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The Role of Insulin Receptors and Receptor Antibodies in States of Altered Insulin Action (40609)

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The first step in the action of insulin is binding to a surface membrane glycoprotein receptor (1). This receptor serves two functions: The first is to recognize insulin from all other substances in blood and bind it tightly. Second, this interaction of the hormone with its receptor then generates some form of transmembrane signal which initiates a series of biochemical pathways that ultimately result in production of insulin's biological effects. Although the insulin receptor has not yet been purified, over the past 8 years, studies have been conducted using radioactively labeled insulin which have given some insight into the nature of the receptor and the biochemical characteristics of its interaction with insulin. One of the most important observations derived from these studies has been that the receptor is not a static entity—rather it can be altered in its number or affinity for insulin. Since the receptor is the crucial link in insulin action at the cellular level, such alterations play an important role in the pathogenesis of a number of disease states.

Characteristics of the insulin receptor. As noted, above, the insulin receptor has not yet been purified and thus most of our information about its structure is indirect. A schematic model of the receptor is shown in Fig. 1. The receptor is an integral membrane protein and can be solubilized in active form only in neutral detergents (2–4). The receptor contains carbohydrate, although there is no evidence that this carbohydrate is important in insulin binding (3). In Triton X-100, the receptor behaves as a molecule with a Stokes radius of 70 Å, although several recent studies suggest that this large complex contains several subunits or closely associated proteins (2, 5–7). Estimates for the size of the insulin binding site itself range from 90,000 to 135,000 (5, 8–10).

The interaction of insulin with its receptor is complex and has been the subject of much controversy. Scatchard analysis of insulin binding data results in a curvilinear plot (Fig. 2) consistent with multiple classes of binding sites or negative cooperativity among receptors (11, 12). Using kinetic experiments, as well as equilibrium experiments with a variety of insulin analogues, De Meyts *et al.* (12) have presented convincing evidence for negative cooperativity in insulin binding, i.e., the affinity of the receptor decreases with increasing occupancy. Although not all workers agree on this point (3, 13), we have found this model useful for data analysis in disease states, since it allows for easy comparison between different groups or individuals.

In the cooperative model (assuming an otherwise homogeneous class of binding sites), the Scatchard plot can be viewed as being formed by a family of lines of decreasing slope originating from the various points of the curve and terminating at the intercept of the plot on the abcissa (Fig. 2) (14). The Scatchard plot can then be described by three parameters: (i) The total receptor concentration (R_0) which is the intercept of the plot on the abcissa; (ii) the limiting high-affinity state of the receptor which is observed as occupancy approaches zero (this has been termed $\bar{K}_e$ or affinity of the empty receptor); and (iii) the limiting low-affinity state of the receptor which is observed as occupancy approaches 100% (this has been termed $\bar{K}_f$ or the affinity of the filled receptor). An even more detailed description of receptor affinity may be had by plotting the changing affinity as a function of occupancy in what has been termed an average affinity profile (Fig. 2, right) (14).

While quantitative analysis of insulin binding data has lead to certain models of insulin action and has been useful for descriptive purposes in disease, it is important to keep in mind several important limitations of this approach. First, whether one chooses the cooperative model or the two-site model, these

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are *only models*, not proven mechanisms, of the insulin–receptor interaction. Second, they are derived analyses which are only as good as the original data from which they were taken. In view of the complex nature of the insulin–receptor interaction, it is not surprising that too often these analyses are made on inadequate data. Even with computer-assisted data analysis and multiple data points, the errors in estimation of binding capacity and affinity are large (15)—much larger than most investigators realize or admit. Finally, it is important to keep in mind the fact that virtually all of the receptor studies are done *in vitro* under conditions designed to minimize the complexity of the binding interaction. At 37°, *in vivo*, there is a considerable number of complicating reactions, including internalization (16–18) and degradation (11, 16) of hormone, which may invalidate all of these *in vitro* analyses.

