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Insulin Antibodies Prevent Insulin-Receptor Interactions

R. de Pirro, A. Fusco, L. Spallone, R. Magnatta, and R. Lauro

2ª Clinica Medica, Policlinico Umberto I°, Roma, Italy

Summary. The study was carried out to investigate whether insulin bound to antibody is able to bind the insulin receptor of target tissues. Three specific rabbit anti-insulin sera as well as sera from eight diabetic patients with insulin antibodies were incubated, free of insulin, with labelled insulin for 48 h at 4 °C; following incubation labelled insulin was employed in binding experiments on monocytes, erythrocytes and placenta membranes. Using rabbit sera, receptor binding was absent when insulin was totally combined with antibody, and appeared in increasing amounts as the percentage of free insulin increased to reach a maximum when no insulin was combined with antibody. The same experiment using sera from diabetic patients showed a close negative relationship ($r = 0.95$) between the amount of insulin bound to the antibody and the amount bound to receptors. The influence of the insulin-antibody complex on the insulin receptor interaction was evaluated by exposing the insulin-antibody complex to the receptor in pH, temperature and competition-inhibition curve experiments. The complex had no effect on receptor affinity or on the pH and temperature relationship influence with insulin-receptor interaction. The findings suggest that insulin resistance in the presence of insulin antibodies is due only to an alteration occurring before the interaction of insulin with its receptor, and demonstrate that the insulin-antibody complex does not influence the insulin receptor interaction.

Key words: Insulin, insulin antibody, insulin receptor, insulin resistance.

It has become increasingly apparent that certain clinical disorders and metabolic states are associated with insulin resistance. Insulin initiates its effects by interacting with receptors located on the plasma membrane [1–3] and altered insulin receptor interaction may be responsible for hormone resistance [4–14].

Insulin resistance due to insulin antibodies may thus be considered a pre-receptor phenomenon [15, 16] with insulin bound to antibody being unavailable for receptor binding [15, 16].

In the present study we have examined the effect of circulating insulin antibodies on insulin-receptor interaction.

Materials and Methods

^{125}I-porcine insulin (120–150 μCi/μg) was purchased from Sorin, Italy, porcine insulin from Organon-Holland, and charcoal (Norit A) from Fisher, USA.

Specific insulin antibodies raised in rabbits were kindly donated by Dr. G. Tamburrano, Rome and sera from diabetic patients with insulin antibodies by Dr. M. Iavicoli, Rome.

Preparation of Insulin-free Serum

Prior to use sera were treated, unless otherwise mentioned, with 1% dextran coated charcoal at pH 3.5 according to Dixon [17], to yield an insulin-free preparation; after this procedure the pH of sera was immediately restored to 7.4 by addition of sodium hydroxide. Approximately 94% of insulin was removed by this treatment.

Erythrocyte, Leukocyte and Placental Preparation

100 ml of venous blood was drawn from normal medical personnel into vials containing 0.129 mol/l sodium citrate. The mononuclear leukocytes and erythrocytes were separated from the other components using a Ficoll-Angiografin gradient [18, 19], according to the procedure of Boyum [20]. They were then suspended in 100 mmol/l Hepes buffer containing 1 g/100 ml bovine serum albumin (BSA) (pH 7.8). The mononuclear cell preparation contained 85% lymphocytes and 15% monocytes [18]; since it has been demonstrated that insulin binding depends primarily upon monocytes [21, 22]

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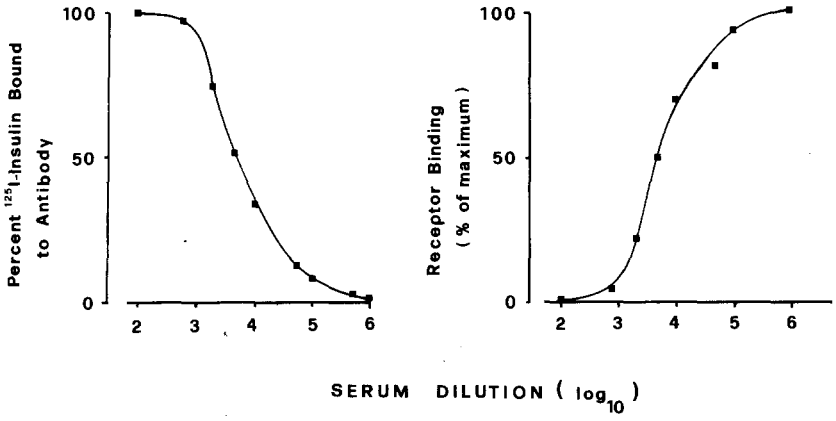

