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Research Article

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Desensitization of the Insulin Receptor by Antireceptor Antibodies In Vivo is Blocked by Treatment of Mice with β-Adrenergic Agonists

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Abstract

In previous studies we reported that immunization of mice with ungulate insulins induced the development of antiinsulin antibodies, which include an idiotype that appeared to recognize the part of the insulin molecule recognized by the hormone receptor. The antiinsulin antibodies of this idiotype were replaced spontaneously by antiidiotypic antibodies. The antiidiotypic antibodies, which persisted for about 14 d, mimicked insulin and functioned as antibodies to the insulin receptor. They induced down regulation, desensitization and refractoriness of the insulin receptor and disturbances in glucose homeostasis in vivo (Shechter, Y., D. Elias, R. Maron, and I. R. Cohen., 1984; Elias, D., R. Maron, I. R. Cohen, and Y. Shechter. 1984, *J. Biol. Chem.* 259:6411–6419). We now report that effects of the antiidiotypic antibodies on the insulin receptor effector system can be modified pharmacologically. Administration of the β-adrenergic agonist isoproterenol during the period of insulin resistance (days 26–40 after primary immunization), largely restored fat cell responsiveness to insulin, and eliminated the appearance of fasting hyperglycemia. This restoration appeared to be caused by inhibition of both insulin receptor desensitization and refractoriness. In contrast, down regulation of insulin receptors was not reversed by isoproterenol treatment in vivo. The effects of treatment with isoproterenol persisted for 2–4 d after termination of treatment. The β-antagonist, propranolol and more so, the β_{1a}-antagonist metoprolol, specifically blocked the effect of isoproterenol at a molar ratio of 3–10:1. Oral administration of the cAMP phosphodiesterase inhibitor, aminophylline, was also effective in inhibiting the development of desensitization in fat cells. These results indicate that treatment with β_1-adrenergic agonists in vivo, or other agents that elevate cellular cAMP levels, can inhibit the development of the "postbinding" defects induced by insulin-mimicking, antireceptor antibodies. These observations have both basic and clinical implications.

Introduction

High concentrations of insulin were shown to decrease the number of insulin receptors (down regulation) in both in vivo and in vitro systems (1–5). Down regulation has been demonstrated in several types of insulin-responsive cells, including rat adipocytes (6–13). The decrease in insulin binding capacity was ~ 50–60% and occurred half-maximally within 2–3 h at 37°C at high concentrations of insulin (reviewed in 14). Receptor loss is primarily the consequence of an accelerated rate of receptor degradation, as the rate of receptor biosynthesis remained unaltered (14). Hyperinsulinemia was also shown to induce refractoriness in target tissues (defined as a decrease in the maximal biological effect) and desensitization (defined as a shift to the right in the dose response curve to insulin). Refractoriness was observed in rat adipocytes (10) and desensitization was observed both in fibroblasts (15) and in hepatoma cells (16). Although down regulation, refractoriness, and desensitization may be induced by common factors, they most likely are caused by different mechanisms. Hepatoma cells after a limited exposure to insulin in vitro recovered fully their ability to respond again to insulin within 2 h, while recovery of their insulin binding capacity required ~ 24 h (16). Thus, desensitization is not caused by down regulation, but must involve a postreceptor mechanism. The possible connection between these processes and insulin resistance in human diabetes (1, 2, 4) makes their study clinically important.

In previous studies we observed that immunization of mice with ungulate insulins induced the development, first of insulin antibodies, and then of antiidiotypic antibodies that recognized and interacted with the insulin receptor, and mimicked the actions of insulin in vitro (17–20). This insulin-like antireceptor antibody was of the IgG2 class and its circulating level was equivalent functionally to ~ 200 ng/ml insulin (17, 18, 20). The appearance of antireceptor antibody was transitory; starting on day 26 after the primary immunization and persisting for about 14 d (18). The presence of circulating antireceptor antibodies induced tissue alterations in the mice in vivo. The adipocytes of antibody positive mice exhibited lower responsiveness to insulin as a consequence of receptor down regulation, refractoriness, and desensitization (18). Marked disturbances in glucose homeostasis were evident; a period of fasting hypoglycemia was initially observed to be replaced by fasting hyperglycemia when insulin resistance developed (18). Thus, in addition to what these mice can teach us about insulin autoimmunity and antiidiotypic networks, they provide a convenient in vivo experimental system of insulin resistance that may be analogous, at least in part, to the insulin resistance developing in humans as a consequence of fluctuations in the circulating levels of insulin and glucose (1).