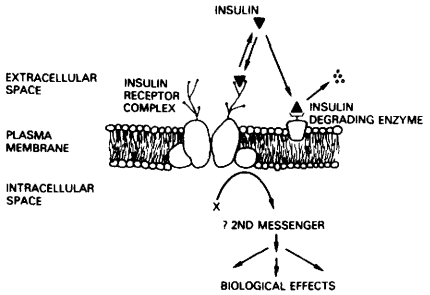

FIG. 1. A schematic model of the insulin receptor. The receptor is viewed as an integral membrane glycoprotein, containing more than one binding site and several subunits. The receptor is chemically distinct from the insulin degrading enzymes.

Obesity as a model of disease with altered receptor concentration. The most common form of insulin resistance in man and animals is that associated with obesity. The insulin resistance of obesity is characterized by hyperinsulinemia, variable degrees of glucose intolerance, and resistance to both endogenous and exogenous insulin (19). The major defect appears to be at the level of the target cell, since the circulating insulin and proinsulin-like materials are normal, and the target tissues demonstrate the same type of subnormal response when challenged with exogenous insulin or when studied *in vitro*. The role of the insulin receptors in disease was first illucidated in studies of obesity, in particular, the obese hyperglycemic mouse (20, 21).

The obese hyperglycemic mouse is an autosomal recessive trait (*ob/ob*) characterized by hyperphagia, excessive deposition of fat, hyperglycemia, hyperinsulinemia, and pancreatic islet cell hyperplasia. These animals have a marked resistance to both endogenous and exogenous insulin, both *in vivo* and *in*

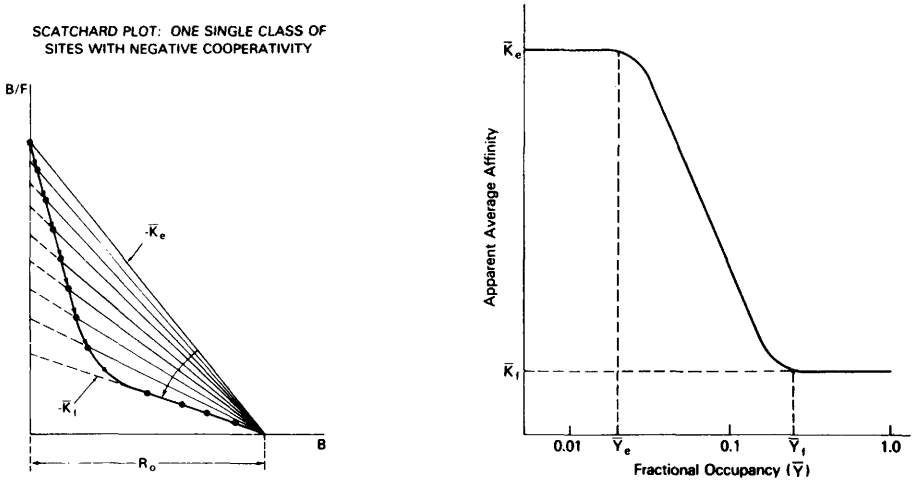

FIG. 2. Data analysis for a single class of binding sites with negative cooperativity. The left panel shows the curvilinear Scatchard plot obtained by plotting the bound/free ratio for insulin (B/F) versus the amount bound (B). R_0 is the total receptor concentration; $\bar{K}_e$ is the affinity of the high-affinity state (empty receptor); and $\bar{K}_f$ is the affinity of the low-affinity state (filled receptor). The right panel shows an average affinity probe obtained by plotting $\bar{K}$ as a function of fractional occupancy ($\bar{Y}$). Adapted from Ref. (14).

vitro. When liver membranes of the *ob/ob* and thin mice were prepared and incubated under identical conditions, the binding of insulin to the membranes of the obese mice was consistently found to be 25–35% of that observed in the thin animals (Fig. 3A) (20). This decrease in insulin binding was not unique to liver, but was also observed in studies using adipocyte membrane (21), myocardial muscle membranes (22), and intact thymic lymphocytes (23).

The difference in observed binding appears to be due to only a decrease in the number of receptors (20, 24). When the bound/free of the labeled hormone is expressed as a function of the bound hormone (Scatchard plot), identically shaped curves are obtained (Fig. 3B). The decrease in insulin binding observed in these mice can be accounted for completely by a decrease in R_0 determined from the intercept of the Scatchard plot, whether one chooses to analyze the data using a cooperative or multisite model.

