JCI The Journal of Clinical Investigation

Clinical disorders associated with autoantibodies to the insulin receptor. Simulation by passive transfer of immunoglobulins to rats.

R F Dons, … , S S Chernick, P Gorden

J Clin Invest. 1983;72(3):1072-1080. https://doi.org/10.1172/JCI111032.

Research Article

Patients with autoantibodies to the insulin receptor (Anti-R) may exhibit either fasting hypoglycemia or hyperglycemia and extreme insulin resistance. Occasionally, both these phenomena are observed in the same patient at different times in the clinical course. In an effort to understand what determines the patient's response to Anti-R, we developed an animal model of these clinical disorders by passive transfer of Anti-R IgG to rats. IgG fractions from the plasma of Anti-R patients and control subjects were prepared by affinity chromatography with staphylococcal protein A-Sepharose. Anti-R IgG, injected into fasting rats, induced severe and persistent hypoglycemia (plasma glucose 30-60 mg/dl). Rats injected with control IgG maintained a plasma glucose within the range of 75 (fasting) to 165 mg/dl (feeding). In comparison with the effects of insulin, the hypoglycemic response to Anti-R IgG had a slower onset (2-4 h) and lasted longer (8-24 h). Similar, dose-dependent hypoglycemic responses were observed in rats whether the Anti-R IgG was derived from an insulin-resistant or hypoglycemic patient. When Anti-R IgG was administered in sufficiently high doses for several days to fed rats, persistent hyperglycemia (plasma glucose 200-400 mg/dl) developed. Based on these in vivo and previous in vitro studies, we attribute the hypoglycemic response to an insulin-like effect of Anti-R, and the hyperglycemic response to a desensitization of host tissues to the effects of […]

Find the latest version:

https://jci.me/111032/pdf

Clinical Disorders Associated with Autoantibodies to the Insulin Receptor

SIMULATION BY PASSIVE TRANSFER OF IMMUNOGLOBULINS TO RATS

ROBERT F. DONS, ROBERT HAVLIK, SIMEON I. TAYLOR, KATHLEEN L. BAIRD, SIDNEY S. CHERNICK, and PHILLIP GORDEN, *Diabetes Branch, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205*

ABSTRACT Patients with autoantibodies to the insulin receptor (Anti-R) may exhibit either fasting hypoglycemia or hyperglycemia and extreme insulin resistance. Occasionally, both these phenomena are observed in the same patient at different times in the clinical course. In an effort to understand what determines the patient's response to Anti-R, we developed an animal model of these clinical disorders by passive transfer of Anti-R IgG to rats.

IgG fractions from the plasma of Anti-R patients and control subjects were prepared by affinity chromatography with staphylococcal protein A-Sepharose. Anti-R IgG, injected into fasting rats, induced severe and persistent hypoglycemia (plasma glucose 30-60 mg/dl). Rats injected with control IgG maintained a plasma glucose within the range of 75 (fasting) to 165 mg/dl (feeding). In comparison with the effects of insulin, the hypoglycemic response to Anti-R IgG had a slower onset (2–4 h) and lasted longer (8–24 h). Similar, dose-dependent hypoglycemic responses were observed in rats whether the Anti-R IgG was derived from an insulin-resistant or hypoglycemic patient.

When Anti-R IgG was administered in sufficiently high doses for several days to fed rats, persistent hyperglycemia (plasma glucose 200–400 mg/dl) developed. Based on these in vivo and previous in vitro studies, we attribute the hypoglycemic response to an insulin-like effect of Anti-R, and the hyperglycemic response to a desensitization of host tissues to the effects of insulin, with more prolonged exposure to higher levels of Anti-R.

INTRODUCTION

Spontaneous autoantibodies directed against cell surface receptors for polypeptide hormones and neurotransmitters are well-known mediators of autoimmune illness (1). Passive transfer of acetylcholine receptor autoantibodies obtained from patients with myasthenia gravis recreates the disease in experimental animals (2). In Graves' disease, passive transfer of thyroid-stimulating autoantibody to rodents (3) produces a delayed and prolonged elevation of thyroxine in the blood (the long-acting thyroid stimulator, [LATS][1] effect).

In patients with autoantibodies to the insulin receptor (Anti-R), three distinct metabolic states are seen: (*a*) most typically, severe fasting hyperglycemia is associated with extreme resistance to endogenous and exogenous insulin; (*b*) fasting hyperglycemia is followed by spontaneous fasting hypoglycemia, or on occasion, there is an alternating pattern of hypoglycemia and hyperglycemia; (*c*) less commonly, hypoglycemia occurs as the sole metabolic manifestation of the disease. In vitro, autoantibodies mimic most, if not all, of insulin's effects and competitively inhibit binding of insulin to its receptor (4).

To fully demonstrate the pathogenicity of an au-

Address reprint requests to Dr. Gorden.
Received for publication 11 February 1983 and in revised form 27 May 1983.

[1] *Abbreviations used in this paper:* Anti-R, autoantibodies to the insulin receptor; LATS, long-acting thyroid stimulator; TSH, thyrotropin.

toantibody, passive transfer of immunoglobulins to animals and recreation of the associated clinical disorder is required. Until now, Anti-R IgG has not been passively transferred to experimental animals. In this study we show that, when this is done, at least part of the clinical illness associated with these antibodies can be reproduced.

METHODS

Patients. Plasma obtained from four patients who spontaneously developed Anti-R was studied. These patients are referred to as B-1, B-6, B-10, and B-12. Their clinical features may be found in Table I and in previous reports (5–10). Patients B-6 and B-10 had received insulin previously, but only patient B-10 had detectable anti-insulin antibodies. Control plasma without Anti-R was obtained and processed in the same manner as the Anti-R plasma. Control subjects included normal volunteers with normal glucose tolerance and a patient with lupus erythematosus under treatment with prednisone and plasmapheresis.

