acetylcholine receptor and in Graves' disease antibodies to the thyrotropin receptor appear to be causally related to the disease. Antibodies to the insulin receptor are responsible for severe carbohydrate intolerance in a form of acanthosis nigricans. Not all subjects with acanthosis and carbohydrate intolerance, however, show evidence of circulating antibodies to the receptor. In some instances receptor binding capacity is diminished even though no antibodies to the receptor are demonstrated. In yet a third category of acanthosis, carbohydrate intolerance with no receptor abnormality is detectable. In this disorder insulin resistance is due to abnormalities in the cell distal to the receptor. Disturbances of insulin recep-

tor binding have also been described in leprechaunism, ataxia telangiectasia and lipoatrophic diabetes.

Patients with cystic fibrosis of the pancreas are known to have disorganization of the pancreas with exocrine insufficiency. Carbohydrate intolerance characterized by abnormal glucose tolerance tests occurs frequently but frank diabetes mellitus is rare. In a study of seven patients we found mild hyperglycemia and diminished insulin secretion. This was associated with increased numbers of receptor sites and some diminution of receptor affinity. Our interpretation of these findings is that the disease state impairs receptor affinity. It has been suggested that alterations in glycoprotein structure of plasma membranes or in concentrations of glycoproteins may affect properties of insulin receptors. Abnormalities in mucous glycoproteins have been reported in cystic fibrosis and it is possible that a more generalized defect in glycoprotein metabolism exists in these patients. In any case, the abnormality in carbohydrate tolerance observed in cystic fibrosis has at least three contributing components: insulinopenia, diminished insulin receptor affinity and increased insulin receptor capacity.

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THE ACTION OF DRUGS ON MEMBRANES

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As far as membrane action is concerned, drugs can be classified as either hydrophilic or hydrophobic. Hydrophilic drugs are likely to bind to sites on membrane proteins much like those on normal water-soluble proteins, and they will show relatively little non-specific binding to the membrane. Most drugs having an effect on membrane proteins, however, are hydrophobic and can be expected to show extensive binding to the membrane.

A number of modes of interaction are possible for the hydrophobic drugs. Firstly, activity could follow indirectly from binding to the lipid component of the membrane. Two ways can be envisaged for linking such binding to an effect on protein function. Binding to the lipid component of the membrane could affect the fluidity of the lipid (either the bulk lipid or the annular lipid around the membrane proteins) and it is known that at least some membrane proteins are sensitive to the fluidity of the surrounding lipid (3). Alternatively, if the drug is charged, then the binding of such a drug will alter the charge on the membrane. Since most enzyme substrates, transmitters etc. are also charged, their concentrations close to the membrane surface will be altered with consequent alterations in enzyme activity, receptor activation etc. Further possibil-