Rapid Regulation of Plasma Membrane Insulin Receptors

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Summary. Injection IP of insulin at a dosage of 1 µg/g body weight into normal rats produced a rapid rise in serum insulin levels from < 1 to 298 ng/ml, and a rapid decrease in specific ¹²⁵I-insulin binding to its receptors in purified liver plasma membranes. A fall in binding was seen as early as 10 minutes after injection and binding remained decreased for up to 60 min. At 10 min, ¹²⁵I-insulin binding had fallen to 59% of controls; in contrast, ¹²⁵I-glucagon binding remained unchanged. Extraction of these plasma membranes followed by radioimmunoassay for insulin did not reveal appreciable amounts of exogenous insulin. The ¹²⁵I-insulin dissociation rate from plasma membranes of control and insulin treated rats was the same, also indicating a lack of exogenous insulin. Scatchard analyses indicated that the decreased binding seen after insulin injection was due primarily to a change in the number of insulin receptors and not their affinity. These studies suggest, therefore, that high doses of insulin in vivo can rapidly regulate the number of plasma membrane insulin receptors in liver.

Key words: Insulin, receptor, liver, diabetic, glucagon, plasma membranes.

Studies in vivo and in vitro demonstrated that, in most tissues, the insulin receptor on the plasma membrane is regulated by insulin itself [1–3]. When extracellular insulin levels are increased, hormone binding to receptors decreases; conversely, when insulin levels are decreased, binding to receptors increases [1–4]. Previously, we reported that 24 hours after the injection of insulin into normal rats, the number of insulin receptors present on liver plasma

membranes is decreased [5]. This finding suggested that insulin has a chronic effect on insulin receptors in vivo. Whether insulin in vivo has more rapid effects on receptor binding is unknown. Studies with lymphocytes in vitro have indicated that 30 min after incubation with unlabelled insulin, ¹²⁵I-insulin binding to receptors is decreased [6]. We have investigated, therefore, whether similar acute regulation of insulin receptors also occurs in vivo.

Materials and Methods

Pork insulin (25.6 U/mg) was purchased from Elanco Products Co., Indianapolis, IN, and glucagon from Sigma Chemical Co., St. Louis, MO. ¹²⁵I-insulin (80–120 µCi/µg) and ¹²⁵I-glucagon (80–100 µCi/µg) were prepared by stoichiometric chloramine T methods [7, 8]. The binding buffer employed in these studies contained 118 mmol/l NaCl, 5 mmol/l KCl, 1.2 mmol/l MgSO₄, 1.2 mmol/l KH₂PO₄, 10 mmol/l Na₂HPO₄, and 5 mg/ml bovine serum albumin, pH 7.5.

Female Sprague Dawley rats, 130–180 g, were allowed free access to food and water. They were injected IP with either 1 µg/g body weight of regular pork insulin (Eli Lilly Co., Indianapolis, IN) or 0.154 mol/l saline, and killed by decapitation after 0, 10, 30 and 60 min. Liver plasma membranes were prepared by the method of Ray [9] and stored at –70 °C. Binding studies were performed with 100 µg/ml of plasma membrane protein and 0.3 ng/ml of labelled ¹²⁵I-insulin in the presence or absence of unlabelled hormone at 23 °C. For time course and Scatchard analysis studies, 1 to 5 ml of incubation mixture was incubated in 17 × 100 Falcon plastic tubes. At various times, 300 µl aliquots were removed, placed into Beckman microfuge tubes and centrifuged at 23 °C for 1 min at 10,000 × g. The supernatants were removed by aspiration, the pellet washed with 300 µl of buffer at 4 °C, and the pellet containing bound hormone counted. Nonspecific binding (in the presence of 100 µg of unlabelled hormone) typically was 2–5% of total binding [5] and was subtracted from total binding to yield specific binding. Specific ¹²⁵I-glucagon binding was performed in the same manner [5].