Immunoglobulin extraction. Freeze-thawed plasma samples of up to 500 ml were diluted with phosphate-buffered saline, pH 8.0 (PBS). The sample was then applied to a 5 × 6-cm staphylococcal protein A-Sepharose column (Pharmacia, Uppsala, Sweden) equilibrated with PBS. Samples were recycled over this column two or three times, washed with ≧10 column vol of PBS, and eluted with 0.1 M citrate-buffered saline, pH 3.0 (11). The acid eluate was concentrated using an ultrafiltration membrane (PM-30, Amicon Corp., Scientific Sys. Div., Danvers, MA) and neutralized with sodium hydroxide. The protein A-Sepharose IgG affinity columns described were capable of absorbing ≧5 g of IgG, but yielded only ~50% of the IgG contained in the samples applied. Further losses were sustained in subsequent procedures, which limited the quantities of IgG available for testing in vivo. To reduce the potential for plasma component carry-over from one IgG preparation to another, a separate protein A-Sepharose column was used to extract IgG from plasma of patient B-10. The content of IgM and IgA in the concentrated eluates from the protein A columns was <1% of the total IgG extracted.

Unlike plasma from patients B-1, B-6, and B-12, which was limited in quantity, plasma from patient B-10 was most abundant for study, but was known to contain a low titer of anti-insulin antibodies (1:10) as well as elevated levels of insulin. Partial removal of these antibodies was achieved by 33% ammonium sulfate precipitation of B-10 plasma followed by affinity chromatography using Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) to which porcine insulin was coupled. The flow-through from this column was then chromatographed on protein A-Sepharose as described.

To be certain that neither insulin bound to anti-insulin antibodies or free insulin was present in significant amounts after protein A-Sepharose chromatography, two additional purification steps were taken. First, the Anti-R IgG fraction was applied to a Sephadex G-50 column equilibrated in 0.05 M ammonium carbonate (pH 8.0) and eluted with the same buffer. At this stage, <10 ng of free insulin/ml of concentrated sample was detected. To dissociate any insulin potentially bound to anti-insulin antibody, a similar gel filtration was carried out in 1.0 M acetic acid (pH 3.0) (12). The void volumes of the alkaline and acid columns were then concentrated for subsequent assay.

Analysis of immunoglobulin preparations. Protein concentration of the immunoglobulin preparations was estimated by adsorption at 280 nm. Human IgG concentrations were estimated by radial immunodiffusion (13). Total and free immunoreactive insulin concentrations were determined using standard radioimmunoassay techniques (14, 15). Anti-insulin receptor antibody titers were determined by their inhibition of ^{125}I-insulin binding to cultured IM-9 lymphocytes (16). Glucose oxidation and insulin binding to rat adipocytes in the presence of B-10 IgG was studied as described (17).

Collection of venous blood specimens from unrestrained rats. Chronic venous access in the unrestrained rat (Sprague-Dawley rats, Charles River Breeding Laboratories, Wilmington, MA) was achieved using aseptic surgical techniques as described in reference 18. Two specially constructed cages each housing a single rat were used in these studies. To facilitate repeated blood sampling, a special venous cannula of 0.051-cm i.d. (Silastic Medical Grade Tubing, Dow Corning Corp., Midland, MI) with tight-fitting tubular collar was fashioned and anchored to the internal jugular vein. When the tip of this cannula was properly situated within the atrial sinus, the collar, encircled by two silicone elastomer bands, was sutured securely in place. Patency of the cannula was maintained for prolonged periods of time by the continuous infusion of a normal saline-heparin solution (5–15 U/ml) at a rate of 1.5–3.5 ml/d (Mill Hill infusion model No. 2703 or Harvard pump model No. 600–900, Harvard Apparatus Co., Inc., S. Natick, MA).

Blood samples (0.16–0.2 ml) were obtained using a syringe and scalp vein infusion set with blunted needle connected to the venous cannula. Care was taken to avoid mixture of sample with infusate. The samples obtained were transferred to lithium heparin tubes and centrifuged immediately in a microfuge for 1.5 min (Beckman Instruments, Inc., Spinco Div., Palo Alto, CA). Plasma was separated and stored at −20°C. Glucose determinations were made in replicate using a glucose analyzer (Beckman Instruments, Inc., Fullerton, CA).

Immunoglobulin administration. The partially purified immunoglobulins were injected into a total of nine rats (eight female and one male) by two basic protocols. The first protocol involved an acutely administered, single intraperitoneal injection given after 15±3 h of fasting. If more than one injection was given to an individual rat, a recovery period of a week or more was allowed during which it was established that plasma glucose values had returned to control levels. This single injection protocol was followed for the data presented in Figs. 3–5. The second protocol involved multiple injections given with short time-intervals between doses. In these experiments, animals were either fed or fasted as indicated in Table II and Figs. 6 and 7. These injections were given intraperitoneally in volumes of 1.0–2.5 ml as in the first protocol or intravenously. These intravenous antibody infusions were given in volumes of 2.5–3 ml over periods of 25–35 min using an infusion device (model AS•2C; Auto-syringe Inc., Hooksett, NH). In some experiments, a continuous infusion of antibody was administered using the Harvard apparatus Mill Hill infuser pump model 2703.

Antibody preparations were exhaustively dialyzed vs. normal saline to ensure isotonicity, and had their pH adjusted to the physiologic range before injection. All immunoglobulin preparations were passed through a sterile 0.45-μm filter before administration to the rats.

Animal evaluation. The rats were weighed two or three times per week. All animals increased in weight independently of the sequence or nature of the intraperitoneal injections they received. In some animals, food intake was measured and was found to fluctuate with changes in body

weight. During fasting periods, the activity and appearance of the animals was carefully observed. If the animal became difficult to arouse or if matted facial hair secondary to protracted periods of hypoglycemia was noted, the fasting period was terminated. Provision of food pellets alone was adequate for the recovery of most hypoglycemic animals, but an intravenous infusion of concentrated dextrose (D20-50%) was required in some instances.

