Proc. Natl. Acad. Sci. USA
Vol. 76, No. 2, pp. 809–813, February 1979
Cell Biology

Desensitization of the insulin receptor at an early postreceptor step by prolonged exposure to antireceptor antibody

(hormone action/insulin resistance/3T3-L1 fatty fibroblasts)

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Communicated by Elizabeth F. Neufeld, November 13, 1978

ABSTRACT **We have used an adipocyte-like cell line, the 3T3-L1 fatty fibroblasts, to compare acute and chronic effects of autoantibodies directed against the insulin receptor. Acute exposure of the cells in tissue culture to the antibodies resulted in a blockade of insulin binding and stimulation of 2-deoxyglucose transport and glucose oxidation. Maximal acute effects were reached within 30–120 min. Subsequently, the stimulatory response decayed and, after 6 hr in the continuous presence of the antibodies, basal glucose metabolism had returned to the level observed with unexposed cells and a state of severe insulin resistance prevailed. In contrast to the decay of bioresponse, no change in insulin binding was detectable over the same time period. The mechanism of desensitization seemed to involve events early after insulin binding to receptor because cells exposed to antibody for prolonged periods of time, although unresponsive to insulin and antireceptor antibodies, responded normally to both spermine and vitamin K_5, agents that stimulate glucose metabolism independently of the insulin receptor. These data suggest that prolonged or continuous occupancy of the insulin receptor by a ligand, in this case antireceptor antibodies, does not produce a continuous biological response. Instead, there is desensitization at some early step in the pathway for insulin action. These observations have important implications with respect to the mechanism of insulin action and to other situations in which there is long-term exposure of cells to antibodies that react with membrane components.**

Antibodies that bind to the insulin receptor have been found in patients with a rare form of insulin-resistant diabetes termed the "syndrome of insulin resistance and acanthosis nigricans type B" (1). These antibodies, which are polyclonal and mainly of the IgG class, block insulin binding (2, 3) and, in the presence of a second antibody, can be used to immunoprecipitate solubilized insulin receptors (4). When tested in short-term *in vitro* experiments, the antireceptor antibodies also have an insulin-like activity. In adipocytes and skeletal muscle, the antireceptor antibodies have been shown to stimulate 2-deoxyglucose transport, glucose incorporation into lipids and glycogen, and glucose metabolism to CO_2 (5, 6). In addition, these antibodies mimic insulin's effect on lipolysis, amino acid transport, and enzymatic activity of glycogen synthase and phosphorylase (7, 8). These *in vitro* findings are in contrast to the chronic *in vivo* effects of these antibodies to produce severe insulin resistance and hyperglycemia.

In the present study, we have investigated the effects of the antibodies on insulin binding and glucose metabolism in more long-term experiments, taking advantage of the recently established adipocyte-like cell line, the 3T3-L1 fatty fibroblasts (9–11). As with adipocytes, the antireceptor antibodies inhibit insulin binding and produce acute insulin-like effects in these cells. This insulin-like action, however, is transient; after 6–40 hr, cells that have been exposed to the antireceptor antibodies are rendered markedly insulin-resistant. This occurs without further change in insulin binding and without a loss in responsiveness to agents that stimulate glucose metabolism by postreceptor mechanisms. These data suggest that chronic occupancy of the insulin receptor by a ligand may produce desensitization beyond the receptor at some step early in the pathway of insulin action.

MATERIALS AND METHODS

Cell Cultures. 3T3-L1 cells, isolated by Howard Green (Massachusetts Institute of Technology), were generously provided to us by him and by Ora M. Rosen (Albert Einstein College of Medicine). Fibroblasts were grown in petri dishes or Linbro six-well tissue culture plates to confluence in Dulbecco–Vogt's minimal essential medium containing 10% fetal calf serum; after confluence they were treated with media supplemented with 1-methyl-3-isobutylxanthine (0.5 mM) and insulin (1 μg/ml) for 2 days and insulin alone for 4 days to enhance the conversion to fatty fibroblasts as described (11). Cultures contained about 80% fatty fibroblasts and were used 3–8 days after completion of insulin treatment.