Since that time, a variety of animals models of obesity, including human obesity, have been studied (Table I) (25–31). These include animals with acquired and genetic obesity (both autosomal recessive and polygenic), animals with hyperplastic and hypertrophic obesity, and animals in which the obesity is associated with normal or abnormal glucose tolerance. With the exception of the Zucker fatty rat (28), cells from each of these animals show decreased insulin binding to membrane receptors in the basal state. In each case, the decrease in insulin binding can be fully accounted for by a decrease in the number of insulin receptors. The receptors which remain are normal in their affinity for insulin, kinetics of association and dissociation, temperature dependence of binding, biological specificity, and site–site negatively cooperative interactions (24).

The major factor regulating the receptor in each case appears to be the basal level of circulating insulin. Thus, the more insulin resistant the animal, the higher the basal insulin and the greater the decrease in receptor number (Table I). Correction of the hyperinsulinemia by diet (31), streptozotocin treatment (21), or diazoxide therapy (32) for only a few weeks in man or a few days with rodents results in correction of the receptor defect, even though significant obesity persists.

The concept that insulin may directly effect the concentration of its own receptors has been substantiated by both *in vitro* studies and experimentally induced hyperinsulinemia *in vivo*. Cells cultured in the presence of increasing concentrations of insulin showed a time-dependent loss of insulin receptors reaching a new steady state in 4–16 hr (33, 34). The steady-state level of receptor is a function of insulin concentration. Similarly, in patients with insulinoma (35) and in rats made chronically hyperinsulinemic by injection of exogenous insulin (oral glucose being given to prevent hypoglycemia) (36), there is a time and dose-dependent decrease in the number of insulin receptors. While the exact mechanism by which insulin regulates its receptor remains unknown, it has been suggested that chronic exposure of cells to high insulin concentrations induces an acceleration in the rate of receptor degradation (37).

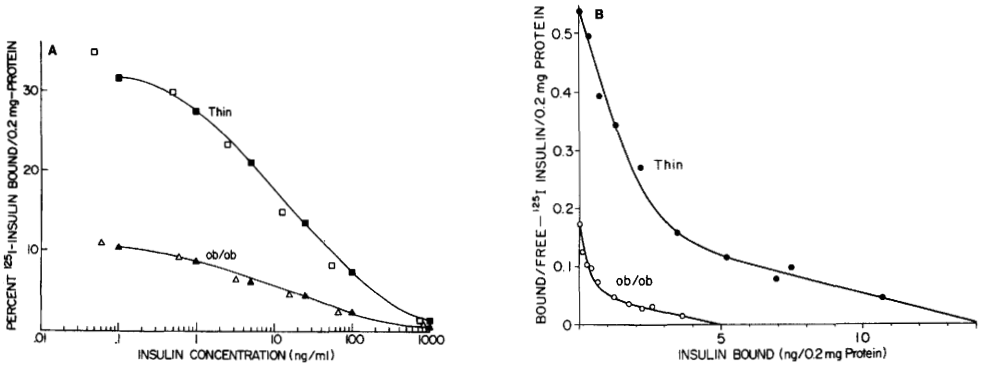

FIG. 3. (A) Insulin binding to purified rat liver plasma membranes of *ob/ob* mice and their thin littermates. (B) Scatchard analysis of these data. Reprinted with permission from Ref. (20).

TABLE I. CHARACTERISTICS OF INSULIN RECEPTOR FUNCTION IN OBESE ANIMALS

Animal Model	Inheritance	Adipocyte hyperplasia	Glucose intolerance	Increased IRI[a]	Decreased receptors
ob/ob mouse	Autosomal recessive	+	++	+++	+++
db/db	Autosomal recessive	−	+++	+++	+++
NZO mouse	Polygenic	+	+	++	++
KK mouse	Polygenic	?	+	+	+
Zucker rat	Autosomal recessive	+	±	+	±
Gold thioglucose-treated mouse	Acquired	−	±	0 to ++	0 to ++
Man	?	+ or −	0 to ++	0 to ++	0 to ++

[a] IRI, immunoreactive insulin.