RESULTS

In vitro effects of Anti-R IgG in isolated adipose cells. It has been demonstrated that Anti-R plasma has effects similar to those of insulin in isolated adipocytes and, in addition, competitively inhibits insulin binding (17). When the partially purified IgG from patient B-10 was incubated with isolated adipocytes, glucose oxidation was stimulated in a dose-dependent fashion (Fig. 1). B-10 IgG also inhibited the binding of ^{125}I-insulin to these cells in a similar, dose-dependent manner. At each concentration of B-10 and B-12 (9) IgG, glucose oxidation was stimulated to a greater extent than it inhibited ^{125}I-insulin binding. These effects of Anti-R are typical of isulin's action under similar conditions (9).

Passive transfer of IgG to the rat. After recuperation from surgery, for periods usually ≧4 d, blood specimens were drawn from unrestrained, cannulated rats. The plasma glucose of both fed and fasted rats was monitored with and without control injections. The plasma glucose did not fall below 75 mg/dl (n = 83) during a 40-h fast in these control experiments. Occasionally, an acute rise in glucose occurred within 1 h of the intraperitoneal injection consistent with the animal's response to stress. When control-injected or noninjected rats were allowed to feed ad lib., their plasma glucose did not exceed 165 mg/dl (n = 26) (Fig. 2).

When Anti-R IgG was injected intraperitoneally into fasted rats, there was a progressive fall in the plasma glucose concentration within a 2–4-h period. The hypoglycemic effect persisted for 8–24 h after injection and, in some instances, the animals required food or glucose infusion to prevent hypoglycemic coma or death (Fig. 3 *A*). The effect was dose-dependent in that IgG obtained from patient B-10, after repeated plasmapheresis treatments (Fig. 3 *B*), showed the same qualitative, prolonged hypoglycemic effect as before

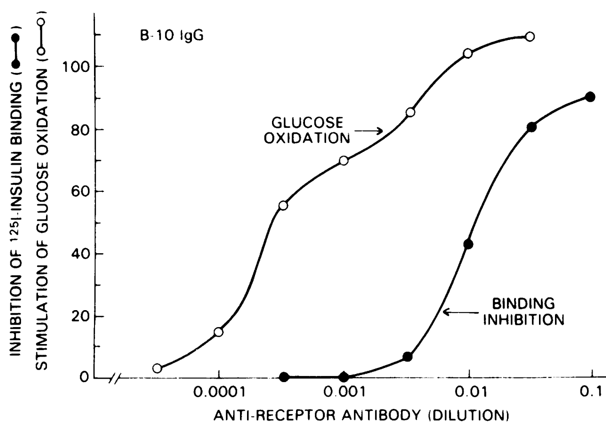

FIGURE 1 Effect of B-10 IgG on glucose oxidation and insulin binding by isolated rat fat cells. Cells were incubated with buffer (17) or the indicated concentration of IgG in buffer. The stimulatory effect of IgG on the oxidation of [U-^{14}C]glucose to $^{14}CO_2$ (O) and its inhibitory effect on ^{125}I-insulin binding (●) are expressed as a percentage of the maximal effect produced by insulin in each system. The titer (16) of undiluted B-10 IgG was ~1:2,000 (50 mg IgG/ml).

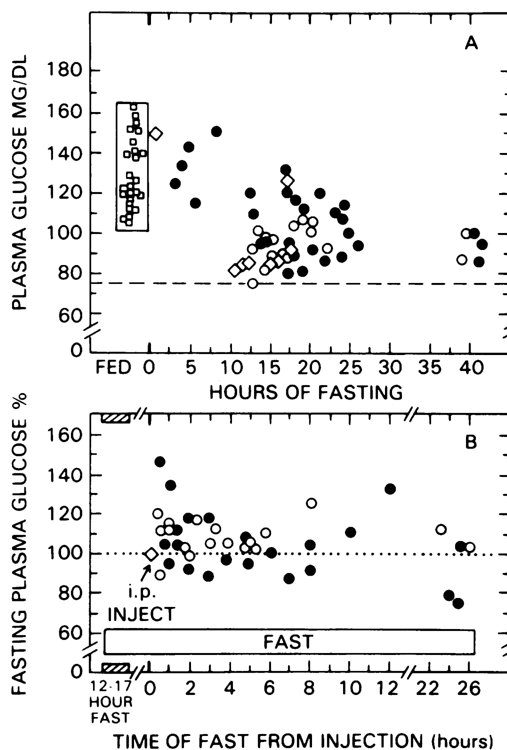

FIGURE 2 Fed and fasting glucose levels of rats in control experiments. (*A*) Plasma glucose (milligrams per deciliter) of rats during feeding *ad lib.* is shown in enclosed box (□; n = 26). Fasting plasma glucose was determined before injection (◇) and after injection with saline (O) or control IgG (●) from normal subjects and the lupus patient. Dashed line indicates lowest fasting plasma glucose value (75 mg/dl) observed with (n = 44) or without (n = 39) an injection. (*B*) Plasma glucose values after injection with saline (O) or control immunoglobulins (●) are replotted from *A* as their percentage excursion from the preinjection base-line (◇) value. The dotted line indicates the base-line value normalized to 100% for each experiment. The hatched area (▨) indicates the 12–17-h fasting period before injection.