Preparation of Antireceptor Antibodies. Plasma from a patient (patient B-2) (1) with insulin resistance of the type B syndrome, containing a high titer of antibodies against the receptor for insulin (1:2000), was treated with 33% ammonium sulfate. The precipitate was dissolved in and dialyzed against 0.05 M sodium phosphate, pH 7.4/0.15 M NaCl to a protein concentration of 10 mg/ml (based on absorbancy measurements assuming $A^{1\%}_{1\,cm,\,280}$ 12). This preparation had an IgG concentration similar to that of the original plasma and is referred to hereafter as antireceptor antibodies (anti-R).

For all studies of glucose oxidation and for some studies of ^{125}I-labeled insulin (^{125}I-insulin) binding, cell suspensions were prepared by detaching the cells from the dishes after incubation at 37°C for 30 min in Joklik's spinner medium supplemented with 2 mM EDTA (11). The cells were then washed three times, and insulin binding was measured as described (11). In brief, cells (1–3 $\times$ 10^6 cells per ml) were incubated at 15°C for 90 min in pH 8.0 Hepes buffer containing bovine serum albumin (20 mg/ml) together with ^{125}I-insulin [0.15–0.20 ng/ml; 140–180 μCi (1 Ci = 3.7 $\times$ 10^{10} becquerels)/μg] (12) alone or with varying concentrations of unlabeled insulin (40 pM to 2 μM). The cells were then separated from the medium by centrifugation in a Beckman Microfuge for 1 min at 10,000 $\times$ *g*, and the ^{125}I-insulin bound was determined. Nonspecific binding was measured as the amount of tracer bound in the presence of unla-

Abbreviation: anti-R, antireceptor antibodies prepared as described herein.

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beled insulin (10 μg/ml) and was subtracted from total binding. Glucose oxidation was assayed by incubating suspended cells in Krebs–Ringer bicarbonate buffer (pH 7.4) containing bovine serum albumin (20 mg/ml) and 0.3 mM [*U*-^{14}C]glucose (about 2×10^5 cpm) for 120 min at 37°C and measuring the amount of $^{14}CO_2$ formed (11).

Transport of 2-deoxyglucose in cells adherent to tissue culture plates was determined in a Krebs–Ringer phosphate buffer (pH 7.4) supplemented with bovine serum albumin (20 mg/ml) and 1.3 mM $CaCl_2$. The cells were preincubated at 22°C for 20 min in the presence or absence of insulin, and transport was measured by a 20-min pulse of 0.2 mM 2-deoxyglucose (0.8 μCi/ml) as described (11). In some experiments, insulin binding also was measured on cells adherent to the plate as described above but with a 120-min incubation terminated by three 3-ml washes with Dulbecco's phosphate-buffered saline at 4°C. Although the rate of hormone degradation is lower with adherent cells, binding is otherwise similar to that obtained on suspended cells. Cell number was measured by a hemocytometer method. DNA content of cells lysed in 0.1% sodium dodecyl sulfate was determined by a fluorometric method (13).

RESULTS

Effect on Insulin Binding. Exposure of the 3T3-L1 cells to anti-R caused a dose- and time-dependent reduction in insulin binding. The maximal inhibition of insulin binding was approximately 90% and was observed with 35 μg of anti-R per ml. This effect was maximal within 2 hr of treatment and remained the same when cells were exposed for 36 hr (Fig. 1). Scatchard analysis of insulin-binding data from experiments with cells exposed to anti-R and control cells resulted in curvilinear plots consistent with negative cooperativity (Fig. 2). The number of insulin receptors, as indicated by the intercept on the abscissa, did not change as a result of either 2 or 36 hr of treatment with anti-R. The decrease in insulin binding caused by antibody was due to a major decrease in the affinity of the receptor for insulin and a loss of negative cooperativity—i.e., curvilinearity of the Scatchard plot. This is similar to the effect of this antibody on insulin binding in cultured human lymphocytes (14) and rat adipocytes (5).

Effect on Glucose Metabolism. As with isolated adipocytes (4), acute exposure of 3T3-L1 cells to anti-R resulted in a stimulation of glucose transport. With these cells, however, this insulin-like effect was only transient. The maximal stimulatory effect of anti-R on 2-deoxyglucose transport (about 70% of the maximal insulin effect) was reached with 60 min of incubation (Fig. 3). By 4 hr of incubation, the stimulation of transport had declined by 50% and by 6 hr, no stimulatory effect of anti-R on 2-deoxyglucose transport was detected. Furthermore, whereas insulin (1 μg/ml) produced about a 3-fold increase in transport rate in control cells, at no time after anti-R treatment did addition of insulin produce any significant increase in transport.