Even normal physiologic concentrations of insulin appear to induce a mild regulatory effect on receptor number. Thus, insulin receptors appear to show a small, but significant, diurnal variation due to changes in insulin level (38) and are significantly increased in hypoinsulinemic, diabetic animals (39). Other diseases with altered receptor concentration are summarized in Table I.

Glucocorticoid excess as a model of disease with altered receptor affinity. It has been well known that when injected into animals under appropriate conditions ACTH and glucocorticoids can produce a form of insulin-resistant diabetes (40). The clinical correlates of these hormonally induced forms of glucose intolerance are also observed in patients with Cushing's syndrome or patients given glucocorticoids for therapy of other diseases (41). Conversely, in the absence of ACTH or glucocorticoids, there is an increase in insulin sensitivity and a tendency toward hypoglycemia (42). The most extreme example of this type of insulin resistance occurs in rats bearing the MtT transplantable pituitary tumor that secretes ACTH, growth hormone, and prolactin. Goldfine and co-workers have shown that in these animals there is a significant reduction in insulin binding to liver membranes, while glucagon remains unchanges (43). This decrease in binding can be corrected by adrenalectomy despite continued high circulating levels of growth hormone and ACTH. More recently, Olefsky *et al.* (44) and Kahn *et al.* (15) have shown that normal rats given high doses of ACTH or dexamethasone show a similar decrease in binding (Fig. 4).

The exact cause for the decrease in insulin binding was initially difficult to ascertain. However, using the negative cooperativity model and computer-assisted data analysis we found that, in contrast to the decrease in binding observed in obesity, this decrease in binding seems to be due to a marked decrease in the affinity of the insulin receptor for insulin (15). The changes on both $\bar{K}_e$ and $\bar{K}_f$ are more or less proportional and the affinity profiles are parallel, suggesting that negative cooperativity is conserved (Fig. 4). Conversely, adrenalectomy produces an increase in insulin binding by increasing the affinity of the insulin receptor, consistent with the increase in insulin sensitivity that occurs in this state. At present, we have not performed sufficient kinetic studies to decide if these changes in affinity are due to changes in the association or dissociation rate constants. It is worth pointing out that without the aid of a number of adequate data points and the computer analysis, it would have been impossible to determine the nature of the receptor defect. Also, in contrast to obesity, it is likely that there are significant changes in intracellular metabolism beyond the receptor which contribute to the insulin resistance (45, 46). Other disorders with major alterations in receptor affinity are summarized in Table II.

Antibodies to the insulin receptor in insulin-resistant states. From the preceding discussions, it is clear that insulin action can be

TABLE II. DISEASES ASSOCIATED WITH CHANGES IN THE CONCENTRATION OF THE INSULIN RECEPTORS

Receptor concentration decreased
- —Obesity
- —Thin, hyperinsulinemic diabetics
- —Growth hormone excess
- —Uremia
- —Insulinoma
- —Syndrome of insulin resistance and acanthosis Nigricans Type A

Receptor concentration increased
- —Hypoinsulinemic diabetes
- —Hypophysectomy (GH deficiency)