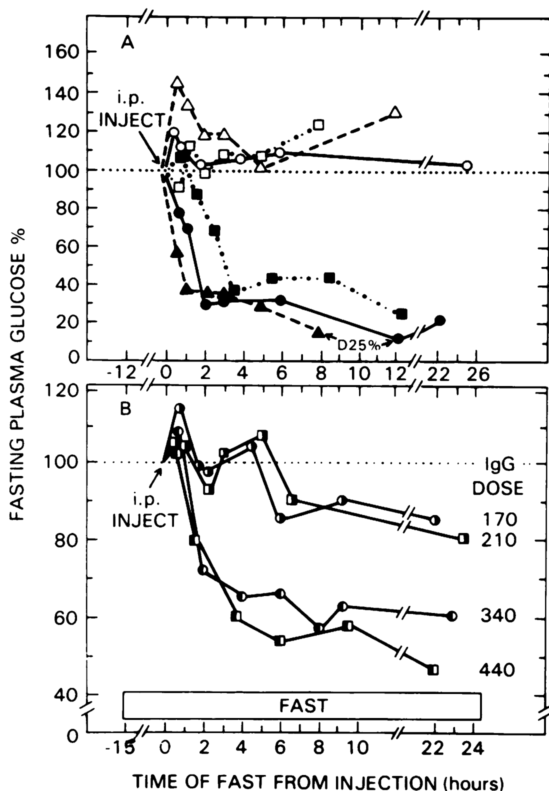

FIGURE 3 Effect of single intraperitoneal injections on the fasting plasma glucose of rats. (*A*) The effect of B-10 IgG given to three individual rats in a dose of 250 mg/kg body weight is shown by the closed figures (●, ▲, ■). The B-10 IgG injected in these experiments was prepared using insulin affinity chromatography followed by elution from protein A-Sepharose. The effect of control injections of saline (○, □) or normal human IgG (△) is shown. (*B*) The effect of B-10 IgG given in varying dosages (milligram per killigram body weight) to rats. Half-closed circles (◑, ◐) indicate effects of different concentrations of B-10 IgG prepared from the void volume of a pH 3, Sephadex G-50 column after elution from protein A-Sepharose. Half-closed squares (◨, ◧) indicate the effect of different concentrations of B-10 IgG prepared by direct elution from protein A-Sepharose. Anti-R titer (16) of the 210 mg/kg dose was <1:640 and that of the 440 mg/kg dose was >1:640.

treatment (Fig. 3 *A*), but was quantitatively less potent.

Passive transfer of IgG from hypoglycemic and hyperglycemic Anti-R patients. Based on the gel filtration studies at acid pH of B-10 IgG, we were confident that its hypoglycemic effect in the rat did not result from insulin slowly released from anti-insulin antibody (Fig. 3). However, we wished to test plasma from other patients in whom anti-insulin antibodies were not present (Table I). Of special note is the effect of Anti-R IgG from patient B-12 (Fig. 4). This patient had never been treated with insulin, had no demonstrable anti-insulin antibodies, and was hypoinsulinemic. The sole clinical manifestation of her anti-R was spontaneous, severe fasting hypoglycemia (9). The Anti-R IgG from patient B-12, tested on three occasions, produced the same persistent hypoglycemic effect in the rat as IgG from patient B-10 (Fig. 4). The Anti-R IgG from patient B-1, tested on only one occasion, produced a less pronounced, but still significant hypoglycemic response (Fig. 4).

The hypoglycemic effect of Anti-R IgG is distinct from that of insulin. The effects of varying doses of regular crystalline insulin and B-10 IgG on the plasma glucose of the fasted rat were compared in Fig. 5. When insulin was administered intraperitoneally to the fasted rat at a dose of 50 ng/kg body wt, there was no effect on plasma glucose. With an insulin dose of 100 ng/kg body wt, a typical, rapidly induced hypoglycemic phase was followed by recovery. With 350 ng insulin/kg body wt, the resultant rapid and continuous fall in plasma glucose prompted the administration of intravenous glucose to prevent death. By contrast, a more slowly induced, persistent hypoglycemic effect occurred with injection of B-10 IgG obtained from the void volume of a Sephadex G-50 column (pH 8.0).

Hyperglycemic effect of Anti-R IgG. While partially purified Anti-R has insulin-like effects when exposed to cultured cells for short intervals, longer exposure to Anti-R desensitizes cells to the effects of both Anti-R and insulin in vitro (19, 20). Therefore, we wished to determine the effect of larger concentrations of Anti-R IgG given repeatedly over longer periods of time on the plasma glucose of our in vivo rat model.

As shown in Fig. 6, injections of Anti-R IgG were begun after a 15-h fast and were accompanied by a decrease in plasma glucose concentration. Following this, the rat was fed and injections were continued. Note that with feeding and continued injections hyperglycemia ensued and was maintained for 5 d (Fig. 6). At this point, with fasting and an injection of Anti-R B-6 IgG, another decline in plasma glucose was induced.

In a series of injections carried out in different animals, hypoglycemia was typically observed in the fasted state after the acute administration of Anti-R IgG while hyperglycemia was typically observed in the fed state (Fig. 7 and Table II). Thus, in addition to the acute administration of the Anti-R IgG, the fasted state is a prerequisite for the induction of hypoglycemia. Neither the presence of the antibody nor fasting per se independently induced hypoglycemia in Anti-R injected rats. Similarly, the repeated administration of the Anti-R IgG to the fed animal is a prerequisite to the induction of hyperglycemia.

TABLE I
Characteristics of Patients

Patient	Sex	Age*	Race	Acanthosis nigricans	Abnormality in glucose homeostasis	Insulin resistance	Anti-R titer‡	Clinical course	Years of followup to 1982	Reference
B-1	F	37	Creole	Moderate	Reactive hypoglycemia-moderate glucose intolerance	Moderate	1:4	Acanthosis decreased	8	7, 8
B-6	F	40	Black	Severe	No hypoglycemia-severe hyperglycemia	Extreme	<1:30§	Complete spontaneous remission	4	5
B-10	F	25–26	Black	Severe	No hypoglycemia-severe hyperglycemia	Extreme	1:1,000–1:250	No change after plasmapheresis	2	6
B-12	F	61	Black	Absent	Severe hypoglycemia-no diabetes	None	1:20	In remission with prednisone but later expired	1	9
Lupus control	F	41	Black	Absent	Impaired glucose tolerance secondary to steroid therapy	—	Negative	No change after plasmapheresis	—	—

* At time plasma was obtained.
‡ In whole plasma.
§ Toward end of plasmapheresis treatment.