Similarly, the stimulatory effect of anti-R on glucose oxidation was transitory. Cells treated with anti-R for 2 hr had an increased basal rate of glucose oxidation, about 25% of maximal insulin effect, whereas with cells treated for 36 hr the basal rate had returned to the level of unexposed cells (Fig. 4). After both 2 and 36 hr of treatment, the insulin dose-response curve was

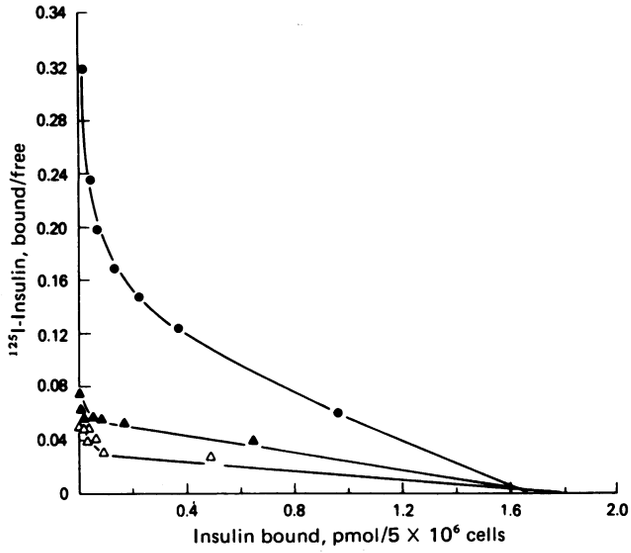

FIG. 2. Scatchard analysis of ^{125}I-insulin binding to 3T3-L1 fatty fibroblasts treated with anti-R. Cells on petri dishes were exposed to anti-R (35 μg/ml) for 2 (▲) and 36 (△) hr; ●, control. Then, cell suspensions were prepared and insulin binding was measured. Detachment of the cells from the plates by calcium-free EDTA-containing buffer produced only a minimal loss of the effect of anti-R, suggesting that little dissociation of antibodies occurred during the period of preparation of cell suspensions required for glucose oxidation studies (data not shown). Nonspecific binding, which has been subtracted, was 2% of the total radioactivity added.

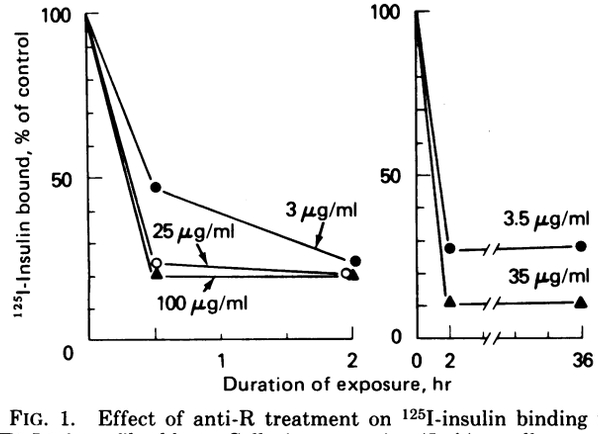

FIG. 1. Effect of anti-R treatment on ^{125}I-insulin binding to 3T3-L1 fatty fibroblasts. Cells, in suspension (*Left*) or adherent to tissue culture plates (*Right*) were exposed to anti-R at the concentrations and for the time periods indicated. The cells were then washed and insulin binding was measured. The data are expressed as percentage of the bound/free ^{125}I-insulin ratio observed with control cells (i.e., cells not exposed to anti-R).

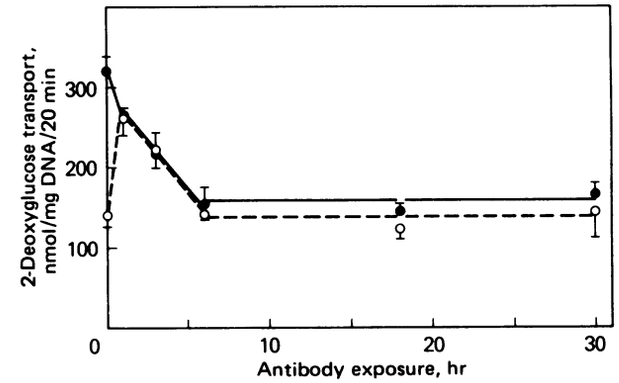

FIG. 3. Effect of anti-R treatment on 2-deoxyglucose transport in 3T3-L1 fatty fibroblasts. Cells on tissue culture plates were exposed to anti-R (35 μg/ml) for the indicated time periods at 37°C, and transport was measured at 22°C over 20 min in the absence (basal) (○) or in the presence (●) of a maximally stimulatory dose of insulin (1 μg/ml).

