Autoantibodies Against the Insulin Receptor

Dissociation of the Acute Effects of the Antibodies from the Desensitization Seen with Prolonged Exposure

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SUMMARY

The effect of antibodies against the insulin receptor (anti-R) found in a patient with the type B syndrome of insulin resistance and acanthosis nigricans was characterized using 3T3-L1 cultured fat cells. Anti-R acutely mimicked the action of insulin by stimulating deoxyglucose uptake. With more prolonged exposure, this insulinomimetic effect decayed, glucose metabolism returned to basal levels, and the cells became severely resistant to the actions of insulin. As seen with anti-R from a previous patient, desensitization consisted of both a dramatic decrease in the maximal responsiveness of the cells to insulin and a shift in the dose-response curve for insulin-stimulated glucose oxidation.

The acute and chronic effects of anti-R were then compared. The concentration of anti-R required to half-maximally inhibit insulin binding averaged more than twice that required for half-maximal stimulation of deoxyglucose uptake, consistent with the amount of spare receptors in 3T3-L1 cells. After prolonged exposure, the insulinomimetic activity was completely lost at all concentrations of anti-R, even at those that did not completely induce insulin resistance. Thus, loss of the insulinomimetic activity of anti-R is necessary, but not sufficient, to cause desensitization.

Less anti-R was required to desensitize cells to insulin than would have been predicted on the basis of the acute inhibition of binding and the number of spare receptors. In addition, the amount of anti-R required to half-maximally inhibit insulin binding after prolonged exposure was an order of magnitude less than that during acute exposure, implying a progressive loss of insulin receptor binding during antibody-induced desensitization. When cells were treated with subsaturating concentrations of anti-R, insulin binding decreased over a period of several hours.

Finally, an acid wash (capable of dissociating antigen-antibody complexes) could not restore insulin binding after desensitization, but could restore insulin binding if cells were exposed to anti-R at 4°C. These results support the theory that insulin receptors are removed from the cell surface during antibody-induced desensitization. DIABETES 1985; 34:205–11.

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The type B syndrome of insulin resistance with acanthosis nigricans is characterized by severe insulin resistance, decreased binding of insulin to the patients' circulating monocytes, and the presence in the patients' serum of antibodies directed against the insulin receptor (anti-R).[1–3] Anti-R blocks the binding of insulin to its plasma membrane receptor.[3] When circulating monocytes from type B patients are treated with a mild acid wash, capable of dissociating antibody-antigen complexes, their insulin binding is "increased toward normal."[4]

In contrast to the insulin resistance produced in vivo, anti-R has insulinomimetic activity when studied acutely in vitro.[5–9] Anti-R has been shown to stimulate deoxyglucose and amino acid transport, glucose incorporation into lipids and glycogen, and glucose metabolism to CO_2. Anti-R activates glycogen synthase and inhibits lipolysis and phosphorylase. These insulinomimetic actions of anti-R are in obvious contrast to the insulin resistance seen in patients with chronic exposure to circulating anti-R.

We have previously used 3T3-L1 cells, a cultured mouse adipocyte line, to develop a model for the insulin resistance seen in the type B syndrome.[10,11] As seen with other insulin-responsive cells, anti-R prepared from the serum of one type B patient (NIH B-2, J.P.) acutely mimicked the action of insulin and inhibited the binding of ^{125}I-insulin in 3T3-L1 cells. With prolonged exposure, however, the insulinomimetic activity of anti-R decayed and 3T3-L1 cells were rendered severely resistant to the actions of insulin, showing both a de-

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creased sensitivity to insulin (shift in the dose-response curve to the right) and a dramatic decrease in the maximal response to high concentrations of insulin.[10] We called this process "antibody-induced desensitization of the insulin receptor."[10] Based on several types of experiments, we have previously postulated that anti-R induced desensitization at "an early post-receptor step."[10,11] First, inhibition of insulin binding per se was not sufficient to induce insulin resistance, as monovalent Fab preparations of anti-R inhibited the binding of insulin and decreased insulin sensitivity acutely but did not produce desensitization; no decrease was seen in the maximal insulin response in contrast to that seen with prolonged exposure to the intact IgG.[11] Second, desensitization could be distinguished from simple "downregulation," as cycloheximide, an inhibitor of protein synthesis that blocks downregulation, had no effect on the induction of insulin resistance.[11] In addition, 3T3-L1 cells do not show downregulation in response to insulin under similar conditions.[12] Finally, the acid wash studies of Muggeo[4] suggested that the insulin receptor remained on the cell surface after desensitization.