DISCUSSION

The major finding in this study is that passive transfer to rats of an IgG fraction from patients with autoantibodies to the insulin receptor produces a prolonged hypoglycemic response. The IgG preparation from patient B-10 was analyzed to show that neither its content of trace quantities of free insulin nor insulin bound to anti-insulin antibodies caused hypoglycemia in the

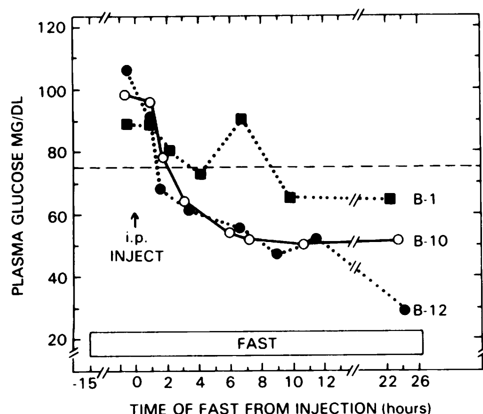

FIGURE 4 Effect of single intraperitoneal injections of anti-insulin receptor IgG from patients B-1, B-10, and B-12 (see Table I) on the fasting plasma glucose of rats. B-1 IgG was given as 80 mg/kg body weight (■); B-10 IgG, as 350 mg/kg body weight (O); and B-12 IgG, as 750 mg/kg body weight (●). All IgG injected in these experiments was prepared by direct elution from protein A-Sepharose.

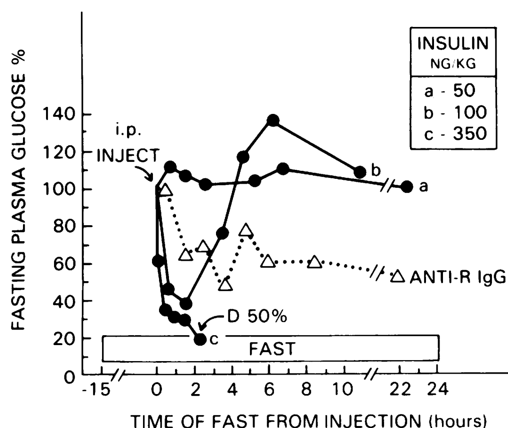

FIGURE 5 Effect of insulin compared with the effect of B-10 IgG on the plasma glucose of the fasted rat. Solid lines (●) indicate the effects of different insulin dosages (nanogram per killigram body weight) injected intraperitoneally into individual rats. Dotted line (△) indicates effect of B-10 IgG injected intraperitoneally in a single dose of 50 mg/kg body weight. B-10 IgG was prepared using insulin affinity and protein A-Sepharose chromatography followed by gel filtration through a Sephadex G-50, pH 8 column.

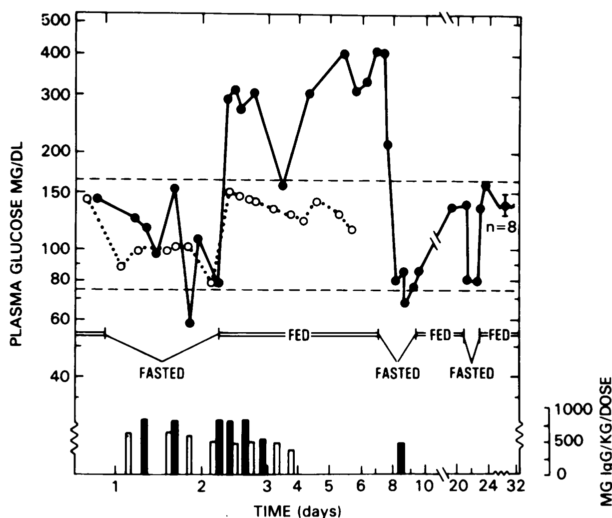

FIGURE 6 Effect of multiple injections of IgG on the plasma glucose of fed and fasted rats. Closed symbols (●) are plasma glucose values of the Anti-R IgG-injected rat (see Experiment 5, Table II). Open symbols (○) are plasma glucose values of the control IgG-injected rat (see Experiment 6, Table II). Closed bars indicate doses of B-10 IgG administered intravenously on days 2–3 and a dose of B-6 IgG injected intraperitoneally after an overnight fast on day 9. Open bars indicate doses of control IgG extracted from the plasma of a lupus patient and administered intravenously on days 2–4. Feeding and fasting periods are labeled. The range of random plasma glucose samples ($n = 8$) obtained from days 24 to 32 is shown.

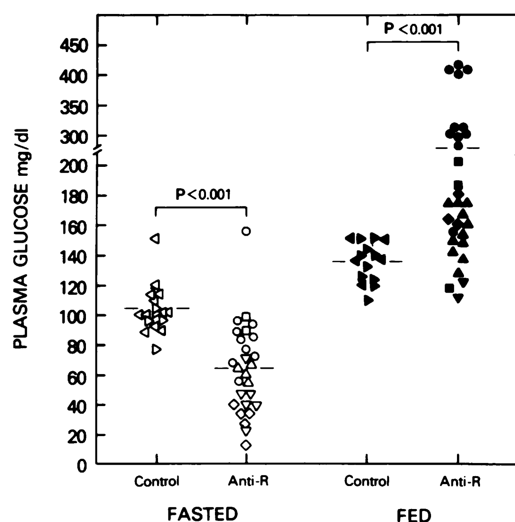

FIGURE 7 Plasma glucose of rats during fasting and feeding after injections with Anti-R IgG or control IgG from a lupus patient. These are pooled data derived from individual experiments of a design similar to that shown in Fig. 6. Open figures indicate fasting plasma glucoses. Closed figures indicate plasma glucoses during feeding. Differently shaped symbols are keyed to the experiments listed in Table II as follows: Experiment 1 (▽, ▼), Experiment 2 (◇, ◆), Experiment 4 (□, ■), Experiment 5, (○, ●) and Experiment 6 (▷, ▶). The symbols (◁, ◀) indicate the effects of control IgG injections not shown in Table II. The levels of statistical significance shown were derived using the t test.

