

Insulin Receptors

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Much of the emphasis in the pathogenesis of diabetes mellitus has justifiably been placed on the endocrine gland, the pancreas. Extensive studies on the biosynthesis and release of insulin from the beta cell, bihormonal control of metabolism by insulin and glucagon, and more recently the role of somatostatin have attracted the attention of students of the subject; but considerable evidence exists to suggest at least some role of tissue resistance to insulin in the pathogenesis of this disorder. There have been many advocates for extra-pancreatic factors causing diabetes. One of the first was Mirsky, who proposed that diabetes might be due to excessive amounts of hepatic insulinase, an enzyme which degrades insulin.¹ Vallance-Owen suggested that a circulating insulin antagonist labeled synalbumin might be the cause of insulin resistance in diabetes.² This factor was later shown to be an artifact. Others, such as Antoniades, proposed that insulin might circulate predominantly in a bound form in diabetic subjects and thus not exert full biologic activity.³

The most articulate spokesman for a role of insulin resistance in diabetes mellitus in recent years has been Gerald Reaven, and his group from Stanford University, who bases his theory on two observations. The first is that a large number, if not the majority, of adult onset diabetics have increased circulating insulin concentrations rather than decreased concentrations as had been expected. This was first observed by Yalow and Berson shortly after the perfection of the radioimmunoassay for insulin.⁴ Hyper-

insulinism in diabetics has since been confirmed by many investigators. A second observation supporting the role of insulin resistance in diabetes is that of "glucose impedance" in diabetic patients. Glucose impedance was demonstrated by Reaven and colleagues⁵ by infusing glucose and insulin at a constant rate in diabetic and non-diabetic subjects whose endogenous insulin release had been shut off by administration of epinephrine and propranolol. New steady states for glucose and insulin were achieved in both groups, with comparable insulin concentrations in diabetics and non-diabetics, whereas the new steady state glucose concentration was considerably higher in diabetic subjects than in non-diabetic subjects. These excellent studies indicated that for a given concentration of insulin, the blood glucose-lowering effect was less in diabetics than in non-diabetic subjects. More recently, Reaven and Olefsky have suggested that insulin resistance in diabetic patients might be due to a decrease in the number of insulin receptors⁶ by showing a decrease in the number of insulin receptors on circulating monocytes in diabetic patients compared to those on monocytes of non-diabetic subjects. Additionally, treatment of their diabetic subjects with an oral hypoglycemic agent resulted in a return to normal of the number of insulin receptors on peripheral monocytes.⁷ A thorough understanding of these latter observations and their obvious, important implications for the pathogenesis of diabetes requires a certain knowledge of the insulin receptor and of recent advances in the field of receptor technology.

Properties of the insulin receptor are shown in Table 1. As is the case with other polypeptide hormones, the receptor for insulin is located on the cell

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TABLE 1
Properties of Insulin Receptors

- A) Located on cell membrane
- B) Unevenly distributed and may occur in clumps
- C) Protein with mol wt approx. 300,000-Tetramer consisting of monomers of 75,000 mol wt each
- D) Continuous synthesis and degradation with relatively slow turnover rate—2%/hr
- E) Presence of insulin receptor on cell membrane requires protein synthesis and microfilament integrity

membrane. Evidence for intracellular distribution of insulin receptors is very scant; they are not evenly distributed over the cell surface but rather occur randomly and in clumps at times.⁸ Present evidence suggests that the insulin receptor is a protein with molecular weight of approximately 300,000. Studies performed at the National Institutes of Health from Dr. Jesse Roth's laboratory suggest that the insulin receptor is a tetramer consisting of monomers of 75,000 dalton units each. Although the turnover rate is low compared to many biologic processes (2% per hour), continuous synthesis and degradation of the insulin receptor occurs. Studies with inhibitors of protein synthesis and microfilaments such as puromycin and cytochalasin respectively indicate that protein synthesis and microfilament integrity are necessary for the presence of insulin receptors on the cell membrane.⁹ Inhibitors of microtubular function surprisingly had no effect on insulin receptors.⁹