Recently, the serum from another patient with type B syndrome has become available in sufficient quantity and titer to be tested in 3T3-L1 cells. In this paper, we demonstrate that anti-R from this patient also acutely mimics the action of insulin, inhibits insulin binding, and, after prolonged exposure, causes desensitization to insulin. The availability of this new anti-R allows us to explore in detail, for the first time, the relationship between the acute effects of anti-R and the ability of anti-R to induce insulin resistance. The data suggest that insulin receptors are lost from the cell surface during desensitization by anti-R.

MATERIALS AND METHODS

Materials. Dexamethasone was purchased from Sigma Chemical Co. (St. Louis, Missouri), 1-methyl-3-isobutylxanthine was obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin), bovine serum albumin was purchased from Armour Co. (Phoenix, Arizona), and pork insulin was obtained from Elanco Products Co. (Indianapolis, Indiana). ^{3}H-2-deoxyglucose (37.3 Ci/mmol), ^{14}C-glucose (1 mCi/mmol), and carrier-free [^{125}I]-NaI were purchased from New England Nuclear Co. (Boston, Massachusetts); Linbro Multiwell plates and fetal calf serum (lot #29101598) were obtained from Flow Laboratories (McClean, Virginia). Anti-R was prepared from serum as previously described.[10,11]

Cell culture. 3T3-L1 cells[13,14] were generously provided by Dr. Howard Green of the Massachusetts Institute of Technology and grown as previously described.[2] Differentiation of confluent cells was enhanced by supplementation of standard Dulbecco-Vogt tissue culture medium (10% fetal calf serum) for 2 days with 0.5 mM 1-methyl-3-isobutylxanthine, 0.25 μM dexamethasone, and insulin (1 μg/ml) as previously described.[15] Cells were then refed with standard medium without additives and used within 5–14 days.

Insulin action and insulin binding. The binding of insulin to its receptor and the biologic activity of insulin were assessed by methods that have been previously characterized in detail.[10–12,15] All procedures were carried out at pH 7.4. Deoxyglucose uptake and insulin binding were assayed on cells adherent to tissue culture plates using Krebs-Ringer phosphate buffer supplemented with bovine serum albumin (20 mg/ml) and 1.3 mM $CaCl_2$.[12,15] Glucose oxidation was studied using cells in suspension in Krebs-Ringer bicarbonate buffer supplemented with bovine serum albumin (20 mg/ml) at pH 7.4 as previously described.[12] Cell suspensions were prepared by briefly incubating the cells in Joklik's Spinner media supplemented with 2 mM EDTA.[12]

Desensitization was carried out by a protocol identical to that used for anti-R from patient B-2.[10,11] Cells were exposed to anti-R in complete Dulbecco-Vogt medium for the indicated times and then prepared for assays as described above.

Acid wash. The acid wash procedure was a modification of that of Muggeo.[4] Cells were exposed to anti-R or control serum either under the conditions for desensitization described above (>6 h at 37°C) or for 3 h at 4°C in Krebs-Ringer phosphate buffer supplemented with 20 mg/ml BSA and 1.3 mM $CaCl_2$. All subsequent steps were performed at 4°C. Cells for acid wash were rinsed once and exposed for 2.5 min to a buffer consisting of 0.85% NaCl, 5 mM KCl, 50 mM glycine, 1.3 mM $CaCl_2$, and 20 mg/ml BSA at pH 3.5. For comparison, cells preexposed in the same manner were treated with Krebs-Ringer phosphate buffer (20 mg/ml BSA, 1.3 mM $CaCl_2$) at pH 7.4 in parallel. An acid wash of 2.5 min gave optimal reversal of the effect of anti-R. No significant increase in binding to anti-R-treated cells occurred if the wash period was extended up to 7.5 min. When exposure to acid exceeded 7.5 min, binding to control and anti-R-treated cells changed. After the acid wash, cells were prepared for measurement of insulin binding as described previously[12,15] except that the incubation was for 3 h at 4°C.

Reproducibility. The data presented in graphs, in general, represent the mean ± SEM of triplicate determinations of a single anti-R preparation. Similar data were also obtained with several other antibody preparations from patient B-8. Although the absolute values for each preparation differed, the relative potency for each event studied remained constant. Average values for three different preparations are reported in the text.