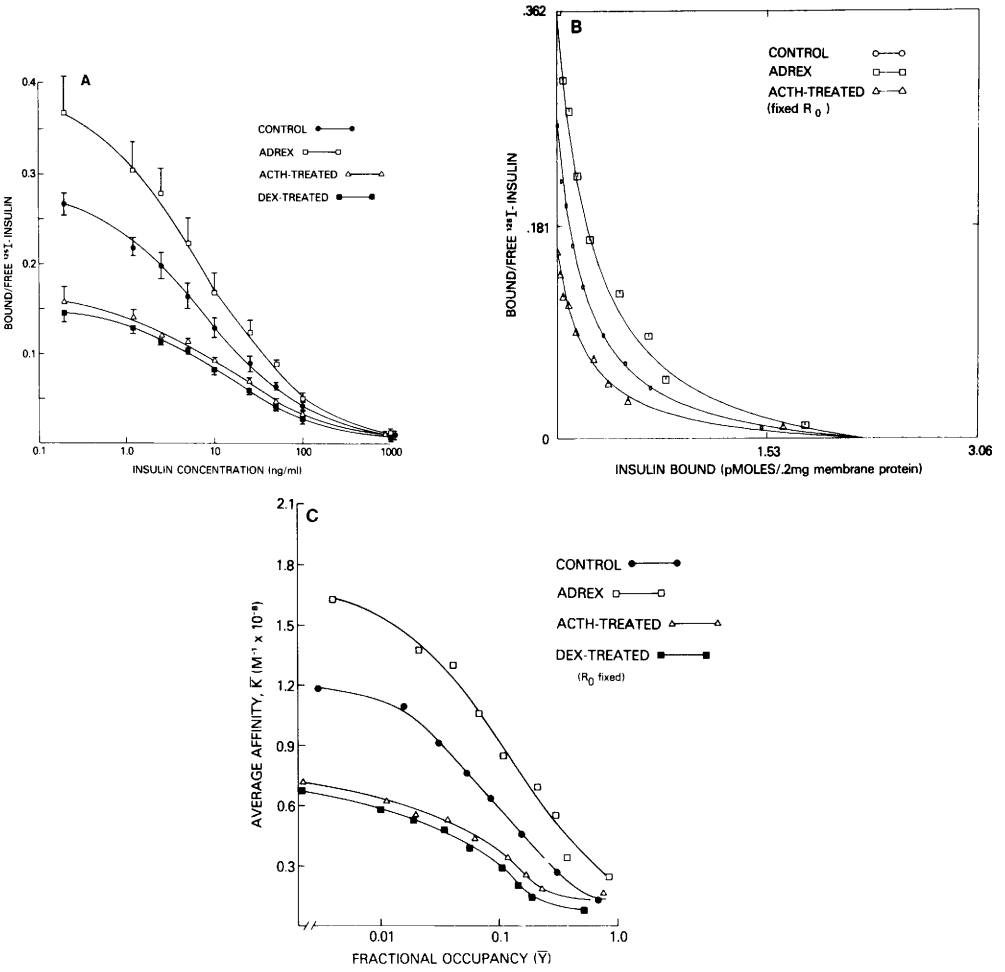

FIG. 4. Effect of steroids on insulin binding. (A) Insulin binding was studied to purified liver plasma membranes of normal rats (control), rats treated with ACTH or dexamethasone (Dex) for 45 days, or adrenalectomized (Adrex) rats. (B) Scatchard plot of the data in (A) fit using a negative cooperative model. (C) Average affinity probe for the data in (B). Adapted from Ref. (15).

TABLE III. DISEASES ASSOCIATED WITH CHANGES IN THE AFFINITY OF THE INSULIN RECEPTOR

Receptor affinity decreased
—Acidosis
—Glucocorticoid excess
—Insulin resistance with anti-receptor antibodies
—Syndrome of insulin resistance and acanthosis nigricans Type B
—Ataxia telangectasia
—Lipoatrophic diabetes (some)
Receptor affinity increased
—Glucocorticoid deficiency
—Isolated GH deficiency (man)

modified by alterations in either receptor number or affinity, and that a variety of factors may regulate the hormone–receptor interaction. An interesting new subset of receptor-related diseases are those in which receptors seem to become the target of a disordered immune response and in which their function is altered by specific antireceptor antibodies. Anti-receptor antibodies have been implicated in the pathogenesis of three diseases: Graves's disease, with antibodies to the thyroid-stimulating hormone receptor; myasthenia gravis, with antibodies to the acetylcholine receptor; and a syndrome of extreme insulin resistance in which antibodies to the insulin receptor have been found (37).