As the mechanism(s) of action of insulin are largely unknown, in particular the events linking the activated receptor to the expression of biological processes (reviewed in 14, 21, 22), it is not currently possible to specify the molecular mechanism responsible for insulin resistance. It is known, however, that hormones and agents that elevate cellular cAMP levels (e.g., catecholamines, glucagon and cAMP-phosphodiesterase

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[PDE] inhibitors) have an antagonistic influence on several insulin-responsive cellular enzyme systems (23–30). As desensitization, down regulation, and refractoriness are insulin-dependent cellular events, we reasoned that factors which elevate cellular cAMP levels may also antagonize the development of insulin resistance in vivo.

Here, we report that administration of either isoproterenol or aminophylline to immunized mice blocks the development of fat cell desensitization and refractoriness to insulin, but does not prevent insulin receptor down regulation.

Methods

Materials. Male mice of the (BALB/c × C57BL/6)F_1 hybrid strain and male Wistar rats (70–100 g) were supplied by the animal breeding center of this Institute or by Jackson Laboratories, Bar Harbor, ME. Bovine insulin and isoproterenol were purchased from Sigma Chemical Co. (St. Louis, MO), and porcine insulin from Eli-Lilly & Co. (Indianapolis, IN). D[U-^{14}C]Glucose (4–7 mCi/mol) was purchased from New England Nuclear (Boston, MA). Collagenase type I (134 U/mg) was from Worthington Biochemicals (Freehold, NJ) and Sepharose-protein A affinity columns from Pharmacia Fine Chemicals, Uppsala, Sweden.

Procedures. Mice were immunized by injecting each hind footpad with 25 μg of bovine insulin emulsified in complete Freund's adjuvant, as described (19). Control mice were immunized with the adjuvant alone. Unless otherwise mentioned a single immunization protocol was used. Isoproterenol (30 μg/mouse, i.p.) or other catecholamines, as specified in the specific experiments, were administered twice a day, for a period of either 7 or 15 d; at 8 a.m. and 3 p.m., starting on day 26 after immunization. Aminophylline (1 mg/d per mouse) was administered orally. Every 2 d several mice were killed and adipocytes were prepared for determination of their responsiveness to insulin and their insulin binding capacity. Blood was collected for determination of the levels of antiinsulin and antireceptor antibodies as described (17–19).

The following methods and procedures were used without modification: Preparation of mouse or rat adipocytes from epididymal fat pads (31); iodination of insulin (32); binding of [^{125}I]iodoinsulin to adipocytes (33); assay of lipogenesis (34); and solid phase radioimmunoassay for measuring antibodies to insulin (35). Glucose was determined in individual sera using a glucose analyzer (type II; Beckman Instruments, Inc., Fullerton, CA) and expressed as mg/100 ml of glucose in the serum.

Percent maximal stimulation of lipogenesis was calculated using the equation V_{ins}-V_{basal}/V_{max}-V_{basal} × 100, where V_{ins}, V_{basal}, and V_{max} are the rates of lipogenesis at a given insulin concentration, either in the absence of insulin or at an insulin concentration of 100 ng ml^{-1}, respectively.

ED_{50} values were derived from the corresponding figures. The results shown in the tables and figures were confirmed by repeating each of the experiments at least three times with similar results. Student's *t* test analysis of the differences between groups showed them to be highly significant ($P < 0.01$).

Results

Isoproterenol prevents fat cell desensitization. In previous studies, we learned that antireceptor antibodies are first detected in the circulation 26 d after immunization. They remain high for 12–14 d and then decline to undetectable levels (18). Therefore, we administered a catecholamine agonist daily from day 26 to day 40 after immunization; the period in which receptor antibodies were present and insulin resistance was developing. Isoproterenol was chosen because of its potency as a β-adrenergic agonist (36). Fat tissue has been shown to be primarily of