RESULTS

Anti-R mimics insulin action and then induces desensitization. Anti-R from patient B-8 was capable of acutely mimicking insulin action as well as inducing insulin resistance (Figure 1). Cells were exposed to anti-R for the number of hours indicated on the abscissa after which basal and insulin-stimulated deoxyglucose uptake were measured. Before exposure to the antibody (time 0), the basal rate of deoxyglucose uptake was approximately 4 nmol/20 min (open circles) and insulin-stimulated uptake (closed circles) was 15 nmol/min. Within 30 min of exposure to anti-R, basal deoxyglucose uptake (in the absence of insulin) had risen to 15 nmol/20 min, indicative of the insulinomimetic activity of anti-R. However, the insulinomimetic activity of anti-R decayed with a $t_{1/2}$ of <2 h; after 6 h of pre-exposure to anti-R, the basal rate of deoxyglucose uptake had decreased to that of unexposed cells (open circles).

Exposure of cells to anti-R resulted in a prompt inhibition of the response to the subsequent addition of insulin (Figure 1, closed circles) at all times. Thus, when the insulinomimetic activity of anti-R had decayed back to baseline (6 h), the

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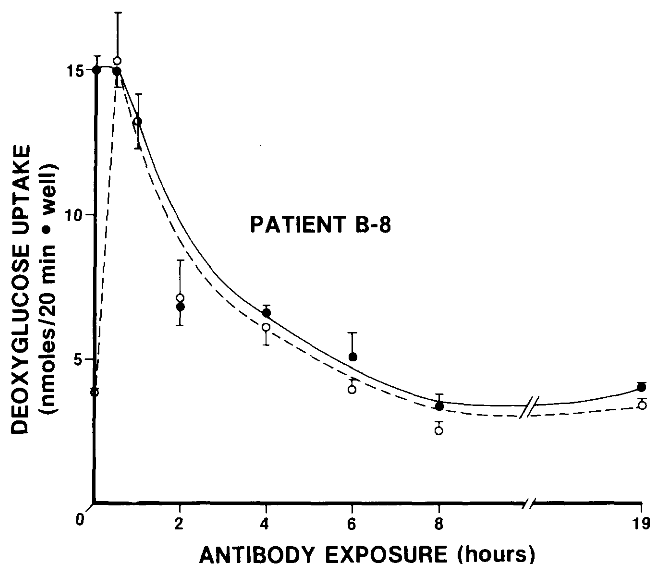

FIGURE 1. The effect of acute and prolonged exposure of 3T3-L1 cells to anti-R on basal and insulin-stimulated deoxyglucose uptake. 3T3-L1 cells adherent to tissue culture plates were exposed to anti-R (610 μg/ml in complete Dulbecco-Vogt tissue culture medium) for the time indicated on the abscissa by the method previously described.[10,11] Cells were then rapidly rinsed and incubated in the absence (open circles) or presence (closed circles) of insulin (1 μg/ml) for 20 min at 24°C. Deoxyglucose uptake was then measured using a 20-min pulse by the method described.[12,15] At the end of the incubation, the cells were processed by rinsing with Dulbecco's phosphate-buffered saline at 4°C and solubilized in SDS for determination of radioactivity.

cells were severely resistant to the action of insulin: deoxyglucose uptake could not be raised significantly above the level of basal deoxyglucose uptake in control cells. When cells were exposed to lower concentrations of anti-R, they retained some ability to respond to insulin (vide infra).

The effect of prolonged exposure to anti-R on insulin-stimulated glucose oxidation. Prolonged exposure to anti-R also resulted in inhibition of the ability of insulin to stimulate glucose oxidation in 3T3-L1 cells (Figure 2). As can be seen in Figure 2, anti-R decreased the maximal ability of insulin to stimulate glucose oxidation in 3T3-L1 cells, which could not be overcome by even very high doses of insulin. In addition, the dose-response curve for the remaining insulin stimulation was shifted dramatically to the right. Approximately 0.4 ng/ml of insulin was required for half-maximal stimulation of glucose oxidation in control 3T3-L1 cells, while 3.3 ng/ml of insulin was required for half-maximal stimulation in the cells pretreated with anti-R. When added acutely, anti-R also stimulates glucose oxidation in 3T3-L1 cells (data not shown).

We have thus demonstrated that anti-R from patient B-8 has the same properties as anti-R from patient B-2 with regard to its acute insulinomimetic action and its ability to induce desensitization.[10,11] We then used anti-R B-8 to explore the relationship between the acute and chronic effects of the antibody on both insulin binding and insulin action. This relationship had not previously been examined.