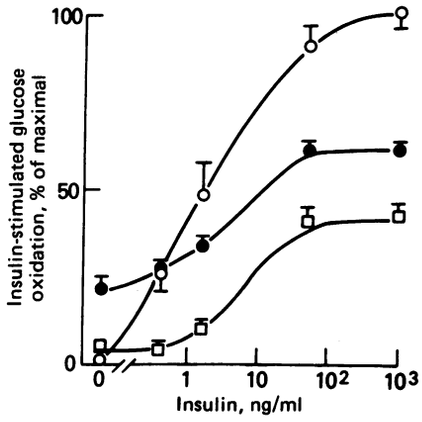

FIG. 4. Effect of anti-R treatment on glucose oxidation in 3T3-L1 fatty fibroblasts. Cells on tissue culture plates were exposed to anti-R (35 μg/ml) for 0 (○), 2 (●), or 36 (□) hr. The cells were then detached from the plates and glucose oxidation was studied as a function of insulin concentration. The data represent mean ± SEM of duplicates from two separate experiments. Maximal glucose oxidation is defined as glucose oxidation of cells not exposed to anti-R (0 hr) in the presence of insulin (1 μg/ml). Basal and maximal rates of glucose oxidation were 8 and 34 nmol per 10^6 cells per 2 hr, respectively.

shifted 3- to 4-fold to the right and the maximal response to insulin was decreased by about 60%. The rightward shift in the dose-response curve observed with anti-R treated cells was consistent with a decrease in receptor affinity produced by anti-R. The disappearance of the insulin-like effect of the anti-R that occurred without noticeable change in the insulin binding (Fig. 1) and the decrease in maximal response to insulin after chronic anti-R treatment, on the other hand, suggested desensitization at some postreceptor site.

Refractory Insulin Receptor. The exact mechanism and site of desensitization are unclear. The decay of the stimulatory effect was not caused simply by consumption of a subpopulation of antibodies with insulin-like activity during the incubation. Medium containing anti-R was fully active on fresh cells even after 35–40 hr of incubation. Conversely, addition of fresh anti-R to the desensitized cells did not produce a second stimulation of 2-deoxyglucose transport or glucose oxidation (Table 1).

The desensitization produced by anti-R appeared to occur at some early postreceptor site in the pathway of insulin action.

Table 1. Stimulation of glucose metabolism by fresh and conditioned anti-R media in 3T3-L1 fatty fibroblasts untreated or treated for prolonged periods of time with anti-R

	Response, % increase above basal	
	2-Deoxyglucose transport	Glucose oxidation
Control cells		
Addition of fresh anti-R	79	105
Addition of conditioned anti-R	75	88
Long-term treated cells		
Addition of fresh anti-R	14	−10
No second addition of anti-R	3	10

Conditioned anti-R media were obtained by incubating cells for 35–40 hr with culture medium containing anti-R (35 μg/ml). Control cells were treated with fresh anti-R medium or conditioned anti-R medium for 1 or 2 hr before measurements of 2-deoxyglucose transport or glucose oxidation, respectively. Basal 2-deoxyglucose transport and glucose oxidation were determined on cells not exposed to either anti-R medium.

Table 2. Glucose oxidation in 3T3-L1 cells treated with anti-R for 2 or 36 hr

	Oxidation, nmol CO_2 produced/ 10^6 cells/2 hr		
	0 hr	2 hr	36 hr
Basal	16.2 ± 0.9	30.6 ± 3.7	20.9 ± 0.9
Spermine	47.8 ± 1.0	87.8 ± 4.7	68.6 ± 2.7
Vitamin K_5	64.3 ± 2.7	103.5 ± 5.3	82.2 ± 6.8

3T3-L1 fatty fibroblasts were treated on dishes with anti-R (35 μg/ml) for the indicated times. Glucose oxidation was then determined after incubation of suspended cells for 2 hr at 37°C in buffer (basal) or in the presence of spermine (10 μM) or vitamin K_5 (15 μM). Qualitatively similar patterns of response were observed in experiments using vitamin K_5 at 30 μM or spermine at 100 or 1 μM. The data represent mean ± SEM of duplicate incubations.