Fig. 1. Effect of insulin antibody dilution on percent labelled insulin bound to antibody (left) and on subsequent binding of labelled insulin (0.2 ng/ml) on 4×10^6 monocytes/ml (right). Insulin antibodies were from a rabbit immunized against porcine insulin; sera from two other rabbits gave similar results. Maximal receptor binding (i. e.; 100%) was evaluated using labelled insulin pre-incubated for 48 h at 4 °C in Hepes buffer and was 2.4% of total radioactivity

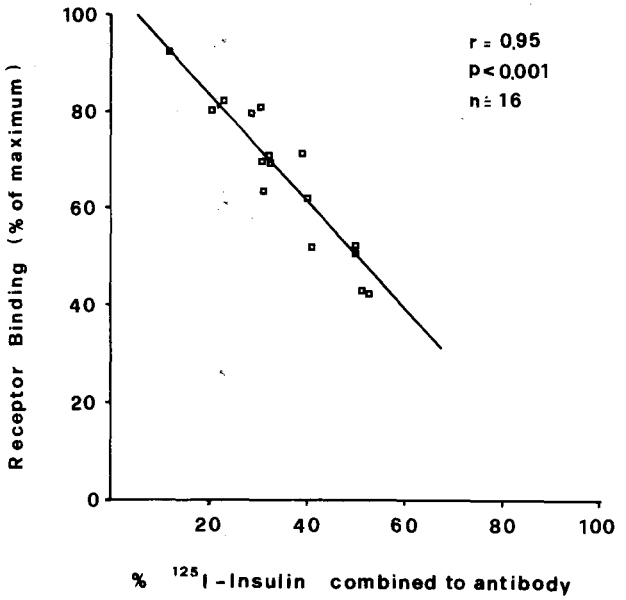

Fig. 2. Relationship between percent labelled insulin combined to antibody and subsequent binding of labelled insulin (0.2 ng/ml) to placenta membranes (150 µg/ml). Maximal receptor binding (i. e. 100%) was evaluated using labelled insulin pre-incubated for 48 h at 4 °C in sodium phosphate buffer and was 31.2% of total radioactivity. Values were obtained using sera from eight diabetic patients with insulin antibodies, at two different dilutions. (five-, twenty- or fifty-fold)

results refer only to the latter cells. In erythrocyte preparations white cells were undetectable.

Normal human placenta collected immediately after delivery, trimmed of amnion and chorion, was washed in 0.25 mol/l cold sucrose and homogenized in the same solution (1:3; w:v) with an Ultra-Turrax (three cycles, 30 s). Membranes were isolated by differential centrifugation according to Cuatrecasas [23] and suspended in 0.05 mol/l sodium phosphate buffer containing 0.5 g/l BSA (pH 7.6) [24]. 1 g of tissue yielded 1 mg of membranes.

Pre-incubation of Labelled Insulin with Serum

Labelled insulin (2 ng/ml) was exposed to rabbit and human sera and to the same buffer used in binding studies (i. e. Hepes for cells and sodium phosphate for membranes) for 48 h at 4 °C. Preincubation produced steady-state equilibrium in 15–36 h depending upon the antibody and the dilution used.

Following incubation 0.1 ml were employed in receptor binding studies and 0.5 ml to evalute the amount of insulin bound to antibody; the percentage bound was determined by 1% dextran coated charcoal precipitation [17].

Binding Studies

Labelled insulin (0.2 ng/ml) previously exposed to serum or buffer (48 h at 4 °C) was incubated with monocytes (4×10^6/ml, 100 min at 15 °C), erythrocytes (3×10^9/ml, 210 min at 15 °C) or placental membranes (150 µg/ml, 60 min at 24 °C) in a final volume of 0.5 ml. Following incubation bound and free insulin were separated by centrifugation [18, 19, 24]. Non specific binding, defined as the amount of radioactivity "bound" in the presence of 7 µmol/l of native insulin was subtracted and found to represent 0.7–1% of total radioactivity [18, 19, 24]. Competition-inhibition curves were performed incubating cells or membranes with labelled insulin and varying amounts of native insulin. Insulin degradation was measured by 10% (v/v) trichloracetic acid precipitation. Labelled insulin incubated in buffer was employed to assay the insulin specifically bound to receptor and the value obtained was taken as maximal binding (i. e. 100%). The effect on insulin binding of preincubation with serum is expressed as the percentage of this value.

Statistical Analysis

Linear regression analysis was used to evaluate the relationship between the percentage of insulin bound to antibody and that bound to receptor.

Results

A preliminary series of experiments, using three different rabbit sera, was carried out to evaluate whether insulin bound to antibody reacts with its receptors.

Receptor binding was absent when insulin was totally combined with antibody and progressively increased as the percentage of free ^{125}I-insulin rose (Fig. 1).