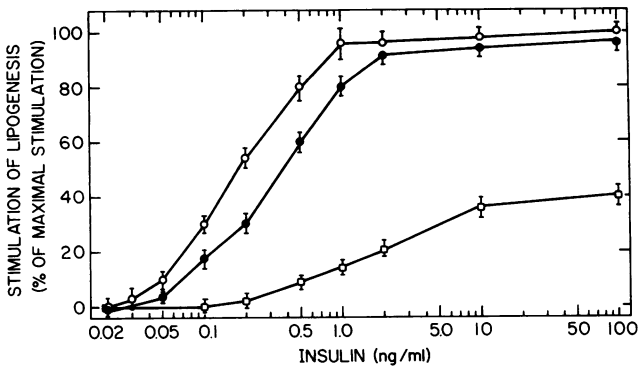

Figure 1. Administration of isoproterenol prevents desensitization of insulin receptors in immunized mice. The lipogenic response to insulin was measured using adipocytes obtained from control mice (that received isoproterenol) (○), from mice that were immunized to insulin (□), and from insulin-immunized mice that received isoproterenol (●). Each group contained adipocytes pooled from five mice. Adipocytes were measured for their responsiveness to insulin 36 d after immunization. Isoproterenol was administered twice daily (30 μg/d), starting on day 26 after immunization to insulin. Results are means±SD of a representative experiment. Maximal (100%) stimulation was assigned to the maximal V_{ins}-V_{basal} of the control group.

β1-subtype-responsive tissue (37). Groups of mice were sacrificed and the lipogenic activity of their adipocytes determined at increasing concentrations of insulin. One group of immunized mice received PBS only, while another group of nonimmunized mice received isoproterenol. Fig. 1 shows that treatment with isoproterenol prevented the development of resistance to insulin. For example, the adipocytes of control mice showed an ED_{50} of 0.15±0.02 ng/ml while those of immunized mice showed an ED_{50} of 1.9±0.2 ng/ml. Treatment of immunized mice with isoproterenol restored the ED_{50} to 0.35±0.01 ng/ml (derived from Fig. 1). In other experiments we found that treating control mice with isoproterenol did not affect the dose response to insulin (ED_{50} and maximal response) compared with mice treated with PBS (not shown). The protective effect of isoproterenol in inhibiting fat-cell desensitization

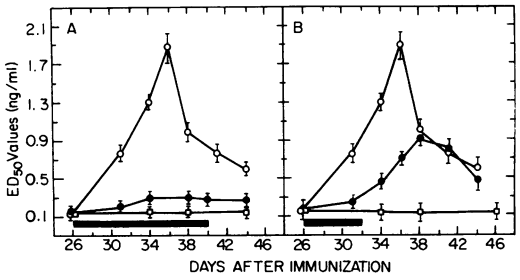

Figure 2. Isoproterenol treatment prevents fat cell desensitization. (*A*) Mice immunized to insulin received isoproterenol twice daily for 15 d (days 26–40, indicated by the bar). At the indicated time points fat cells were taken and examined for their lipogenic activity at increasing concentrations of insulin. Immunized mice receiving no isoproterenol (○); immunized mice receiving isoproterenol (●); nonimmunized mice receiving isoproterenol (□). (*B*) The same experimental design as in A, but a shorter duration of isoproterenol therapy (7 d, day 26–34) was used. Results are means±SD of a representative experiment. Each time point represents the ED_{50} value of pooled adipocytes obtained from five mice.

Table I. Circulating Levels of Antiinsulin and Antireceptor Antibodies on Day 36 in Insulin Immunized Mice Receiving Isoproterenol

	Antibodies to native insulin	Receptor antibodies
	titer*	Serum dilution to produce 50% of maximal stimulation of lipogenesis‡
Immunized mice	10^4	$1.2\pm0.1 \times 10^4$
Immunized mice plus isoproterenol (days 28–40)	10^4	$1.2\pm0.12 \times 10^4$

* Determined by A solid phase radioimmunoassay (35).
‡ Lipogenesis was performed in KRB buffer containing 0.2 mM [U-^{14}C]glucose, 1% bovine serum albumin and about 2×10^5 rat adipocytes. Reaction was carried out for 90 min at 37°C. Maximal lipogenesis obtained by incubation with 100 ng/ml of insulin was 340% of the basal lipogenesis (obtained in the absence of added hormone). Values are the means±SE of six mice. Each serum was measured for its antibody content individually.