Vitamin K_5 and spermine, agents known to act on glucose metabolism independently of the insulin receptor (15, 16), stimulated glucose oxidation to the same degree in control cells and cells treated with anti-R for 2–36 hr (Table 2).

Restoration of Insulin Binding and Action. Cells chronically treated with anti-R recovered insulin binding and insulin stimulation of glucose metabolism after removal of anti-R from the medium (Fig. 5). The inhibition of the insulin receptor-mediated glucose metabolism was relatively rapidly reversible; stimulation of 2-deoxyglucose transport by maximal doses of insulin was restored with a $t_{1/2}$ of 2 hr and returned to the control level within 6 hr. Insulin binding was restored more gradually ($t_{1/2}$ = 14–18 hr) with full restoration after 48 hr. The rate of recovery of insulin binding was slightly faster than the turnover time of the insulin receptor in these cells ($t_{1/2}$ = 20–24 hr), as determined by the inhibition of protein synthesis (11).

The more rapid return of bioactivity than of binding is consistent with our previous observation that these cells have spare receptors (11) and thus a maximal bioresponse occurs with less than 100% occupancy of the receptors. Furthermore, this observation suggests that the insulin receptors that became available either by dissociation of antibodies or by *de novo* synthesis display at least normal coupling and ability for signal generation.

DISCUSSION

Autoantibodies to the insulin receptor, obtained from patients with a rare form of insulin-resistant diabetes (1), display striking acute insulin-like properties when studied *in vitro*. In adipocytes, the antibodies fully mimic insulin's ability to stimulate

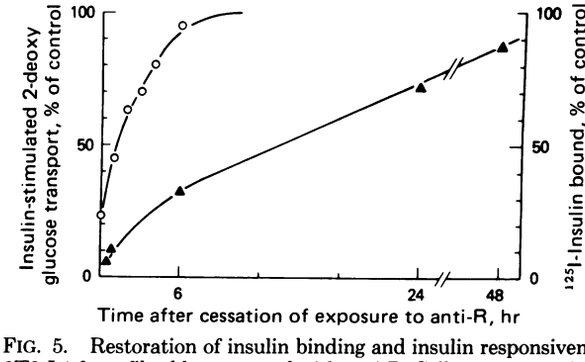

FIG. 5. Restoration of insulin binding and insulin responsiveness in 3T3-L1 fatty fibroblasts treated with anti-R. Cells on tissue culture plates were exposed to anti-R (35 μg/ml) for 36 hr, washed twice with culture medium at 37°C, and then studied on the plates for insulin binding (▲) and insulin-stimulated (1 μg of insulin per ml) transport (○). Cells not exposed to anti-R, but studied in parallel, represent the control. Control levels of insulin binding and 2-deoxyglucose transport were similar to those shown in Figs. 2 and 3, respectively.

2-deoxyglucose transport, glucose incorporation into lipids, glucose oxidation, and amino acid transport (5), to inhibit lipolysis (7) and phosphorylase (8), and to activate glycogen synthase (8). In isolated muscle, similar insulin-like effects have been observed (6). On a molar basis, anti-R is equipotent with insulin in many of these effects (17). The stimulatory activity of the antibodies has seemed at variance with the clinical picture of the patients, which is characterized by severe insulin resistance and hyperglycemia. However, previous experimental systems have not permitted a study of the long-term effects of the antibodies upon target cell metabolism.