rat. By way of additional confirmation, we found that Anti-R IgG from patients with very low endogenous insulin concentration and no detectable anti-insulin antibody produced a similar, prolonged hypoglycemic effect. Further, we demonstrated qualitatively that the rapidly induced, transient hypoglycemic effect of insulin is different from the slowly induced, prolonged hypoglycemic effect of Anti-R IgG. The effect of insulin vs. Anti-R IgG is analogous to the effect of thyrotropin (TSH) vs. LATS in the rodent. TSH produces a rapid, but brief, rise in thyroid hormone secretion, whereas the LATS effect is slower in onset and produces a prolonged stimulation of hormone secretion (3). Another intriguing observation made in this study is that, under appropriate conditions, administration of Anti-R IgG can lead to a hyperglycemic state in the rat.

The present study is the first report of the effects of Anti-R IgG passively transferred to experimental animals and provides further information on the pathogenesis of the metabolic aspects of the humoral disease associated with Anti-R. Previous in vitro studies have demonstrated that Anti-R plasma mimics most of insulin's biologic effects (4). The results obtained in this study are a direct demonstration that this same type of response occurs in vivo.

Mechanism of Anti-R-induced hypoglycemia. Anti-R IgG when injected intraperitoneally into the fasted rat typically produces a progressively more intense hypoglycemic response requiring several hours to reach a nadir. Once the nadir of the response is reached, hypoglycemia, induced by a single pulse injection, may persist for several hours. In vitro, binding of the Anti-R IgG and the resultant biologic response is of rapid onset and similar to insulin's effect. The slow onset of the in vivo response most likely results from delayed transport of the Anti-R IgG from the peritoneal cavity and intravascular space to its sites of action. The prolongation of the response would be anticipated since the dissociation rate of the Anti-R IgG from the cell is slow. To induce an in vitro response, the Anti-R IgG must be bivalent with optimal activation of the cell occurring at physiologic temperatures in a concentration-dependent fashion (16). All of these conditions are present in vivo in the rat. Thus, the major initial effect of the Anti-R IgG in vivo is to act as an insulin agonist.

Mechanism of the hyperglycemic response to Anti-R. When Anti-R IgG is given repeatedly in large doses

TABLE II
Effects of IgG Passively Transferred to Fed and Fasted Rats

Experiment	IgG per injection	Route of injection	Total injections per observation period	Period of observation	Human IgG in rat plasma	Feeding status	Plasma glucose
	mg/kg			*d*	*mg/ml*		*mg/dl*
1	250*	i.p.	1	1	—	Fasted (24 h)	26 (nadir)
			—	1.5	2.3	Fed (12 h)	124
			2	2	2.3	Fasted (10 h)	42 (nadir)
2	250*	i.p.	1	1	2.4	Fasted (20 h)	16 (nadir)
			—	1.5	—	Fed (16 h)	160–179
			2	2	1.8	Fasted (7 h)	41 (nadir)
3	370‡	i.p.	1	1.5	5.9	Fasted (40 h)	56 (nadir)
			7	10	2.4–5.9	Fed (8.5 d)	125–174
			9	15	4.9–8.4	Fed (5 d)	142–175
4	575‡	i.v.	1	2	4.9	Fed (2 d)	116
			5	6	>21	Fed (4 d)	214
			—	7	>21	Fasted (20 h)	89–99
			—	9	18	Fed (2 d)	183
5	775‡	i.v.	2	1.5	25.6	Fasted (39 h)	58 (nadir)
			6	2.5	16.8–36.4	Fed (1.0 d)	156–312
			—	7	6.3–15	Fed (4.5 d)	300–415
	500§	i.p.	1	8.5	6.3–12.2	Fasted (44 h)	68 (nadir)
6	600‖	i.v.	3	1.5	19	Fasted (39 h)	77 (nadir)
			8	4.5	4.5–36	Fed (3 d)	120–152
			—	9.5	3–>21	Fed (5 d)	110–140

Each experiment was performed in an individual rat after a control period. Experiments 4 and 6 were performed in the same rat, with an intervening 3-wk control period. Experiments 5 and 6 are shown in Fig. 6.
* B-10 IgG after insulin affinity and protein A-Sepharose chromatography (see Fig. 3 A).
‡ B-10 IgG-eluted and concentrated directly from the protein A-Sepharose column.
§ B-6 IgG prepared as in *.
‖ Lupus control IgG prepared as in *.

to a fed rat, a hyperglycemic state supervenes. We infer, but do not directly demonstrate, that the hyperglycemic animals are insulin resistant under these conditions. The experimental preparation and the amount of Anti-R IgG available to us has precluded all the experiments that would be necessary to fully demonstrate an insulin-resistant state. The possibility that the hyperglycemia might be a result of anti-insulin antibodies present in B-10 IgG seems most unlikely since only brief periods of hyperglycemia can be induced by high-titer guinea pig anti-insulin antibodies (21). As shown by Fig. 6, an extended period of hyperglycemia could be induced by B-10 IgG.