Table II. Treatment with Isoproterenol Inhibits Receptor Refractoriness in Insulin Immunized Mice

	Lipogenesis		
	cpm incorporated* per 3×10^5 cells/h		
Source of adipocytes	No insulin	Insulin§	Percent maximal stimulation‡
Control mice	3,000±100	7,200±200	100
Immunized mice‖	2,800±50	4,500±100	40.5
Isoproterenol-treated immunized mice¶	2,900±100	6,700±150	90.5

* Lipogenesis was performed for 1 h at 37°C.
‡ Hundred percent stimulation was taken as $V_{ins} - V_{basal}$ of the control group.
§ Final concentration in the assay was 100 ng ml^{-1}.
‖ Adipocytes were taken on day 36 after immunization. Each group contained adipocytes pooled from 5 mice.
¶ Adipocytes were taken on day 36 after immunization; treatment with isoproterenol began on day 26.

could be observed after 7–8 d of treatment and persisted as long as this β-adrenergic agonist was administered (Fig. 2 *A*). ED_{50} values of the insulin-immunized mice receiving isoproterenol were about twofold higher than the ED_{50} of control adipocytes (not shown) or those taken from control mice receiving isoproterenol (Fig. 2 *A*).

Effect of isoproterenol is transient. In the experiments summarized in Fig. 2 *B*, isoproterenol was administered for 7 d only (days 26–32 after immunization). The shorter duration was also effective (although to a lesser extent) in preventing fat cell desensitization to insulin (Fig. 2 *B*). It is important to note that the effect of isoproterenol persisted 2–4 d after terminating treatment. Thus, cells obtained on days 34 to 36 had much lower ED_{50} values than cells obtained from untreated immunized mice (Fig. 2 *B*).

Administration of isoproterenol does not affect antireceptor antibodies. To investigate the possibility that isoproterenol prevented desensitization by decreasing the relevant antibodies, we measured the levels of both antiinsulin and receptor antibodies (Table I). No significant differences could be observed in the levels of either of these antibodies in immunized mice, as a result of isoproterenol administration (Table I). Thus, maintenance of the sensitivity of the fat cells to insulin was not due to a decrease in the level of the antibodies responsible for triggering desensitization.

Isoproterenol prevents refractoriness to insulin. In addition to desensitization, adipocytes taken from immunized mice exhibited considerably lower responsiveness to high (100 ng/ml) concentrations of insulin, a phenomenon termed refractoriness (18). The results shown in Fig. 1 and Table II demonstrate that the administration of isoproterenol in vivo also prevented refractoriness to insulin. Thus, the treatment inhibited the development of both desensitization and refractoriness.

Isoproterenol does not prevent receptor down regulation. In contrast to desensitization and refractoriness, the administration of isoproterenol in vivo could not block receptor down regulation (Table III). About 50% of the sites were lost whether or not the immunized mice had received isoproterenol or were untreated. Thus, the mild degree of insensitivity to insulin demonstrate in treated mice (Figs. 1 and 2 *A*) may be attributed predominantly to decreased receptor binding capacity. These results support the notion that down regulation and desensitization involve separate mechanisms and that desensitization does not occur as a result of receptor down regulation (16).

Administration of isoproterenol prevents fasting hyperglycemia. Because insulin resistance was accompanied by fasting hyperglycemia (2), we tested the effect of isoproterenol treatment on the concentration of blood glucose in fasting mice. Fig. 3 shows that, unlike the immunized mice, those receiving isoproterenol did not develop fasting hyperglycemia. This indicates that the fasting hyperglycemia seen in mice with antireceptor antibodies was due to desensitization and refractoriness, rather than to receptor down regulation.

Effects of B_1 receptor antagonists or aminophylline. To determine whether the effect of isoproterenol was mediated via B_1 receptor sites, isoproterenol was administered together with propranolol, a known B_1 antagonist (38). Propranolol, which by itself had no effect (Table IV), inhibited the effect of isoproterenol at a 10:1 molar ratio (Table IV). Metoprolol, a more specific B_1 antagonist (38) inhibited the effect of isoproterenol at a 3:1 molar ratio (Table IV). Thus the effect of isoproterenol seems to be mediated primarily by way of the B_1 subtype adrenergic receptor.

Table III. Treatment with Isoproterenol Does Not Inhibit Receptor Down Regulation

Source of adipocytes	[^{125}I]Iodoinsulin specifically bound
	fmol/10^6/cells
Control mice	23±2
Immunized mice*	11±1
Isoproterenol-treated immunized mice‡	12±1

* Adipocytes were taken on day 36 after immunization to insulin. Each group contained adipocytes pooled from four mice.
‡ Binding was performed for 1 h at 22°C using 1 nM [^{125}I]iodoinsulin, in the presence and the absence of 5 μM unlabeled insulin.

