sugars and amino acids is caused by binding of inhibitors to the active sites of the lectins. Alternatively, those inhibitors could prevent thrombin activation and, thereby, block the exposure of a cryptic form of the surface protein that mediates aggregation. This alternative appears unlikely, however, since (i) the aggregation induced by adenosine diphosphate (ADP), a nonproteolytic stimulus, was also inhibited by the amino sugars (Fig. 3); (ii) the amino sugars and basic amino acids caused no more than a 20 percent inhibition of thrombin-induced serotonin secretion (data not shown); and (iii) inhibition of the thrombin-enhanced lectin activity was observed after platelet activation had occurred. Therefore, we conclude that the amino sugars and basic amino acids did not block platelet aggregation by preventing platelet activation. Although neutral sugars had no effect on thrombin-induced aggregation, galactose (Fig. 3) had a slight effect on ADP-induced aggregation. This difference may reflect some inherent difference between ADP- and some thrombin-induced aggregation of platelets.

The data presented here demonstrate that platelet activation causes the expression of a previously inactive lectin that appears to mediate at least the initial phases of platelet aggregation by specifically binding to receptors on adjacent platelets. Since aggregation of thrombin-stimulated platelets can be blocked by both amino sugars and basic amino acids, the lectin receptor could be either protein or carbohydrate. Further work is required to determine whether platelet cohesiveness is derived entirely from the expression of platelet lectin activity, or whether activation also affects the expression of lectin receptors.

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References and Notes

1. G. Gregg, *J. Gen. Physiol.* **39**, 813 (1956); D. R. Sonneborn, M. Sussman, L. Levine, *J. Bacteriol.* **87**, 1321 (1964); H. Beug, F. E. Katz, G. Gerisch, *J. Cell Biol.* **56**, 647 (1973).
2. S. D. Rosen, J. A. Kafka, D. L. Simpson, S. H. Barondes, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2554 (1973).
3. T. K. Gartner and T. R. Podleski, *Biochem. Biophys. Res. Commun.* **67**, 972 (1975).
4. T. P. Nowak, P. L. Haywood, S. H. Barondes, *Biochem. Biophys. Res. Commun.* **68**, 650 (1976); H. Den, D. A. Malinsak, A. Rosenberg, *ibid.* **69**, 621 (1976); T. P. Nowak, D. Kobiler, L. E. Roch, S. H. Barondes, *J. Biol. Chem.* **252**, 6026 (1977).
5. S. D. Rosen, D. L. Simpson, J. E. Rose, S. H. Barondes, *Nature (London)* **252**, 148 (1974); D. L. Simpson, D. R. Thorne, H. H. Loh, *ibid.* **266**, 367 (1977).
6. A. Marcus and M. B. Zucker, *The Physiology of Blood Platelets* (Grune & Stratton, New York, 1965); J. F. Mustard and M. A. Packham, *Pharmacol. Rev.* **22**, 97 (1970).
7. T. K. Gartner, D. C. Williams, D. R. Phillips, *Biochem. Biophys. Res. Commun.* **79**, 592 (1977).
8. M. M. BoyleKay and H. H. Fudenberg, *Nature (London)* **244**, 288 (1973).
9. R. L. Kinlough-Rathbone, M. A. Packham, J. F. Mustard, *Br. J. Haematol.* **19**, 559 (1970).
10. P. Ganguly and W. J. Sonnichsen, *ibid.* **34**, 291 (1976).
11. N. L. Baenziger, G. N. Brodie, P. W. Majerus, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 240 (1971); K. Grette, *Acta Physiol. Scand.* **56**, (Suppl. 195) 1 (1962); J. G. White, W. Krivit, R. L. Vernier, *Blood* **25**, 241 (1965).
12. W. C. Boyd, *Ann. N.Y. Acad. Sci.* **169**, 168 (1970).
13. The following compounds at a final concentration of 30 m*M* were without effect on both platelet lectin activities: *N*-acetylgalactosamine, *N*-acetylglucosamine, *N*-acetylmannosamine, galactose, glucose, mannose, fructose, arabinose, fucose, ribose, xylose, lactose, maltose, sucrose, glutamine, alanine, and asparagine.
14. The following reagents (0.015*M*) inhibited thrombin-induced aggregation: galactosamine, glucosamine, mannosamine, arginine, and lysine.
15. G. Millonig, *J. Appl. Physiol.* **32**, 1637 (1961).
16. T. F. Anderson, *Ann. N.Y. Acad. Sci.* **13**, 130 (1951).
17. G. V. R. Born and M. J. Cross, *J. Physiol. (London)* **168**, 178 (1963).
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Antibodies to Purified Insulin Receptor Have Insulin-Like Activity