The insulinomimetic activity of anti-R. The ability of anti-R from patient B-8 to acutely mimic the action of insulin on deoxyglucose uptake is presented in Figure 3 (upper panel). Forty-five micrograms per milliliter of anti-R was required for half-maximal stimulation of deoxyglucose uptake. Maximal stimulatory biologic activity was obtained when anti-R was used at a concentration approaching 200 μg/ml.

Insulin binding. The ability of anti-R from patient B-8 to acutely inhibit the binding of ^{125}I-insulin to 3T3-L1 cells is presented in the lower panel of Figure 3. Eighty-four micrograms per milliliter was required for 50% inhibition of insulin binding. Greater than 600 μg/ml of anti-R was required to completely inhibit insulin binding. Thus, for this preparation of B-8, 70% more anti-R was required to acutely inhibit insulin binding than to acutely stimulate deoxyglucose uptake. When the results from three different preparations of anti-R B-8 were averaged, 120% more anti-R was required to acutely inhibit binding (185 μg/ml) than to acutely stimulate deoxyglucose uptake (84 μg/ml). This difference was significant to $P < 0.05$ in a paired *t*-test. The difference between the ability of anti-R to inhibit insulin binding and to stimulate deoxyglucose uptake is consistent with the presence of between 40% and 70% spare receptors in 3T3-L1 cells.[10,12]

Concentration dependence for desensitization. Cells were then exposed to varying concentrations of anti-R for 6–8 h at 37°C under the conditions required for desensitization, after which basal and insulin-stimulated deoxyglucose uptake were measured (Figure 4). After these prolonged exposures, the insulinomimetic activity of anti-R (open circles) decayed at all concentrations tested. Thus, the insulinomimetic activity was lost even at concentrations that were below that required for maximal biologic activity or inhibition of binding, and this occurred with a similar time course at all concentrations. When the ability of anti-R to decrease the maximal response to insulin was measured (closed circles), 75 μg/ml of antibody was required for 50% inhibition of insulin action. Thus, *less* anti-R was needed to

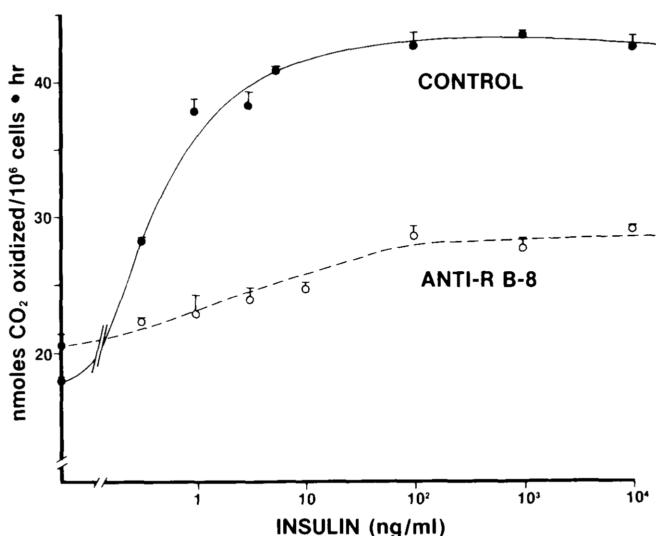

FIGURE 2. The effect of anti-R pretreatment on insulin-stimulated glucose oxidation. 3T3-L1 cells were incubated in the absence (closed circles) or presence (open circles) of anti-R for 16 h as previously described.[10] The cells were then removed from plates using 2 mM EDTA and glucose oxidation was measured in the absence or presence of increasing concentrations of insulin as indicated on the abscissa. The rate of glucose oxidation was measured over 1 h using ^{14}C-glucose. $^{14}CO_2$ was collected after completion of the incubation using hyamine.[12]