In the present work, we have investigated the effects of more prolonged exposure to anti-R, using cells in tissue culture. The 3T3-L1 cell is particularly useful for these experiments because it possesses insulin receptors and a number of insulin-stimulated biological responses (9–11). Furthermore, this cell line does not show "down-regulation" of receptors after prolonged exposure to insulin (11), suggesting that it is ideal for the study of possible postreceptor desensitization. The current experiments revealed that the insulin-like bioactivity of anti-R was only transient. The activity of the antibodies changed within hours from that of a potent agonist to that of a pure antagonist, rendering the cells insulin-resistant. This desensitization to the insulin-like bioactivity of anti-R occurred without any change in the ability of the antibodies to inhibit insulin binding. Furthermore, at this time, the response to added insulin was markedly diminished; maximal response in glucose transport was decreased by more than 90%, and glucose oxidation revealed a 60% decrease in maximal response and a shift of the dose-response curve to the right. The decrease in maximal response seems to be due to a desensitization at an early postreceptor step because vitamin K_5 and spermine, agents that activate beyond the receptor, still stimulated glucose oxidation normally. The slightly different results obtained in the studies of insulin sensitivity of 2-deoxyglucose transport and glucose oxidation in cells chronically exposed to anti-R may be due to differences in the ability of the antibodies to block each of these actions of insulin or to methodological differences in the two bioassays. It should be noted that transport was measured for 20 min at 22°C whereas glucose oxidation was carried out for 2 hr at 37°C. Because dissociation of the antibody occurs more rapidly at higher temperatures (3), the increased response during glucose oxidation may simply reflect increased dissociation of anti-R.

After removal of anti-R from the medium, the insulin binding was restored with a $t_{1/2}$ of 14–18 hr. In preliminary experiments, iodinated and receptor-purified anti-R dissociated at 37°C from the 3T3-L1 cells with a $t_{1/2} \approx 10$–12 hr, suggesting that the restoration of insulin binding was due to the dissociation of antibodies from the receptors. Insulin, in contrast, dissociates much more rapidly with $t_{1/2} \approx 15$ min. After removal of anti-R from the medium, maximal insulin-stimulated 2-deoxyglucose transport capacity was rapidly restored ($t_{1/2} \approx 2$ hr) during a period when only a fraction of total insulin binding was recovered.

The mechanism by which the antireceptor antibodies lose their ability to stimulate glucose metabolism is not known. There are many levels at which the metabolic response of a target tissue can be modulated, including alterations at the level of the hormone receptor in either receptor affinity or number and alterations at any of the multiple steps distal to the receptor (18, 19). Antibodies against some cell-surface antigens may cause a loss of that antigen by increasing its degradation, sometimes via a mechanism involving patching and capping (20, 21). In the present experiments, it seems unlikely that the desensitizing event is related to a change at the receptor level because *in vitro* exposure of cells to anti-R both acutely and for prolonged periods of time resulted in a similar reduction in receptor affinity. Likewise, circulating monocytes from the patients with insulin receptor antibodies show primarily a decrease in the affinity of the receptor for insulin with no change in receptor number, and the receptor affinity of these cells can be restored toward normal by treatment of the cells with acidic buffer to remove cell-bound antibodies (M. Muggeo and C. R. Kahn, unpublished observations).

Irreversibility of ligand binding may predispose to desensitization. This has been suggested to be a factor in the desensitization of glucagon and β-adrenergic receptors (22–24). Preliminary studies have shown that anti-R dissociates slowly from the 3T3-L1 cells, making irreversible binding a candidate to explain the induction of the refractory state. Consistent with this notion, prolonged exposure of these cells to insulin itself, which can rapidly dissociate from the receptor, does not induce any receptor down-regulation or any desensitization in response to reexposure to insulin (11). Preliminary experiments also suggest that the desensitization by anti-R requires energy.

Because the bioactivity of the antibodies is related to their bivalency (17), another potential mechanism of desensitization would be the conversion of the cell-bound antireceptor antibodies to monovalent fragments. Although we think that this is unlikely, if it did occur, it would have important implications with respect to the interaction of antibodies with cells.

In summary, we have shown that the insulin-like activity of antibodies directed at the insulin receptor is of short duration. The end result of prolonged exposure of insulin-responsive cultured cells to the antibodies is a state of severe insulin resistance, analogous to the clinical picture observed in patients with these antibodies. The current study suggests that alterations in biochemical events close to the receptor play a role in this desensitization. These observations may have relevance to understanding the mechanisms by which various ligands stimulate cells, because they suggest that occupancy of receptors alone is not sufficient for sustained signal generation and that the kinetics or reversibility of binding may play an important role (25). Furthermore, study of the desensitization process in 3T3-L1 cells may provide important clues to the mechanism of insulin action. In other tissues, insulin itself may produce some form of postreceptor desensitization (26–28), although this is difficult to evaluate because prolonged exposure of many cells to insulin results in a reduction in the number of receptors as well (19, 27, 29). Whether antibodies that interact with other cell membrane receptors are capable of producing similar transient biological responses and postreceptor desensitization is not known.