The syndrome of insulin resistance due to anti-receptor antibodies is a rare disorder, but has provided many useful insights into the role of receptors in disease, as well as basic information about the receptor itself. The

hallmark of the syndrome is extreme resistance to both endogenous and exogenous insulin (47). The tissue resistance results in compensatory hyperinsulinemia, with basal and stimulated insulin levels from 10 to 100 times greater than normal. In some patients, the endogenous hyperinsulinemia is sufficient to overcome the insulin resistance, and glucose tolerance may be normal or only mildly impaired. In others, the hyperinsulinemia does not compensate for the insulin resistance, and clinical diabetes results. In these diabetic patients, the resistance to exogenously administered insulin can be extraordinary; one probable case in the literature (48) received up to 177,500 units in one day. None of the known causes of insulin resistance, such as high titers of anti-insulin antibodies, obesity, lipoatrophy, Cushing's disease, or acromegaly, accompany the disorder. In addition, these patients have features suggestive of an immunologic disease, including high erythrocyte sedimentation rate, positive antinuclear and anti-DNA antibodies, leukopenia, alopecia, and arthralgias. Acanthosis nigricans also is present in most patients.

To investigate the possible role of the insulin receptor in this disease, we studied the binding of insulin to its receptor on circulating monocytes in these patients. Like animals with glucocorticoid excess, patients with insulin resistance and anti-receptor antibodies have decreased binding of insulin to its membrane receptors, and this decrease is due to a decrease in receptor affinity (Fig. 5) (47, 49). However, the defect differs from that due to steroids in that only the high-affinity state of the receptor is altered. The receptors behave as if they are "locked" in the low-affinity ($\bar{K}_f$) conformation. All existing data suggest that the underlying receptor is normal. Thus, the defect can be corrected by removing the anti-receptor antibodies by acid wash of the cells or plasma exchange and is absent in cultured fibroblasts from the patients and during remission (49).

The antibodies to the insulin receptor have been detected using two basic assays (Fig. 6). The binding-inhibition assay, initially conceived of by Flier *et al.* (50), is the simplest and may be performed with intact cells, membranes, or even solubilized receptors. The immunoprecipitation assay is theoretically more sensitive, but is considerably more tedious and requires solubilized receptors (51). Thus far most of the antibodies have been IgG in nature, although one patient had IgM antibodies, and in one family with insulin resistance due to anti-receptor antibodies and ataxia telangectasia, the antibodies appeared to be a low molecular weight, monoclonal IgM (52, 53).

Attempts to understand the mechanism by which these anti-receptor antibodies produce an insulin-resistant state were initially confusing. When studied in a variety of systems acutely, the anti-receptor antibodies mimicked insulin action (Table IV) (54–57). This insulin-like effect, however, is transient. Exposure of cells to anti-receptor antibody for 36 hr results in a state of insulin resistance (Fig. 7) (58). This desensitization of the cells occurs without a further change in insulin binding and appears to be an active metabolic process.

Both the acute biological effects and chronic state of desensitization appear to require antibody bivalence (59). Monovalent antibody fragments bind to the cell and block insulin binding and action, but are without biological effect (Fig. 8). Addition of a second

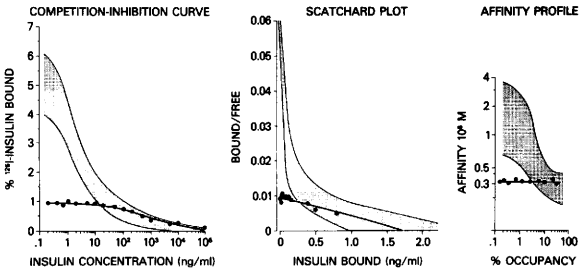

FIG. 5. Insulin binding to circulating monocytes from a patient with anti-receptor antibodies. Insulin binding was studied as previously described. The shaded area is the mean ± SD for 35 normal controls. Note the flat affinity profile which contrasts that seen after glucocorticoids, although in both cases receptor affinity is decreased.