In the cultured 3T3-L1 adipocyte, it has been shown in vitro that after the initial stimulation of glucose transport, the cell becomes less responsive to further stimulation by the Anti-R IgG and by insulin (19). This phenomenon is referred to as "desensitization." Desensitization is a receptor-mediated response in that it relates only to receptor-mediated stimuli such as insulin, Anti-R IgG, or concanavalin A, but not to agents that act independently of the receptors, such as spermine or vitamin K_5 (19). The requirements for desensitization by Anti-R IgG are similar to the requirements for its agonistic effect. These include bivalency, physiologic temperature, and the presence of a sufficient concentration of antibody (20). In addition, however, desensitization requires a latency period as well as a particular milieu during in vitro incubation. For desensitization to occur, the incubation medium must include glucose or pyruvate, but 2-deoxyglucose, glucosamine, or mannose may substitute as well (20). Whether these small molecules serve as cofactors or act in some other way is unknown.

In patients, the most typical clinical feature of the disease associated with Anti-R IgG is hyperglycemia

and insulin resistance. All these patients demonstrate the characteristic low affinity receptor defect on their circulating blood cells (7). Thus, one mechanism whereby the Anti-R IgG may cause insulin resistance is by occupancy of the insulin receptor and competitive inhibition of insulin binding. Such a phenomenon has been demonstrated in vitro and is operative analogous to other circumstances in which the number or affinity of hormone receptors is reduced. In addition, it seems reasonable to speculate that hyperglycemia in the patient and in the rat with Anti-R IgG results, in part, from a desensitization process analogous to that seen in the tissue culture system.

Other potential mechanisms associated with Anti-R-related diseases. A variety of different pathogenic mechanisms have been demonstrated to mediate other anti-receptor antibody effects. In patients with myasthenia gravis, antibodies to the acetylcholine receptor are known to decrease the number of available receptors both by increasing their degradation rate and blocking their binding to neurotransmitter (22). This same type of mechanism may be relevant to the anti-insulin receptor IgG, but this has not been demonstrated at the present time. In patients with Graves' disease, heterogenous subpopulations of antibodies that either stimulate or inhibit the TSH receptor may be isolated with monoclonal techniques. These antibodies appear to interact with different subunits of the TSH receptor to mediate their different effects (23). Monoclonal antibodies to the IM-9 insulin receptor have been produced in rodents (24). However, these antibodies do not mimic the action of insulin in vitro even though they block insulin binding and inhibit insulin action. It is possible that polyclonal Anti-R IgG circulating in patients may be comprised of two differently functioning antibodies, but this has not been demonstrated. By analogy with circulating antibodies found in Graves' disease patients (24), one type of Anti-R IgG may associate with the primary binding site of the insulin receptor and simulate insulin's action, while another binds to a different portion of the receptor and blocks insulin binding. Other mechanisms, perhaps involving non-IgG immunoglobulins, could be envisioned and may be important in the pathogenesis of the clinical conditions experienced by individual patients.

Different metabolic states associated with Anti-R IgG. The varied metabolic states (i.e., hypoglycemia, hyperglycemia, or both) seen with autoantibodies to the insulin receptor continue to pose a mechanistic enigma. The present study adds a new dimension to our understanding, however, in that it shows directly that Anti-R IgG is biologically active in vivo and that the different clinical states seen in patients can be simulated in the rat.

The simplest formulation is to suggest that hypoglycemia results from the direct insulinlike agonistic effect of the Anti-R IgG. These insulinlike effects seen in freshly isolated and cultured cells, as well as in vivo in the rat, and in hypoglycemic Anti-R patients are all consistent with this type of mechanism.

In patients, however, the most typical clinical picture is hyperglycemia and insulin resistance. Thus, prolonged exposure of the Anti-R IgG to cultured cells, or to rats in vivo, leads to a desensitization effect in vitro, and hyperglycemia in vivo. If we speculate that desensitization occurs in vivo, then the combination of this effect with competitive inhibition of insulin binding could explain the insulin-resistant hyperglycemic state. Another important variable in both the rat and the patient is food intake. Caloric restriction is necessary to demonstrate hypoglycemia in the rat and feeding is necessary for the hyperglycemic response. Even when Anti-R patients are extremely resistant to insulin, a 24-h fast may bring their blood glucose values down to normal. However, resolution of hyperglycemia by fasting these patients is unaccompanied by any change in insulin binding (7).

The fact that Anti-R IgG from hypoglycemic patient B-12 and hyperglycemic patient B-10 have essentially the same effects on isolated fat cells in vitro (compare our Fig. 1 with Fig. 2 of reference 9) and the same acute response in vivo in the rat (see Fig. 4) suggests that the net metabolic response is not determined by heterogeneity of the Anti-R IgG, but is determined by the host tissue response. In instances where desensitization does not occur, then hypoglycemia prevails. In the more typical situation where desensitization predominates, an insulin-resistant hyperglycemic state appears. The factors that mediate this presumably postreceptor desensitization state or prevent it from occurring are unknown at present. The factors involved, however, are likely to be closely related to the mechanisms by which the insulin receptor transduces its biological signal.

ACKNOWLEDGMENTS

The authors give special thanks to M. McKenna and the Laboratory of Nutrition for assistance with implementation of the unrestrained, cannulated rat system; R. Scow for helpful discussions and use of animal facilities; M. Wu for care and weighing of the animals; A. Adams for help with glucose assays; C. M. Hendricks for the insulin assays, and J. Ryan and E. Klima for help with the quantitative immunoglobulin assays. Excellent secretarial assistance of C. Culwell is gratefully acknowledged.