Knowledge of three concepts involving the insulin receptor (Table 2) is of critical importance in interpreting studies in which receptor number and affinity have been determined. The number of receptors per cell varies with the particular cell being studied. However, for the peripheral monocyte, which is the most commonly studied cell in man because of its accessibility, the numbers of receptors vary between 15,000 and 30,000 per cell. Clearly, only a fraction of the receptor sites must be occupied for biological activity, and the number of occupied sites required for the different activities of insulin may vary. For example, dose response data suggest that fewer sites must be occupied to inhibit lipolysis than to stimulate glucose oxidation. Thus, many of the insulin receptors on the cell surface will be spare or unused receptors. Recent investigations have even shown that some of these receptors may serve as a peripheral reservoir for insulin, releasing intact insulin under appropriate circumstances.¹⁰

A second concept which is probably the most

important in understanding current receptor studies is that insulin inhibits insulin receptor number. Experiments by Gavin et al¹¹ demonstrated that pre-incubation of cultured lymphocytes with physiological concentrations of insulin reduces the number of insulin receptors on these cells. An obvious corollary of this finding would be the presence of decreased insulin receptors in states of hyperinsulinism such as obesity and some forms of diabetes. Although not reported yet, reduced insulin receptors would be anticipated in patients with islet cell tumors. Thus, reduced receptor number might offer some protection to the patient with an islet cell tumor.

The third important concept in understanding insulin receptors is that of negative cooperativity.¹² Simply stated, this concept refers to site interactions on the cell surface by which affinity of the receptor for insulin is decreased as increasing numbers of receptors are occupied. This phenomenon might also be considered a homeostatic mechanism which protects the individual from the effects of hyperinsulinism.

The insulin receptor perceives and either directly or through a transducer substance influences the effector for a specific activity. Insulin binding is the first step in biological activity of the hormone. Although not all receptors are required for biological activity, more receptors increase the likelihood of binding for a given concentration of insulin. Binding of insulin to its receptor is therefore determined by insulin concentration, receptor number, and receptor affinity. Radioimmunoassay techniques for the measurement of insulin have been available for years; now methods are available to measure insulin receptor number and affinity.

As mentioned the most accessible cells for measuring insulin receptors in vivo are peripheral mon-

TABLE 2
Important Concepts Involving Insulin Receptors

- A) Spare receptors
 - 1) Number of receptors per cell varies with cell type
Peripheral monocytes have 15,000-30,000 receptors per cell
 - 2) Only small percentage of receptors must be occupied for biologic activity
 - 3) Spare receptors may serve as peripheral insulin reservoir
- B) Feedback inhibition of insulin receptor number by insulin
- C) Negative cooperativity

ocytes. These cells are obtained by Ficoll-Hypaque separation of the buffy coat of centrifuged blood. To determine receptor number and affinity, these cells are incubated with labeled insulin and increasing amounts of cold insulin, resulting in a binding curve (Fig 1). Applying Scatchard analysis to this data results in a curvilinear plot (Fig 2B). Similar studies with the growth hormone receptors, or other hormones not showing negative cooperativity, produce a linear Scatchard plot (Fig 2A). Although the favored interpretation of the curvilinear Scatchard plot for insulin receptors is negative cooperativity, the possibility that two types of insulin receptor sites (broken lines Fig 2) exist cannot be eliminated from present data. Employing Scatchard analysis, the number of receptors is calculated from the amount of bound insulin where the plot crosses the X axis. The slope of the plot reflects affinity, and new graphic analyses are available to express affinity even from a curvilinear plot.¹³

Employing these techniques, insulin receptor number and affinity can be determined. The factors influencing affinity and receptor number are shown in Table 3. Some of these have already been discussed. One of the most important determinants of affinity is pH; its effect on insulin binding to receptors is shown in Figure 3. For both human monocytes and cultured lymphocytes, reducing pH from 7.4 to 6.8 results in greatly depressed insulin binding and may contribute to the insulin resistance observed in severe diabetic ketoacidosis.

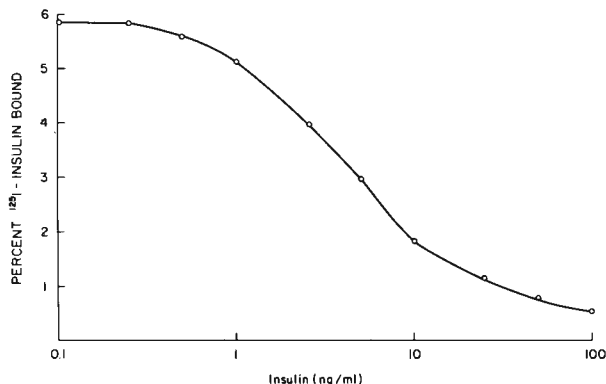


Fig 1—Insulin binding to peripheral monocytes. 20×10^6 mononuclear cells (14% monocytes) were incubated in 0.5 ml buffer containing 50–100 pg ^{125}I -insulin and increasing amounts of unlabeled insulin to give the final concentration indicated in the figure. After 3 hours incubation, 200 μl aliquots were centrifuged, aspirated, and the sediment counted.