Abstract. *Antibodies to insulin receptors purified from rat liver membranes do not complete with [^{125}I]insulin for binding to the insulin receptor but do precipitate solubilized receptors labeled with [^{125}I]insulin. These antibodies have the insulin-like activities of enhancing glucose oxidation and inhibiting epinephrine-induced lipolysis in rat adipocytes. Thus, antibody binds to the receptor at a different site from that to which insulin binds, yet the interaction can initiate an effective biological response. These results indicate that the previously studied insulin-binding sites are the physiological macromolecular receptors for insulin.*

Antibodies to the insulin receptor, which compete with insulin for binding to the receptor, occur spontaneously in patients with the Kahn type B syndrome of acanthosis nigricans and insulin resistance (*1*). In three patients with this syndrome, these antibodies have been shown to have insulin-like biological activity (*2*). We now report the production of rabbit antibody to purified insulin receptors that bind to the receptor at a site distinct from that which binds insulin, but can stimulate conversion of glucose to CO_2 and inhibit epinephrine-stimulated lipolysis in isolated fat cells in a manner similar to that of insulin.

Insulin receptor was solubilized from rat liver membranes with 2 percent Triton X-100 and purified by DEAE-cellulose chromatography and affinity chromatography on insulin-agarose and concanavalin A–agarose (*3*, *4*). A rabbit was immunized with this highly purified receptor preparation, which, as revealed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, contained a major Coomassie blue staining band with an apparent molecular weight of 135,000 and minor bands of lower molecular weight (*4*).

The antiserum to the receptor did not inhibit [^{125}I]insulin binding to rat liver membranes or to rat fat cell membranes, even when high concentrations of antiserum were used (1:5 dilution), or when the membranes were incubated with antiserum for up to 6 hours before [^{125}I]insulin was added (data not shown). A direct interaction between the antiserum to the receptor and the insulin receptor was demonstrated by indirect immunoprecipitation (Fig. 1). Solubilized receptor from rat liver membranes was labeled with [^{125}I]insulin and incubated with antiserum to receptor. The receptor-antibody complex was then precipitated with an antiserum to rabbit immunoglobulin G (IgG) (Fig. 1A). When an excess of native insulin was added to the incubation mixture to prevent receptor labeling, the radioactivity in the precipitate was greatly diminished (Fig. 1B). No [^{125}I]insulin-labeled receptor was precipitated when anti-insulin receptor serum was replaced by serum obtained from the rabbit before immunization or buffer alone (Fig. 1, C and D) or when solubilized receptor was omitted from the incubation mixture (Fig. 1E), a procedure that excluded the possibility that the specific precipitation of [^{125}I]insulin (Fig. 1A) was due to antibodies to insulin rather than to the receptor. The amount of [^{125}I]insulin-labeled receptor precipitated by varying concentrations of antiserum to the receptor was compared with the amount of labeled receptor precipitated by the polyethylene glycol assay (*3*). Both methods resulted in the precipitation of similar amounts of la-

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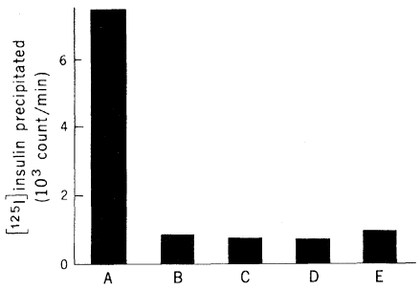