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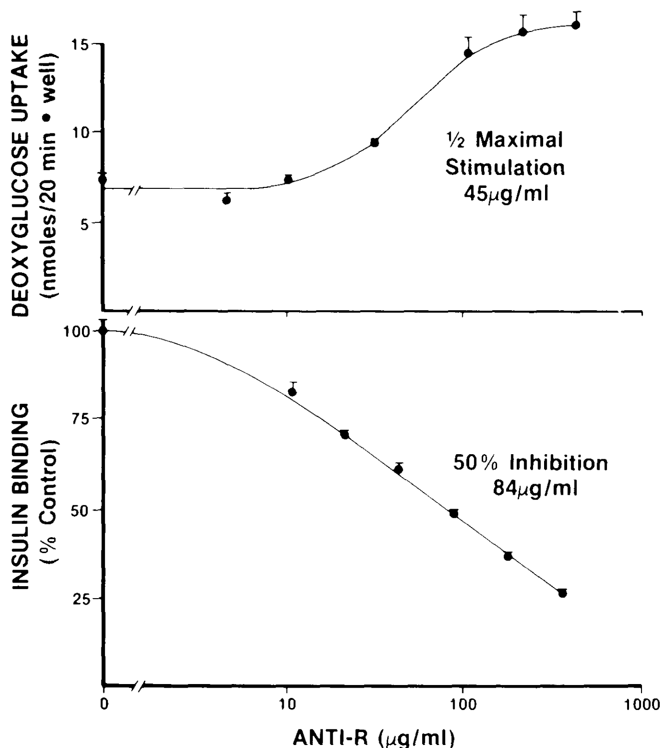

FIGURE 3. Acute effects of anti-R on insulin action and insulin binding. (Upper panel) 3T3-L1 cells adherent to tissue culture plates were preincubated in the presence or absence of anti-R at the indicated concentration for 75 min. Deoxyglucose uptake was then measured using a 20-min pulse as in Figure 1.[12,15] (Lower panel) Inhibition of insulin binding: 3T3-L1 cells adherent to the tissue culture plates were incubated with ^{125}I-insulin (0.2 ng/ml) in the presence or absence of anti-R at the indicated concentrations for 75 min under identical conditions to those used in the upper panel.[15] Nonspecific binding (in the presence of 10 μg/ml unlabeled insulin) has been subtracted.

induce desensitization than would be expected on the basis of the presence of spare receptors and the ability of anti-R to acutely inhibit insulin binding (cf. Figure 3). If the acute inhibition of binding directly correlated with desensitization, one would expect that *more* anti-R would be required to produce desensitization than to inhibit binding, as there are spare receptors in 3T3-L1 cells (cf. refs. 10 and 11 and Figures 1 and 2). For example, with 50% spare receptors, a 50% reduction in insulin action would require approximately a 75% reduction in insulin binding. The concentration of anti-R required for half-maximal desensitization was *lower* than that for half-maximal inhibition of insulin binding for all three preparations of anti-R B-8 that were tested.

The effect of prolonged exposure to anti-R on insulin binding. We therefore sought to determine whether even less anti-R was required to inhibit insulin binding after prolonged exposure (8 h). As can be seen in Figure 5, only 19 μg/ml of anti-R was needed to inhibit insulin binding by 50% after this pre-exposure. Seventy-five percent inhibition of insulin binding was achieved after incubation with 78 μg/ml of anti-R. This represents a significant increase in the ability of anti-R to inhibit insulin binding when cells were exposed for prolonged periods of time at 37°C compared with the acute inhibition seen at lower temperatures. When all three preparations were compared, 16.8 μg/ml of anti-R B-8 were required for half-maximal inhibition of binding after prolonged incubation, strikingly less than that required for acute inhibition of binding. This difference was significant in a paired *t*-test ($P < 0.05$).

Progressive loss of cell surface insulin receptors by exposure to anti-R. In contrast to the rapid effects of anti-R on insulin binding and mimicking insulin action (Figures 1 and 3), the data in Figures 4 and 5 are consistent with a progressive loss of insulin receptor binding during desensitization. This was confirmed by studying the time course of inhibition of insulin binding when 3T3-L1 cells were exposed to subsaturating amounts of anti-R at 37°C (Figure 6). Insulin binding continued to decay over several hours.

Acid wash does not reverse inhibition of insulin binding after desensitization by anti-R. To determine the fate of the cell surface insulin receptor during desensitization, we tested the ability of an acid wash (capable of dissociating antibody from antigen) to restore insulin binding to anti-R-treated cells. When cells were treated with anti-R (370 μg/ml) under the conditions required for induction of desensitization, the acid wash could not restore insulin binding to normal (Figure 7, left panel). However, when cells were exposed to anti-R at reduced temperature (4°C) to prevent internalization, the acid wash was able to restore binding to normal. Intermediate values were obtained when cells were exposed to 15°C or 24°C indicating that some internalization occurs at these temperatures (data not shown).