For dissociation studies of ¹²⁵I-insulin, 1.0 mg/ml of plasma membrane protein was incubated with 0.6 ng/ml ¹²⁵I-insulin for 1 hour in a volume of 50 µl. The mixture was then diluted to 5 ml in

Fig. 1. The effect of insulin on specific ^{125}I -insulin binding to receptors on liver plasma membranes (top), serum insulin levels (middle), and serum glucose levels (bottom). Each value is the mean $\pm$ SEM

Fig. 3. Scatchard plots [32] of specific ^{125}I -insulin binding to liver plasma membranes. Time of incubation was 120 min. Each value is the mean of triplicate determinations

Fig. 2. Time course of specific ^{125}I -insulin binding to liver plasma membranes. Each value is the mean $\pm$ SD of triplicate determinations

Fig. 4. Dissociation of ^{125}I -insulin from plasma membranes of control rats and rats 10 min after insulin injection. Each value is the mean of duplicate determinations

insulin-free buffer and dissociation followed by removing 300 μl aliquots and processing the bound hormone as described above.

Insulin was measured by a double antibody radioimmunoassay [10] employing guinea pig anti-insulin antibody purchased from Dr. Peter Wright, Indianapolis, IN. With a tracer of 10 pg/ml and a

first antibody titre of 1:1,000,000, the assay was routinely capable of detecting 10 pg/ml of unlabelled hormone.

Insulin was extracted as previously described from 1 mg of fresh liver plasma membranes [5] by incubation with 1.0 ml of 1.0 mol/l acetic acid for 30 min at 23°C. The supernatant was

Table 1. Studies on liver plasma membranes 10 min after insulin injection

	5'Nucleotidase [3] ($\mu\text{mol}/\text{mg}/\text{min}$)	^{125}I -glucagon binding [3] (bound/free)	Insulin extracted [5] (ng/0.1 mg protein)
Control	1.3 ± 0.1	13.8 ± 1.0	0.014 ± 0.005
Insulin	1.6 ± 0.3	14.8 ± 5.0	0.078 ± 0.050

Values are mean $\pm$ SD, values in parentheses are the number of studies performed

removed and the plasma membranes again extracted by the same procedure. The two supernatants were then combined, lyophilized, and measured by radioimmunoassay. This procedure extracts 85–100% of the insulin as intact hormone [5]. ^{125}I -insulin degradation was measured by precipitation in 10% (w/v) trichloroacetic acid [11]. 5'Nucleotidase [12], protein [13], and glucose [14] were determined by published techniques.

Results

Insulin and Glucose Values

Injection of rats IP with $1 \mu\text{g}/\text{g}$ insulin produced a marked increase in serum insulin levels (Fig. 1) from a pretreatment value of $0.86 \pm 0.08 \text{ ng}/\text{ml}$ to a peak of $298 \pm 48 \text{ ng}/\text{ml}$ (mean $\pm$ SEM) 10 min after injection. Sixty min after injection insulin levels were still elevated at $153 \pm 68 \text{ ng}/\text{ml}$. At the same time blood glucose values had fallen from a mean value of $128 \pm 4 \text{ mg}/\text{dl}$ (Fig. 1) to $64 \pm 5 \text{ mg}/\text{dl}$ after 10 min, with a further fall to $32 \pm 4 \text{ mg}/\text{dl}$ after 60 min.

Insulin Binding to Receptors

When purified liver plasma membranes were prepared 10 min after the injection of insulin into rats, ^{125}I -insulin binding to receptors at steady state had fallen to $59 \pm 6\%$ (mean $\pm$ SEM, $n = 4$) of controls (Figs. 1–3); 60 min after injection binding was at $81 \pm 8\%$ of controls. This decreased binding was seen throughout the time course of ^{125}I -insulin binding (Fig. 2). In contrast, when animals were injected with insulin and studied immediately, insulin binding was the same as that measured in controls injected with $0.154 \text{ mol}/\text{l}$ saline (Figs. 1–3).

Scatchard plots (Fig. 3) were constructed to determine whether the decreased insulin binding was due to a change in either binding affinity or binding capacity. The plots suggested that the major effect of insulin injection was to decrease the insulin binding capacity from approximately $6\text{--}7 \text{ ng}/100 \mu\text{g}$ protein to approximately $3 \text{ ng}/100 \mu\text{g}$ protein.

De Meyts has demonstrated that occupancy of insulin receptors by insulin leads to an enhanced rate of hormone dissociation (i. e., negative cooperativity) [15]. Accordingly, to rule out occupation of receptors by exogenous insulin, we bound ^{125}I -insulin to liver plasma membranes obtained from both control rats and rats treated with insulin, and then measured hormone dissociation. No difference in the dissociation of ^{125}I -insulin was noted (Fig. 4).