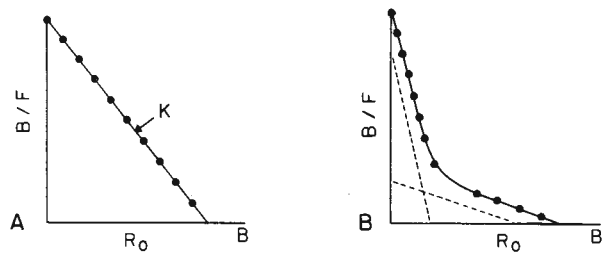


Fig 2—Scatchard analysis of binding data. B/F = bound/free radioactive ligand. Horizontal axis (R_0) is amount of ligand bound to receptor in molar quantities. *A*. Scatchard plot for hormone not exhibiting negative cooperativity; *B*. Plot is for hormone exhibiting negative cooperativity or having two different receptor sites. Insulin receptor studies show curvilinear plot as in *B*.

The major factor so far uncovered altering insulin receptor number is insulin acting in a type of feedback mechanism to inhibit insulin receptor number. Thus, as previously pointed out, reduced insulin receptors are anticipated in obesity where insulin resistance and hyperinsulinism exist. Reduced insulin receptors have indeed been shown to occur in obese humans and animals.^{14–16} In addition, dieting and weight reduction result in normalization of the numbers of insulin receptors.¹³ It is debatable whether reduced receptor concentration is primary, resulting in insulin resistance and hyperinsulinism, or whether insulin resistance due to some other factor is primary, causing hyperinsulinism and, secondarily, reduced insulin receptors.

Returning to Reavan and Olefsky's observations in non-obese diabetic subjects,⁶ it is not clear whether the reduced insulin receptor number is due to the hyperinsulinism exhibited by this group [fasting immunoreactive insulin (IRI) 20 ± 2 versus 10 ± 1 in normals] or whether it might be primary and thus be important pathogenetically. Nevertheless the decreased insulin receptors observed in the diabetic sub-

TABLE 3
Determinants of Insulin Binding to Tissues

- | |
|---|
| I. Receptor affinity |
| pH |
| Temperature |
| Ionic strength |
| Receptor occupancy (Negative cooperativity) |
| II. Receptor number |
| Insulin decreases receptor number |
| III. Insulin concentration |

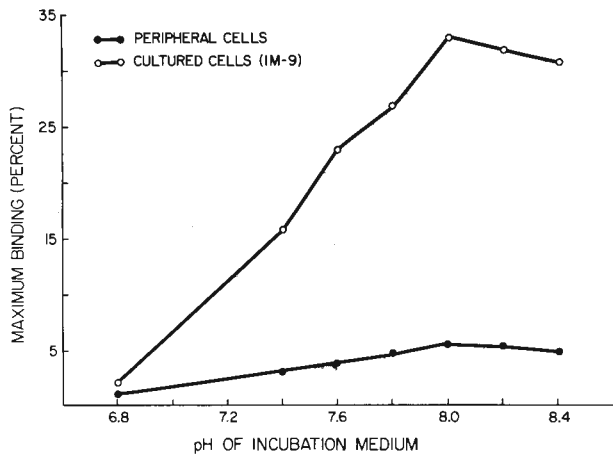


Fig 3—Effect of pH on insulin binding to receptors. Maximum binding refers to percent of ¹²⁵I-insulin bound to receptors in the absence of unlabeled insulin. For peripheral cells 20 × 10⁶, mononuclear cells (14% monocytes) were used; cultured lymphoblastoid cells (IM-9) were used at 3.0 × 10⁶ cells per ml concentration.

jects in Reaven and Olefsky's study would certainly contribute to the insulin resistance observed. The return of receptor number to normal with chronic sulfonylurea treatment⁷ might in similar fashion be attributed to the reduced insulin concentrations in well-controlled diabetics on chronic sulfonylurea therapy.