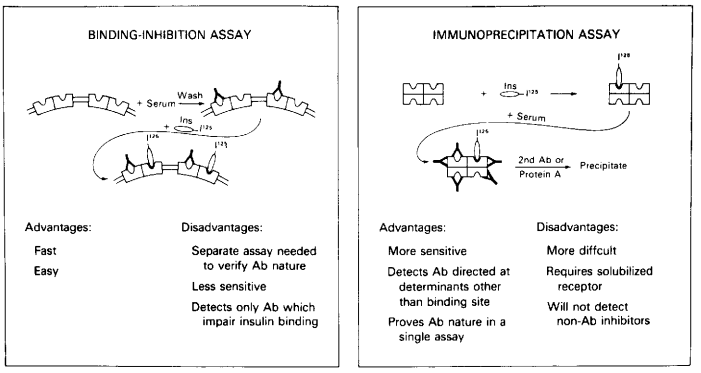

FIG. 6. Two direct assays for antibodies to the insulin receptor. In the binding-inhibition assay, the cells or membranes are first exposed to serum (usually for 60 min at 22°) and washed and then insulin binding is studied. In the immunoprecipitation assay, solubilized receptors are prelabeled with ^{125}I-insulin, then serum is added, and immunoprecipitation of the complex is effected by addition of a second antibody or protein A-Sepharose.

TABLE IV. BIOLOGICAL EFFECTS OF THE ANTI-RECEPTOR ANTIBODIES

Adipocytes
Stimulation of 2-deoxyglucose transport, glucose incorporation into lipid and glycogen, and metabolism to CO_2
Activation of glycogen synthase (in the presence and absence of glucose)
Stimulation of pyruvate dehydrogenase and acetyl-CoA carboxylase
Stimulation of leucine incorporation into protein
Inhibition of lipolysis
Inhibition of phosphorylase
3T3-L1 Preadipocytes
Stimulation of 2-deoxyglucose transport and glucose metabolism to CO_2
Stimulation of lipoprotein lipase
Muscle
Stimulation of 2-deoxyglucose transport and glucose incorporation into glycogen
Stimulation of glycolysis
Stimulation of glycogen synthase (partial)
Liver
Stimulation of AIB transport

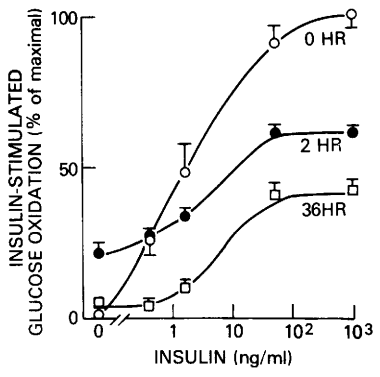

FIG. 7. Effect of prolonged treatment of 3T3-L1 cells with anti-receptor antibody. Cells were pretreated for 0, 2, or 36 hr with anti-receptor antibody, and insulin-stimulated glucose oxidation was measured in the usual fashion. Adapted from Ref. (58).

antibody which crosslinks the monovalent antibody fragments restores bioactivity.

Summary and perspectives. The insulin receptor is at a crucial step in insulin action serving as the link between extracellular insulin concentration and the metabolic pathways for insulin action. Changes in receptor number and affinity may occur under a variety of influences and play an important role in many disease states. The discovery of antibodies to the receptor has not only proved to be of interest with respect to illucidation of a mechanism for disease, but the antibodies themselves have been a unique probe of receptor structure and function. Recently, using these antibodies we have developed an immunoassay for the solubilized insulin receptor (60). With this assay we should be able to

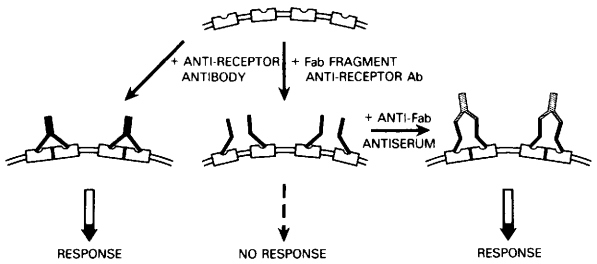

FIG. 8. Proposed mechanism for the biological effects of antibodies to the insulin receptor.

further characterize the receptor, as well as uncover new defects in the receptor which may not be manifest by alterations in insulin binding. The future identification of other factors (including drugs) which regulate receptor concentration or affinity will also be important in our understanding of the hormone–receptor interaction, as well as of the management of disease states.

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