DISCUSSION

The actions on 3T3-L1 cells of anti-R purified from the serum of a second patient (B-8) with type B syndrome of insulin resistance and acanthosis nigricans are similar to those found with the first patient studied.[10,11] These similarities are seen despite the fact that the titer of patient B-8's antibody is approximately one-eighth that of patient B-2 and patient B-8 was significantly less resistant to insulin than was patient B-2.

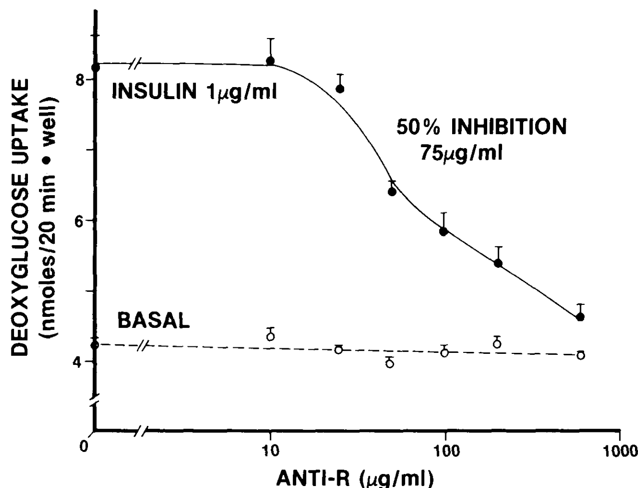

FIGURE 4. The concentration of anti-R required to induce insulin desensitization. 3T3-L1 cells adherent to tissue culture plates were pre-exposed for 8 h to anti-R at the concentration indicated on the abscissa under the same conditions as used for Figure 1. Cells were then rinsed and basal deoxyglucose uptake (open circles) and maximal insulin-stimulated deoxyglucose uptake (1 μg/ml) (closed circles) were measured by the pulse method as in Figures 1 and 3.[12,15]

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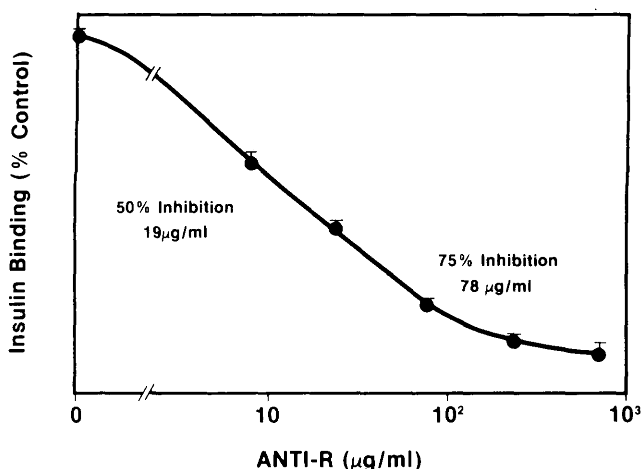

FIGURE 5. The effect of prolonged exposure to anti-R on insulin binding. 3T3-L1 cells adherent to tissue culture plates were pre-exposed for 8 h to anti-R at the concentrations indicated on the abscissa, under identical conditions as those for Figures 1 and 4 (8 h, 37°C). Cells were then rinsed and insulin binding measured as in Figure 3.[12,15]

The insulinomimetic activity of anti-R B-8 was rapid, with maximal effect being seen in less than 30 min (Figure 1). With prolonged exposure of 3T3-L1 cells to each anti-R, the insulinomimetic activity of anti-R declined and a state of insulin resistance ensued (refs. 10, 11, and this paper). The time course of desensitization found with anti-R from patient B-8 resembled that from patient B-2.[10] Coincident with the decay in insulinomimetic activity, a profound insulin resistance develops when using saturating concentrations of anti-R. Thus, at any given time after exposure to high concentrations of anti-R, insulin could not stimulate deoxyglucose uptake above the basal rate found in anti-R-treated cells. This desensitization process involves both a shift in the dose-response curve to insulin as well as a decrease in the maximal response to insulin (Figure 2). Very high concentrations of insulin (100-fold higher than that required in control cells for maximal stimulation) cannot overcome the block in deoxyglucose uptake or glucose oxidation produced by anti-R (ref. 10 and this paper).