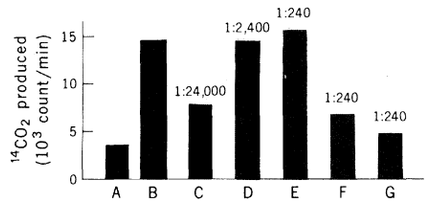

Fig. 1 (left). Immunoprecipitation of [^{125}I]insulin-labeled insulin receptor by antiserum against the purified receptor. (A) Insulin receptor with a total insulin-binding capacity of about 1 ng of insulin, solubilized from rat liver membranes with Triton X-100 and partially purified with DEAE-cellulose, was incubated with [^{125}I]insulin (23,000 count/min; 180 μCi/μg) in 0.2 ml of 50 m*M* sodium phosphate buffer with 0.1 percent albumin, *p*H 7.4, at 22°C. After 45 minutes, the antiserum to the receptor was added to achieve a final dilution of 1:20, and the incubation continued for 18 hours at 4°C. The mixture was then centrifuged at 2500*g* for 20 minutes, and the radioactivity in the precipitate was counted. (B) Unlabeled insulin (2 μg) was added to the reaction mixture before the addition of labeled insulin. (C) Antiserum to the receptor was replaced by serum obtained from the rabbit before immunization. (D) Rabbit serum was omitted from the incubation mixture. (E) Solubilized insulin receptor was omitted from the incubation mixture. Fig. 2 (right). Stimulation of glucose oxidation in isolated fat cells by antiserum to the receptor. Isolated rat fat cells (10^5 per milliliter) were incubated with (A) no additions, (B) insulin (1.7 ng/ml), (C to E) antiserum to the receptor, (F) antiserum to the receptor that was first incubated with donkey antiserum to rabbit IgG and the resulting precipitate removed, and (G) serum from the rabbit before immunization. Incubations were performed for 90 minutes at 37°F in Krebs-Ringer bicarbonate buffer with 1 percent albumin and 0.2 m*M* D-[U-^{14}C]glucose (5 Ci/mole), *p*H 7.4. The CO_2 produced was measured by absorption by hyamine hydroxide.

beled receptor at concentrations of antiserum to the receptor higher than a 1:100 dilution. A final serum dilution of 1:600 was required to precipitate 50 percent of the labeled receptor.

The antiserum to the receptor, like insulin, stimulated the conversion of glucose to CO_2 in isolated rat fat cells (Fig. 2). Half-maximal stimulation occurred at about a 1:20,000 dilution of antiserum. More than 99 percent of the insulin-like activity of the antiserum could be removed by precipitation with antiserum to rabbit IgG. The insulin-like activity of the antiserum copurified with IgG upon ammonium sulfate fractionation and DEAE-cellulose chromatography (data not shown), thus establishing its immunoglobulin nature.

To determine whether an antibody to the receptor for insulin that is capable of mimicking the cell surface effect of glucose transport could also cause one of insulin's cytoplasmic effects, we studied the regulation of lipolysis in fat cells (Fig. 3). The antiserum to the receptor, like insulin, inhibited epinephrine-stimulated lipolysis, but had little effect on the basal lipolytic activity. This antilipolytic effect of the antiserum to the receptor was not present in serum obtained from the rabbit before immunization, could be removed by precipitation with antiserum to rabbit IgG, and copurified with IgG. Thus, the effects of insulin on both glucose transport and epinephrine-stimulated lipolysis were simulated by perturbation of the same or immunologically similar receptors. Whether some of the delayed actions of insulin, such as stimulation of DNA or protein synthesis, can also be elicited by anti-insulin receptor antibodies remains to be determined.