Other Studies

Degradation of ^{125}I -insulin was measured by its precipitability in 10% trichloroacetic acid 120 min after incubation with plasma membranes. Control plasma membranes degraded $28.1 \pm 5.4\%$ ($n = 4$) of the labelled hormone. In the plasma membranes from insulin-treated animals there was no significant change in insulin degradation; membranes obtained 10 min after insulin injection degraded $23.7 \pm 6.4\%$ of the label and those obtained 30 min after injection degraded $22.9 \pm 3.3\%$ of the label.

Plasma membranes from both control and insulin-treated rats were treated with acetic acid and the extracted insulin measured by radioimmunoassay. Control membranes contained $0.014 \pm 0.005 \text{ ng}$ insulin/ $100 \mu\text{g}$ of protein; membranes obtained from animals 10 min after injection contained only $0.078 \pm 0.050 \text{ ng}$ insulin/ $100 \mu\text{g}$ of protein (Table 1). This amount of insulin was approximately 10–20 fold less than the amount of insulin (i. e., $0.5\text{--}1.0 \text{ ng}/100 \mu\text{g}$ protein) needed to cause the observed decrease in the bound/free ratio (Fig. 3).

5'Nucleotidase activity and specific ^{125}I -glucagon binding were measured on both control plasma membranes and plasma membranes obtained 10 min after insulin injection. There was no significant change in either enzyme activity or glucagon binding (Table 1).

Discussion

The present studies demonstrate that after the injection of large amounts of insulin IP into normal rats there is a very rapid decrease in insulin binding to its receptors on purified liver plasma membranes. Four lines of evidence indicate that this decrease in binding was not due to either the occupation of receptors by exogenous insulin or changes in the purity of these membranes. First, extraction of plasma membranes revealed that insufficient amounts of insulin were present to account for the lowered binding. Second, Scatchard analyses revealed that the decreased binding was due to a change in binding capacity rather

than a change of binding affinity. If the decreased binding was due to contamination, there should have been a change in affinity rather than capacity [6]. Third, dissociation studies provided no evidence of prior occupancy of insulin receptors by injected insulin. Fourth, while insulin binding was decreased, glucagon binding and 5'nucleotidase activity remained unchanged.

These *in vivo* studies are counterparts to prior *in vitro* studies which demonstrate that high levels of extracellular insulin can rapidly lower insulin receptor binding [6]. These concentrations of insulin would not normally be encountered *in vivo*; however, the levels obtained are comparable to those levels of insulin produced in humans by the intravenous injection of high doses of insulin for the treatment of diabetic ketoacidosis [16]. This observation raises the possibility, therefore, that high dose insulin therapy for diabetic ketoacidosis may regulate insulin receptors. In contrast, with low dose insulin therapy for diabetic ketoacidosis, insulin levels remain in the physiologic range [17] and receptors may not be effected.

The mechanism whereby insulin regulates its receptors on the plasma membrane is at present unknown. Studies *in vitro* suggest that there may be several mechanisms involved in this process. With lymphocytes, hepatocytes, and adipocytes in culture, lower concentrations of insulin (10^{-8} mol/l or less) decrease insulin binding but the effect may take 24 hours or longer [6, 18, 19]. This delayed regulation may be mediated through new protein synthesis [20]. In contrast to this delayed regulation, with lymphocytes and adipocytes, high concentrations of insulin ($> 10^{-7}$ mol/l) rapidly decreases insulin binding within 1 hour [6, 18].

Studies from our laboratory [12, 21–23] and others [24–28] have indicated that insulin enters several target cells including hepatocytes and that this entry is a receptor-mediated process. Studies of other systems in which ligand-surface receptor interactions are followed by internalisation of the ligand, such as the uptake of low density lipoproteins [29], toxins [30] and even hormones [23, 25, 27], have revealed that these receptors also enter cells with the resultant depletion of surface receptors. It is also possible, therefore, that the receptor-mediated entry of insulin into hepatocytes leads in turn to the subsequent rapid depletion of insulin's plasma membrane receptors.

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