With a new source of antibody against the insulin receptor, we were then able to compare the concentration dependence of acute effects of anti-R with that of the induction of insulin resistance. Previous studies had suggested that 3T3-L1 cells are more sensitive to the insulinomimetic effects of anti-R from patient B-2 than to inhibition of insulin binding.[10] However, direct comparisons were not possible, since in that study, the assays for inhibition of binding and biologic activity were performed in different buffers at widely disparate pHs.[10] In this paper, both assays were performed at pH 7.4 in the same buffer. Approximately 50% less anti-R from patient B-8 was required to half-maximally stimulate deoxyglucose uptake than was necessary to half-maximally inhibit insulin binding. This figure is consistent with the number of spare receptors that we have found in 3T3-L1 cells, which varies from 40% to 70% between batches of cells. We have previously confirmed this estimate of spare receptors by demonstrating that, during recovery from anti-R-induced desensitization, the full biologic response to insulin is restored when 40–50% of insulin binding is recovered.[10]

As seen with previous studies,[10,11] the data in this paper demonstrate that desensitization is not due merely to the blockage of insulin binding, but requires loss of the initial insulinomimetic activity. When we then examined the effects of exposure to anti-R for prolonged periods of time, we found that the insulinomimetic activity of anti-R decayed, even at low concentrations of anti-R (Figure 4). Thus, neither maximal biologic response nor full occupancy of insulin receptors, as measured by acute inhibition of insulin binding, is necessary for the decay in the insulinomimetic action of anti-R. We have also demonstrated here that the loss of insulinomimetic activity of the antibody is necessary but is not sufficient for the emergence of insulin resistance, as the insulinomimetic effect is lost at concentrations that do not produce full insulin resistance. This is in marked contrast to the action of insulin, which persists for many hours in 3T3-L1 cells (ref. 12 and unpublished data). In our hands, insulin does not cause significant downregulation in these cells.[12] Furthermore, when insulin is acutely washed off of 3T3-L1 cells, basal glucose metabolism returns to normal,[12] while several hours are required to recover from antibody-induced desensitization.[10,11]

Based on the estimate of approximately 50% spare receptors under these conditions (Figure 1), one would have expected that a higher concentration of anti-R than we found would be required to induce insulin resistance. The concentration of anti-R required to acutely reduce binding by 50% should still leave adequate receptors to allow for full biologic activity. Indeed, we have previously shown that recovery from the effect of antibody-induced desensitization on insulin action is complete when approximately 50% of insulin binding is restored.[10] Thus, one would predict that, under these conditions, approximately 75% of insulin binding would have to be lost to block insulin action by 50%.

Yet, despite the rapid initial effect of anti-R (Figure 1 and ref. 10), less anti-R was required to desensitize the cells than would have been predicted on the basis of *acute* inhibition of binding. The cells were an order of magnitude more sensitive to inhibition of binding when exposure took place at 37°C for several hours than when inhibition was studied in an acute equilibrium assay. This difference was reproducibly

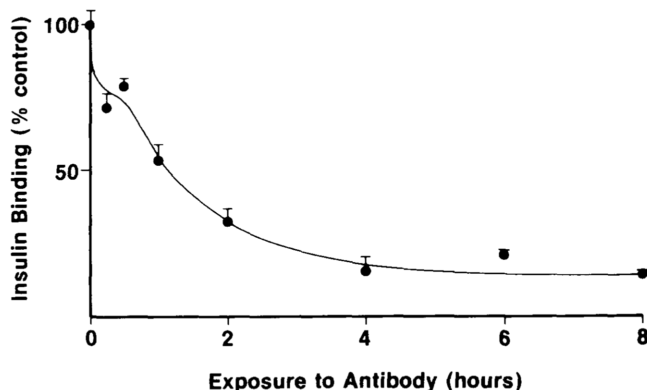

FIGURE 6. The effect of exposure of cells to subsaturating anti-R at 37°C. 3T3-L1 cells were exposed to anti-R (63 μg/ml) for the time period indicated on the abscissa under conditions similar to the desensitization protocol in Figures 1 and 4. Cells were then rinsed and insulin binding measured as in Figure 3.[12,15]

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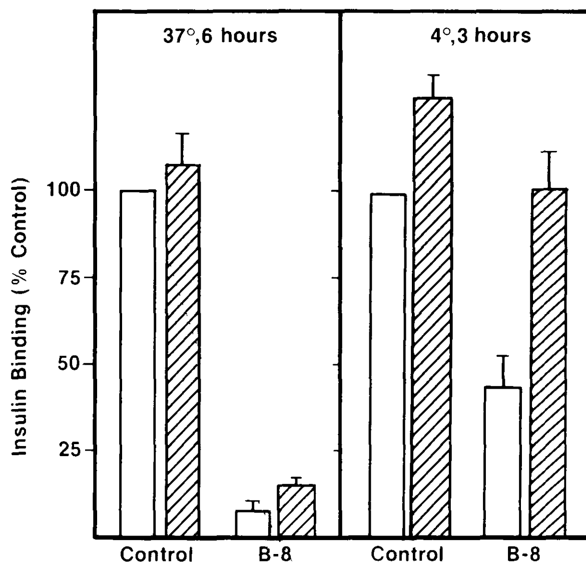