REFERENCES

1. Fink, J. N., and G. N. Beall. 1982. Immunologic aspects of endocrine diseases. *J. Am. Med. Assoc.* 248:2696–2700.

2. Toyka, K. V., D. B. Drachman, D. E. Griffin, A. Pestronk, J. A. Winkelstein, K. H. Fischbeck, and I. Kao. 1977. Myasthenia gravis. Study of humoral immune mechanism by passive transfer to mice. *N. Engl. J. Med.* 296:125–131.
3. McKenzie, J. M. 1958. Delayed thyroid response to serum from thyrotoxic patients. *Endocrinology.* 62:866–878.
4. Harrison, L. C., E. Van Obberghen, C. Grunfeld, G. L. King, and C. R. Kahn. 1980. Modulation of the insulin receptor by insulin receptor autoantibodies. *In* Membranes, Receptors, and the Immune Response. Alan R. Liss, Inc., New York, 109–126.
5. Muggeo, M., J. S. Flier, R. A. Abrams, L. C. Harrison, A. B. Deisserroth, and C. R. Kahn. 1979. Treatment by plasma exchange of a patient with autoantibodies to the insulin receptor. *N. Engl. J. Med.* 300:477–480.
6. Taylor, S. I., R. F. Dons, E. Hernandez, J. Roth, and P. Gorden. 1982. Insulin resistance is associated with hirsutism, polycystic ovaries, and autoantibodies to the insulin receptor. *Ann. Intern. Med.* 97:851–855.
7. Bar, R. S., M. Muggeo, C. R. Kahn, P. Gorden, and J. Roth. 1980. Characterization of insulin receptors in patients with the syndromes of insulin resistance and acanthosis nigricans. *Diabetologia.* 18:209–216.
8. Kahn, C. R., J. S. Flier, R. S. Bar, J. A. Archer, P. Gorden, M. M. Martin, and J. Roth. 1976. The syndromes of insulin resistance and acanthosis nigricans. Insulin receptor disorders in man. *N. Engl. J. Med.* 294:739–745.
9. Taylor, S. I., G. Grunberger, B. Marcus-Samuels, L. H. Underhill, R. F. Dons, J. Ryan, R. F. Roddam, C. E. Rupe, and P. Gorden. 1982. A new clinical syndrome of hypoglycemia associated with antibodies to the insulin receptor. *N. Engl. J. Med.* 307:1422–1426.
10. Flier, J. S., R. S. Bar, M. Muggeo, C. R. Kahn, J. Roth, and P. Gorden. 1978. The evolving clinical course of patients with insulin receptor autoantibodies: spontaneous remission or receptor proliferation with hypoglycemia. *J. Clin. Endocrinol. Metab.* 47:985–995.
11. Ey, P. L., S. J. Prowse, and C. R. Jenkin. 1978. Isolation of pure IgG, IgG_{2a}, and IgG_{2b} immunoglobulins from mouse serum using protein A-Sepharose. *Immunochemistry.* 15:429–436.
12. Kilpatrick, J. M., and G. Virella. 1980. Isolation and characterization of soluble insulin and anti-insulin immuno-complexes formed *in vitro* and *in vivo* in sera from patients with diabetes mellitus. *Clin. Exp. Immunol.* 40:445–452.
13. Fahey, J. L., and E. McKelvey. 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.* 94:84–90.
14. Nakagawa, S., H. Nakayama, T. Sasaki, K. Yoshino, Y. Y. Yu, K. Shinozaki, S. Aoki, and K. Mashimo. 1973. A simple method for the determination of serum-free insulin levels in insulin-treated patients. *Diabetes.* 22:590–600.
15. Kuzuya, H., P. M. Blix, D. L. Horwitz, D. Steiner, and A. H. Rubenstein. 1977. Determination of free and total insulin and C-peptide in insulin-treated diabetics. *Diabetes.* 26:22–29.
16. Flier, J. S., C. R. Kahn, D. B. Jarrett, and J. Roth. 1976. Characterization of antibodies to the insulin receptor. A cause of insulin-resistant diabetes in man. *J. Clin. Invest.* 58:1442–1449.
17. C. R. Kahn, K. Baird, J. S. Flier, and D. B. Jarrett. 1977. Effects of autoantibodies to the insulin receptor on isolated adipocytes. *J. Clin. Invest.* 60:1094–1106.
18. Popp, M. B., and M. F. Brennan. 1981. Long-term vascular access in the rat: importance of asepsis. *Am. J. Physiol.* 241:H606–H612.
19. Karlsson, F. A., E. Van Obberghen, C. Grunfeld, and C. R. Kahn. 1979. Desensitization of the insulin receptor at an early post-receptor step by prolonged exposure to anti-receptor antibody. *Proc. Natl. Acad. Sci. USA.* 76:809–813.
20. Grunfeld, C., E. Van Obberghen, F. A. Karlsson, and C. R. Kahn. 1980. Antibody-induced desensitization of the insulin receptor. Studies of the mechanism of desensitization in 3T3-L1 fatty fibroblasts. *J. Clin. Invest.* 66:1124–1134.
21. Armin, J., R. T. Grant, and P. H. Wright. 1960. Experimental diabetes in rats produced by parenteral administration of anti-insulin serum. *J. Physiol. (Lond.).* 153:146–165.
22. Drachman, D. B., R. N. Adams, L. F. Josifek, and S. G. Self. 1982. Functional activities of autoantibodies to acetylcholine receptor and the clinical severity of myasthenia gravis. *N. Engl. J. Med.* 307:769–775.
23. Valente, W. A., P. Vitti, Z. Yavin, E. Yavin, C. M. Rotella, E. F. Grollman, R. S. Toccafondi, and L. D. Kohn. 1982. Monoclonal antibodies to the thyrotropin receptor: simulating and blocking antibodies derived from the lymphocytes of patients with Graves' disease. *Proc. Natl. Acad. Sci. USA.* 79:6680–6684.
24. Roth. R. A., D. J. Cassell, K. Y. Wong, B. A. Maddux, and I. D. Goldfine. 1982. Monoclonal antibodies to the human insulin receptor block insulin binding and inhibit insulin action. *Proc. Natl. Acad. Sci. USA.* 79:7312–7316.