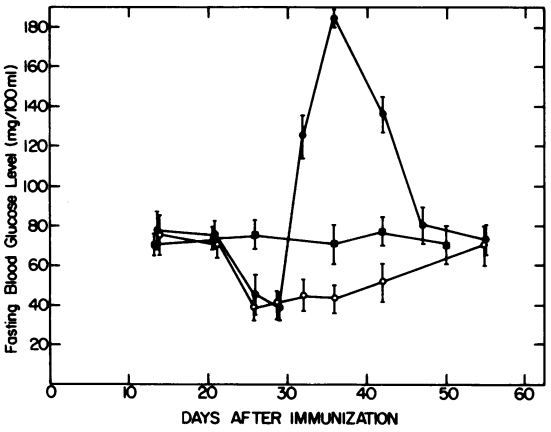

Figure 3. Administration of isoproterenol inhibits development of fasting hyerglycemia in insulin immunized mice. Fasting blood glucose levels were determined after removing food for 16 h (4 a.m.–8 p.m.) at the indicated time points in immunized mice receiving no isoproterenol (●); in immunized mice receiving isoproterenol (○); and in nonimmunized mice receiving isoproterenol (■). Each group consisted of seven mice. Isoproterenol was administered for 15 d (days 25–40).

As the cAMP-phosphodiesterase inhibitor, aminophylline, can be absorbed orally (38), we evaluated its effect on desensitization and found that oral treatment of immunized mice for several days with aminophylline equally restored sensitization. Thus, developed desensitization can also be blocked by nonhormonal oral agents known to elevate cellular cAMP levels.

Discussion

Earlier studies from our laboratory revealed that mice immunized with ungulate insulins exhibit a critical and transitory period of 12–14 d (day 26–40 after primary immunization) during which antireceptor antibodies present in the circulation are associated with alterations in insulin receptor physiology and disturbances in glucose homeostatis (17–20). This study was done to establish conditions that might prevent the development of receptor-effector malfunction. Here, we report that administration of either a β-adrenergic agonist or aminophylline, prevented the development of fat cell desensitization and refractoriness (Figs. 1, 2 *A* and *B*, Tables II and IV). In contrast, the accompanying loss of insulin receptor sites (down regulation) was not inhibited (Table III). As the isoproterenol treatment eliminated fasting hyperglycemia (Fig. 3), the latter state can be attributed to postreceptor events (namely, desensitization and refractoriness), rather than to a loss of insulin binding capacity.

It is generally accepted that effects of β-adrenergic agonists are mediated through the production of cyclic AMP as a second messenger (39–41). This intracellular signal provides a general mechanism for protein phosphorylation catalyzed by the activation of cAMP-dependent protein kinases (39–41). It is also well documented that certain agents and/or conditions that elevate cAMP levels have an antagonistic influence on some insulin-responsive cellular enzyme systems, and vice versa. Examples are glycogen synthase and phosphorylase in muscle (23, 24), liver (25, 26), and adipocytes (27, 28), and hormone-sensitive lipase in adipocytes (29, 30). These antagonistic effects occur most likely at points distal to the production or de-esterification of the nucleotide itself, as insulin has been shown to either have no effect (42–45) or even to increase (46, 47) cAMP levels in muscle or adipocytes, while still activating glycogen synthase in muscle (47, 48), or inhibiting lipolysis in adipocytes (42–45). In adipocytes, agents that elevate cAMP levels lead to an enhanced rate of lipolysis that can be inhibited by insulin. These, however, are rapid processes. Both initiation and termination occur within minutes after the addition or the removal of the respective hormones (42–45). In this study, however, the effect of isoproterenol persisted for 2–4 d after administration was terminated (Fig. 2 *B*). Therefore, it is conceivable that the effect might be mediated by a modification of longer duration, such as might be attributed to protein synthesis. In cultured cells insulin stimulates protein synthesis (49) and inhibits protein degradation (50). Insulin may regulate rates of transcription and translation and may have either positive (51) or negative (52) effects on the levels of

Table IV. Effect of Various Agents in Restoring Fat Cell Desensitization of Immunized Mice In Vivo