FIGURE 7. The effect of temperature during exposure to anti-R on the ability of acid wash to restore insulin binding. 3T3-L1 cells were exposed to antibody (370 μg/ml) for either 6 h at 37°C (left panel) or 3 h at 4°C (right panel). Results from control serum (left pair) is contrasted to anti-R (right pair). Cells were then rinsed once and then exposed for 2.5 min to either acid wash buffer, pH 3.5 (hatched bars), or to Krebs-Ringer phosphate buffer, pH 7.4 (open bars) for 2.5 min as described in the text. The cells were then prepared for measurement of insulin binding as in previous figures[12,15] except that the assay was performed at 4°C for 3 h. The data represent the average of three identical experiments performed in triplicate.

seen with three different anti-R preparations in multiple assays. The partial desensitization seen at submaximal concentrations of anti-R was not due to recovery from antibody-induced desensitization, as the time required for recovery[10] far exceeds that used in this short bioassay. This observation led to the prediction that a progressive loss of binding over several hours would be seen when subsaturating quantities of anti-R were used (Figure 6). We then postulated that insulin receptors were lost from the cell surface during the course of desensitization. This conclusion was confirmed by demonstrating than an acid wash capable of dissociating antibody-antigen complexes could restore insulin binding when cells were incubated with anti-R at low temperatures but that the same wash was ineffective when cells were allowed to be desensitized at 37°C.

It is important to point out the differences between the conclusions of this study and those of previous studies of anti-R-induced desensitization of the insulin receptor, which had concluded that anti-R induced insulin resistance at "an early postreceptor step in insulin action."[10,11] The previous implication of a postreceptor defect was based on several lines of evidence. Because Muggeo had demonstrated that an acid wash of monocytes from the type B patients restored binding "towards" normal,[4] it was concluded that the receptor remained on the surface in anti-R-induced insulin resistance. However, careful examination of the acid wash data from the paper by Muggeo[4] reveals that, although insulin binding was significantly increased when monocytes isolated from type B patients were acid washed, the loss of binding was only reversed by 36%, while insulin binding could be *fully* restored by the acid wash when control monocytes had been preincubated with anti-R at lower temperatures. Based on our data and that of Muggeo, we would conclude that acid wash cannot fully restore insulin binding after cells are exposed to anti-R for prolonged periods at 37°C in vivo or in vitro. In addition, the previous experiments of anti-R desensitization of the insulin receptor in 3T3-L1 cells used saturating amounts of anti-R so that maximal inhibition of insulin binding was seen immediately; therefore, binding remained unchanged throughout the course of desensitization.[10,11] However, here we also show that cells are more sensitive to the inhibition of insulin binding by anti-R during the prolonged exposure at 37°C required for the desensitization process than after acute exposure, although the conditions of the acute assays were more than adequate to allow equilibrium at the lower temperature.[12,15] These data demonstrate that a progressive loss of insulin binding occurs during desensitization.

Once desensitization occurs, insulin binding cannot be restored by an acid wash capable of dissociating antibody from antigen. These results imply that loss of the cell surface insulin receptors occurs during anti-R desensitization in 3T3-L1 cells. They are consistent with recently published studies from several laboratories.[16-19] We have demonstrated that antibody raised against the rat insulin receptor, which does *not* acutely inhibit insulin binding, also causes desensitization to insulin in 3T3-L1 cells, and the process of desensitization results in a striking loss of insulin receptors.[16] This antibody has also been shown to deplete insulin receptors from rat hepatocytes.[17] In addition, both human anti-R and monoclonal anti-R have been shown to induce downregulation in IM-9 lymphocytes.[18,19] However, anti-R-induced desensitization in 3T3-L1 cells can be distinguished from simple downregulation.[10-12] Since the insulin resistance seen with anti-R in the type B syndrome is so much more severe than that seen with insulin-induced downregulation,[3] further studies on 3T3-L1 cells may shed light on the unique properties of anti-R that produce this severe syndrome of insulin resistance.

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