A subsequent series of experiments was performed using sera from diabetic patients with insulin

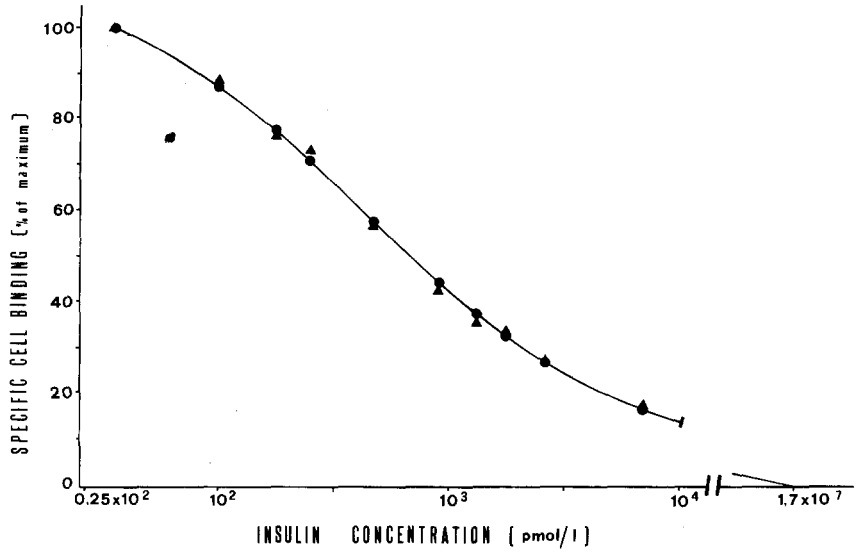

Fig. 3. Competition-inhibition effect of native insulin on labelled insulin (0.2 ng/ml) binding on 3×10^9 erythrocytes/ml. Labelled insulin was pre-incubated with Hepes buffer (●——●) or human insulin antibodies (▲——▲) for 48 h at 4 °C; specific cell binding is the percentage of labelled insulin bound at any given concentration of total insulin (B/T) minus the percentage bound at the concentration of 7 μmol/l (nonspecific binding)

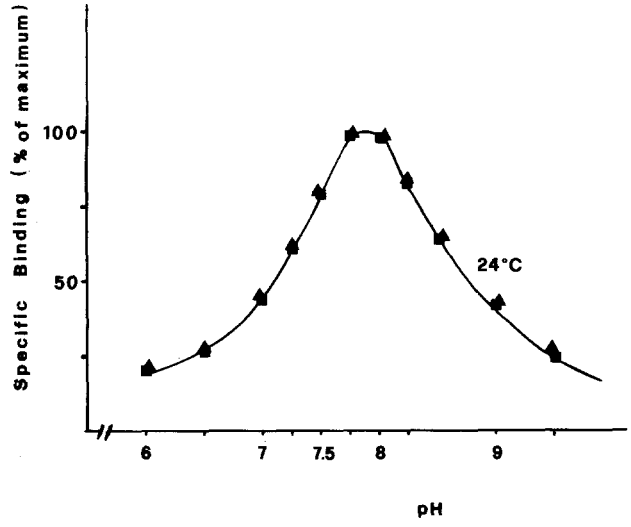

Fig. 4. pH effect on labelled insulin (0.2 ng/ml) binding (cpm × 10^{-2}) on 200 μl of placenta membranes (150 μg/ml). Labelled insulin was pre-incubated with sodium phosphate buffer (●——●) or human insulin antibodies (▲——▲) for 48 h at 4 °C. Nonspecific binding, radioactivity bound in the presence of 7 μmol/l of native insulin, was substracted

antibodies, diluted 5–50 fold. These sera bound a percentage of labelled insulin thus inducing a reduction in binding to receptor. A close negative relationship was demonstrated (r = 0.95) between the percentage of insulin bound to antibody and bound to receptors (Fig. 2).

Using sera treated with neutral charcoal, instead of acid charcoal, the percentage of insulin bound to antibody was slightly lower. Conversely a more marked decrease in receptor binding was observed probably due to a native insulin displaced from antibody following pre-incubation with labelled insulin.

Insulin degradation, measured at the end of each incubation with receptors (monocytes, erythrocytes, placenta membranes) was 3–5%.

Using sera from diabetic patients on oral therapy (whose fasting insulin levels ranged from 18–12 μU/ml) no insulin was bound to serum components and pre-incubation failed to induce any variation in insulin binding on receptors.

The influence of the insulin antibody complex on the insulin receptor interaction was evaluated. Labelled insulin was exposed to rabbit or human sera to obtain combination with antibody from 25 to 70%. This was used to examine pH and competition-inhibition curves and temperature studies. Competition-inhibition (Fig. 3), pH (Fig. 4) and temperature dependence curves of insulin binding (Fig. 5) were not influenced by the presence of the insulin antibody complex in the medium. All experiments were performed either with monocytes, erythrocytes or placenta membranes and results from the different tissues were identical.