All previous biochemical studies of insulin receptors have been directed toward identifying receptor molecules through [^{125}I]insulin binding. However, structures other than receptors can potentially bind the hormone, and many nonreceptor materials bind insulin in a manner similar to true receptor binding (*5*). In order to demonstrate that a binding site is a true physiological receptor, binding at the site must be associated with a known physiological response. Immunization with the solubilized and purified insulin-binding site produces an antibody with insulin-like activity, thus providing evidence that this binding site is a physiologically important receptor.

Some antibodies to the receptor that occur spontaneously in patients with type B acanthosis nigricans and with insulin resistance stimulate glucose transport by interacting with the receptor at a site different from that at which insulin binds (*2*). The antibody described in our report is similar in that it does not compete with insulin for binding to membranes from isolated fat cells or from liver, but has insulin-like activity. Although this antibody does not directly compete with insulin for binding, it causes a time-dependent decrease in the number of insulin-binding sites when incubated with intact fat cells in contrast to membranes derived from these cells (*6*), suggesting internalization, shedding, or other loss of available receptor sites. A similar phenomenon occurs with certain antibodies to the acetylcholine receptor, which do not compete directly with α-bungarotoxin binding but appear to increase the rate at which the acetylcholine receptor is cleared from the surface of target cells grown in culture (*7*). The availability of specific antiserums to the purified insulin receptor should permit studies on the structure of the receptor and on the mechanism by which it elicits biological responses.

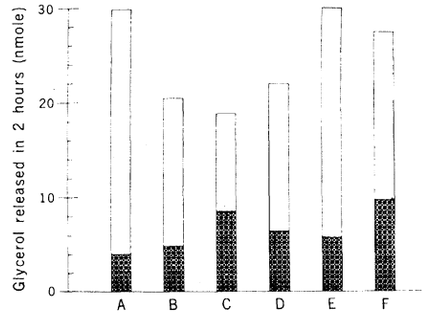

Fig. 3. Antilipolytic effect of antiserum to receptor. Isolated rat fat cells (10^6 per milliliter) were incubated in the presence (□) or absence (■) of epinephrine (0.3 μg/ml) and either (A) no other addition; (B) insulin (1.7 ng/ml); (C) antiserum at a final dilution of 1:1200, resulting in a final concentration of rabbit serum protein of 67 μg/ml; (D) the IgG fraction (8.5 μg/ml) from the antiserum to receptor purified by ammonium sulfate precipitation and DEAE-cellulose chromatography; (E) serum from the rabbit before immunization; or (F) antiserum to the receptor that was first incubated with donkey antiserum to rabbit IgG and the precipitate removed by centrifugation. The incubations were performed in Krebs-Ringer bicarbonate buffer with 3 percent albumin, *p*H 7.4 at 37°C. After 120 minutes, the fat cells were removed by flotation, and the glycerol released into the medium was determined by an enzyme-coupled assay (*8*).

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References and Notes

1. J. S. Flier, C. R. Kahn, J. Roth, R. S. Bar, *Science* **190**, 63 (1975); J. S. Flier, C. R. Kahn, D. B. Jarrett, J. Roth, *J. Clin. Invest.* **58**, 1442 (1976).
2. C. R. Kahn, K. Baird, J. S. Flier, D. B. Jarrett, *J. Clin. Invest.* **60**, 1094 (1977).
3. P. Cuatrecasas, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 318, 1277 (19 → ____ and G. P. E. Tell, *ibid.* **70**, 485 (1973).
4. S. Jacobs, Y. Schechter, K. Bissell, P. Cuatrecasas, *Biochem. Biophys. Res. Commun.* **77**, 981 (1977).
5. P. Cuatrecasas and M. Hollenberg, *ibid.* **62**, 31 (1975); *Adv. Protein Chem.* **30**, 251 (1976).
6. S. Jacobs, K.-J. Chang, P. Cuatrecasas, in preparation.
7. S. Heinemann, S. Benam, R. Kullberg, J. Lindstrom, J. Rice, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3090 (1977).
8. S. S. Chernic, *Meth. Enzymol.* **14**, 627(1969).

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