The authors thank Dr. Jesse Roth for continued advice throughout this study and Ms. C. Shinn and Mrs. B. Knight for excellent secretarial assistance.

1. Kahn, C. R., Flier, J. S., Bar, R. S., Archer, J. A., Gorden, P., Martin, M. M. & Roth, J. (1976) *N. Engl. J. Med.* **294,** 739–745.
2. Flier, J. S., Kahn, C. R., Roth, J. & Bar, R. S. (1975) *Science* **190,** 63–65.
3. Flier, J. S., Kahn, C. R., Jarrett, D. B. & Roth, J. (1976) *J. Clin. Invest.* **58,** 1442–1449.
4. Harrison, L. C., Flier, J. S., Karlsson, F. A., Kahn, C. R. & Roth, J. (1978) *J. Clin. Endocrinol.*, in press.
5. Kahn, C. R., Baird, K. L., Flier, J. S. & Jarrett, D. B. (1977) *J. Clin. Invest.* **60,** 1094–1106.
6. Le Marchand-Brustel, Y., Gorden, P., Flier, J. S., Kahn, C. R. & Freychet, P. (1978) *Diabetologia* **14,** 311–318.
7. Kasuga, M., Akanuma, Y., Tsushima, T., Suzuk, K., Kosaka, K. & Kitata, M. (1978) *J. Clin. Endocrinol.* **47,** 66–77.
8. Lawrence, J. C., Larner, J., Roth, J. & Kahn, C. R. (1978) *Mol. Cell. Biochem.*, in press.

9. Green, H. & Kehinde, O. (1974) *Cell* **1,** 113–116.
10. Rubin, C. S., Lai, E. & Rosen, O. M. (1977) *J. Biol. Chem.* **252,** 3554–3556.
11. Karlsson, F. A., Grunfeld, C., Kahn, C. R. & Roth, J. (1978) *Endocrinology,* in press.
12. Roth, J. (1975) *Methods Enzymol.* **37,** 223–233.
13. Hinegardner, R. T. (1971) *Anal. Biochem.* **39,** 197–201.
14. Flier, J. S., Kahn, C. R., Jarrett, D. B. & Roth, J. (1977) *J. Clin. Invest.* **60,** 784–794.
15. Czech, M. P., Lawrence, J. C., Jr. & Lynn, W. S. (1974) *J. Biol. Chem.* **249,** 5421–5427.
16. Livingston, J. N., Gurny, P. A. & Lockwood, D. H. (1977) *J. Biol. Chem.* **252,** 560–562.
17. Kahn, C. R., Baird, K. L., Jarrett, D. B. & Flier, J. S. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4209–4213.
18. Roth, J. (1973) *Metabolism* **22,** 1059–1073.
19. Kahn, C. R., Megyesi, K., Bar, R. S., Eastman, R. C. & Flier, J. S. (1977) *Ann. Intern. Med.* **86,** 205–219.
20. Drachman, D. B. (1978) *N. Engl. J. Med.* **298,** 136–142.
21. Raff, M. (1976) *Nature (London)* **259,** 265–266.
22. Lin, M. C., Nicosia, S., Lad, P. M. & Rodbell, M. (1977) *J. Biol. Chem.* **252,** 2790–2792.
23. Williams, L. T. & Lefkowitz, R. J. (1977) *J. Biol. Chem.* **252,** 7207–7213.
24. Lefkowitz, R. J., Mullikin, D. & Williams, L. T. (1978) *Mol. Pharmacol.* **74,** 376–380.
25. Kahn, C. R. (1975) in *Methods in Membrane Biology,* ed. Korn, E. D. (Plenum, New York), Vol. 3, pp. 81–146.
26. Livingston, J. N., Purvis, B. J. & Lockwood, D. H. (1978) *Nature (London)* **273,** 394–396.
27. Olefsky, J. M. (1976) *Diabetes* **25,** 1154–1162.
28. Czech, M. P. (1976) *J. Clin. Invest.* **57,** 1523–1532.
29. Gavin, J. R., III, Roth, J., Neville, D. M., Jr., De Meyts, P. & Buell, D. N. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 84–88.