Discussion

The data presented clearly demonstrate that insulin combined with antibody does not react with insulin receptors and that the insulin antibody complex does not influence the insulin receptor. The proportion of insulin combined with antibody correlated closely with the percentage of insulin receptor binding; furthermore the presence of the insulin antibody complex in the incubation medium did not influence receptor affinity or the pH and temperature characteristics of the reaction. It is noteworthy that the decrease in binding was not due to insulin degrada-

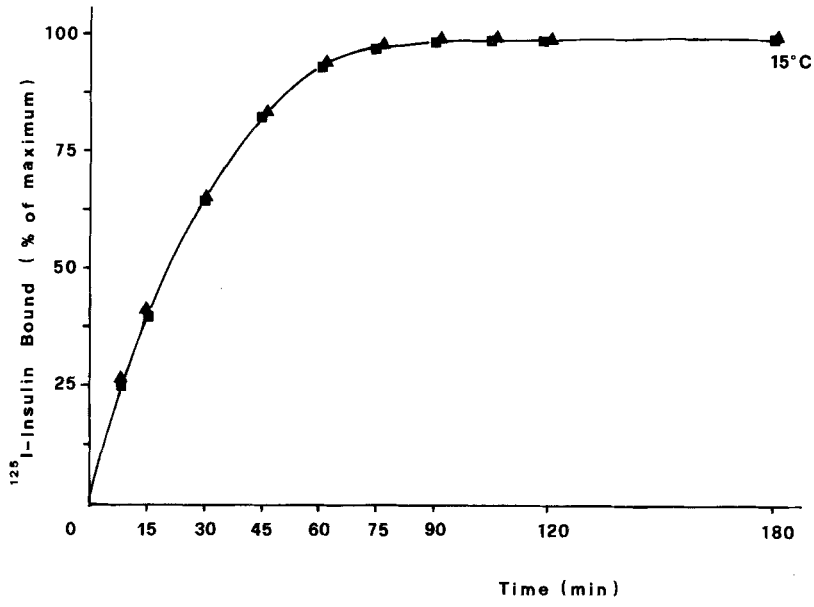

Fig. 5. Time and temperature dependence of labelled insulin (0.2 ng/ml) binding on 8×10^6 monocytes/ml. Labelled insulin was pre-incubated with Hepes buffer (●——●) or human antibodies (▲——▲) for 48 h at 4 °C. Nonspecific binding (radioactivity bound in the presence of 7 μmol/l of native insulin) was subtracted. The same experiment performed at 24 °C did not show any difference in time-course presented by labelled insulin pre-incubated in buffer or with insulin antibodies

tion, since this was very low and that the amount of labelled insulin used was within the physiologic range (~ 5 μU/ml).

The phenomenon occurred with rabbit and human immunoglobulins but not with sera from patients without insulin antibodies, thus confirming that it does not depend upon the source of antibodies or upon unknown components of diabetic sera. Furthermore, the phenomenon occurred using sera treated with neutral charcoal making it unlikely that alterations in serum components produced by acid treatment are responsible.

All experiments were performed at sub-physiological temperatures (15 °C for monocytes and erythrocytes, and 24 °C for placenta membranes) as insulin is known to be rapidly degraded at 37 °C and we assume that the influence of insulin antibodies on insulin-receptor binding also occurs at physiological temperatures.

Recently Kahn et al. [25] demonstrated that highly diluted anti-insulin antibodies added after binding of insulin to its receptors enhanced insulin stimulated adipocyte glucose oxidation.

In view of the different experimental conditions it is not possible to make a direct comparison between our results and those of Kahn. These authors did however observe an effect of added insulin antibodies to decrease insulin action possibly analogous to the reduction in insulin binding noted in our experiments.

Monocytes, erythrocytes and placenta membranes were employed since these are the only human tissues with insulin receptors which may be obtained without biopsy. The fact that results were very similar in all tissues examined suggests that the effects observed are applicable to other target tissues.

In diabetic patients insulin antibodies are induced only after exogenous insulin injections [26] although occasional patients have insulin antibodies, without having had previous insulin injections [27, 28]. The present data indicate that insulin antibodies may theoretically contribute to the insulin resistance shown by some patients thus confirming previous hypotheses [15, 16]. Kahn's results, however suggest that anti-insulin antibodies might also potentially enhance insulin action [25]. Further physiological experiments are necessary to resolve this question.

Authors' note: Since submitting this paper Shechter et al. [29] have shown that anti-insulin antibodies enhance insulin binding on liver membranes and fibroblasts but not on adipocytes, thus suggesting that tissue specific phenomena may occur in the presence of antibodies.

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Roberto de Pirro, M. D.
2ª Clinica Medica
Policlinico Umberto I°
Viale del Policlinico
I-00161 Roma
Italy