Group of mice	Type of treatment	Class of agent	ED_{50} values* (day 36)	(ED_{50} control/ED_{50} treated) × 100
			ng/ml	
Control	None	—	0.15±0.02	100
Immunized‡	None	—	2.0±0.3	7.5
Immunized	Isoproterenol (30 μg/d)§	β-agonist	0.35±0.05	42
Immunized	Propranolol (300 μg/d)	β-antagonist	1.9±0.2	7.8
Immunized	Isoproterenol + propanolol (300 μg/d)	β-agonist + β-antagonist	2.0±0.3	7.5
Immunized	Isoproterenol + metoprolol (50 μg/d)	β-agonist + β_1-antagonist	1.3±0.2	11.5
Immunized	Isoproterenol + metoprolol (100 μg/d)	β-agonist + β_1-antagonist	2.2±0.3	6.8
Immunized	Aminophylline (1 g/d)‖	PDE inhibitor	0.36±0.03	42

* Determined by the assay of lipogenesis. ‡ Mice were immunized by inoculating each hind footpad with 25 μg of bovine insulin emulsified in complete Freund's adjuvant (according to reference 19). Each group consisted of 7–10 mice. § Administered twice daily at 8 a.m. and 3 p.m. Isoproterenol was administered at 30 μg/d in all experiments summarized in the table. ‖ Administered orally, twice daily 1 mg/d using a soft rubber catheter.

specific mRNA synthesis. With respect to translational control, insulin and growth factors on the one hand and hormones that elevate levels of intracellular cAMP on the other, may initiate cellular events that ultimately result in the phosphorylation of ribosomal protein S6 (reviewed in 53). Thus, antagonistic actions of insulin to hormones known to elevate cAMP levels may also exist on the regulatory level of protein synthesis and protein degradation.

Further studies in vitro are required in order to elucidate the molecular mechanisms involved in insulin desensitization and its prevention by β-adrenergic stimulation. Care, however, should be taken in choosing a suitable in vitro system, as more than one mechanism can lead to desensitization. In rat hepatoma cells, for example, insulin causes desensitization to the induction of tyrosine aminotransferase by IGF-I and IGF-II, which mediate this activity via their own distinct receptor sites (54). Thus, desensitization in this cell type may be distal to the insulin receptor itself. Also, studies of insulin desensitization in cultured cells are usually applied to the intermediate and the long-term actions of insulin. These types of desensitization may not necessarily coincide with desensitization of the immediate, or short-term actions of insulin, such as the stimulation of glucose uptake and its metabolism.

Several studies have demonstrated a partial reduction in insulin binding and/or in stimulating hexose uptake as a result of β-adrenergic stimulation in in vitro systems. These effects are presumably mediated via cAMP (55–58) and are shown to be both rapid and transient, namely they proceed and are reversed within minutes after the addition or the removal of isoproterenol (55). The effects observed in our study seem to involve synchronous cAMP-dependent events, which induce alterations of longer duration (Fig. 1). A link, however, between these two cAMP-dependent actions is conceivable and is currently being studied.

However, the clinical application of our observations need not await a complete biochemical characterization; β-adrenergic agonists are widely and safely used. Aminophylline and related methyl-xanthines can be administered orally. These agents are now thought to increase cAMP levels primarily by virtue of their interaction with adenosine receptors (59). This can result from displacement of adenosine from inhibiting receptors or from direct activation of stimulatory adenosine receptors (59). Our results suggest that these agents could be tried to treat patients suffering from insulin resistance. The most striking insulin resistance is demonstrable in patients suffering from type B acantosis nigricance with antiinsulin receptor antibodies, usually of the IgG type (60). However, we have observed that antiinsulin receptor antibodies of the IgM type are found in patients with type I diabetes (61), where a mild resistance to insulin is often present (62). Insulin resistance is the hallmark of type II diabetes (6), which may also have an autoimmune component. Thus, agents that could prevent or reverse antibody-mediated receptor desensitization might be of value. The results presented here in addition indicate that the pathological significance of autoantibodies to hormone receptors is influenced greatly by how the endocrine system reads and implements the signals transmitted by the antibodies. As shown in Fig. 3, preventing adaptive desensitization led to prolonged hypoglycemia in place of hyperglycemia in the immunized mice. Indeed, we have observed persistent hypoglycemia in a child with antireceptor antibodies of the same antiidiotypic specificity as the mouse antibodies apparently responsible for receptor desensitization and hyperglycemia (63). Thus, the insulin receptor of certain individuals may respond to anti-receptor antibodies without undergoing adaptive desensitization.

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