Although the role of reduced insulin receptors in the pathogenesis of diabetes mellitus is equivocal, a rare diabetic syndrome recently reported is clearly related to decreased insulin receptors.¹⁷ In several patients with other evidence of immunologic disease associated with severe insulin resistance, an antibody

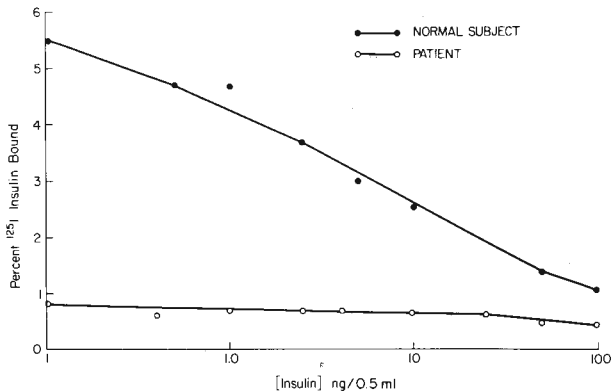


Fig 4—Insulin binding to peripheral monocytes from normal subject and patient with antibodies to the insulin receptors. Details as for Fig 1.

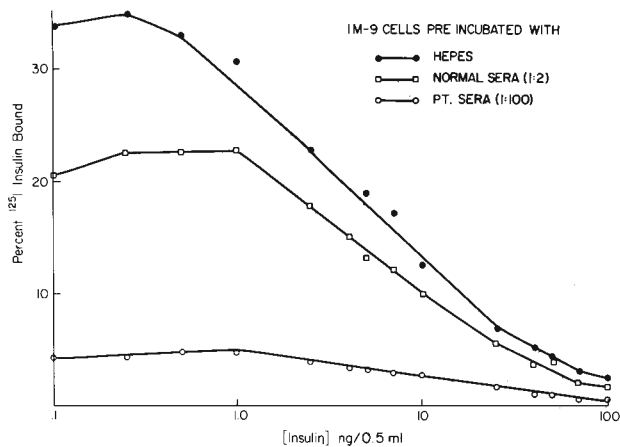


Fig 5—Effect of serum preincubation on insulin binding to cultured lymphoblastoid cells (IM-9). IM-9 cells were preincubated with buffer, normal serum, or serum from insulin-resistant patient for 60 min, washed twice, and resuspended in 0.5 ml buffer at final concentration of 3 × 10⁶ cells per ml. Binding curves were then obtained on these cells.

to the insulin receptor has been demonstrated. Since this report we at the Medical College of Virginia have had the opportunity to study two patients with this syndrome. One of the patients requiring over 2,000 units of insulin daily had a strongly positive antinuclear antibody as the only other manifestation of autoimmunity; the second patient had a scleroderma-like illness and required 1200 units of insulin daily. Insulin binding curves by peripheral monocytes from one of these patients is shown in Figure 4. That a serum factor was responsible for the decreased binding was indicated by studies in which cultured lymphocytes (IM-9) were preincubated with the patient's sera (1:100) and then used for binding studies (Fig 5). Scatchard analysis (Fig 6) revealed the decreased binding to be due to a reduction in numbers of insulin receptors. Studies, not shown, in which

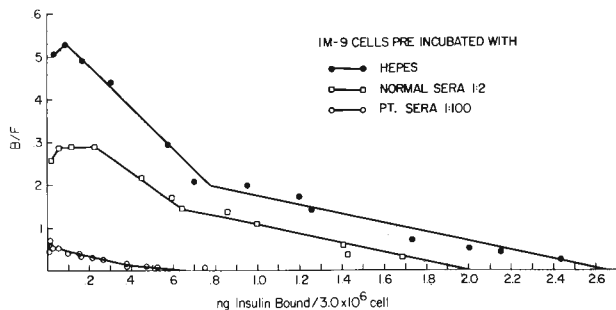


Fig 6—Scatchard analysis from binding data shown in Fig 5.

cultured lymphocytes were preincubated with IgG fraction of the patient's sera exhibited the same phenomenon, suggesting that the serum contained an antibody to the insulin receptor.

In summary, techniques are now available for measuring insulin receptors in vivo. So far, reduced insulin receptors have been observed in obese persons and in a selected group of adult onset diabetic patients. The pathogenetic significance of the latter observation is uncertain and may possibly be a manifestation of the high insulin concentrations in these diabetics. However, a rare diabetic syndrome in which severe insulin resistance due to antibodies to the insulin receptor has been reported and is now corroborated by our findings in two patients.

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