Insulin Receptors on Erythrocytes and Hepatocytes from Streptozotocin Induced Diabetic Rats

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Abstract

Insulin receptors on hepatocytes and erythrocytes were studied in rats two and eight weeks after the injection of streptozotocin (50 mg/kg) to see if erythrocyte insulin receptors change parallel with hepatocyte insulin receptors in response to hypoinsulinemia.

Insulin binding to hepatocytes increased two (14.0±2.5% v.s. 7.7±0.7%; P<0.025) and eight weeks (15.9±1.9% v.s. 6.6±1.1%; P<0.005) after the streptozotocin injection. Scatchard analysis revealed that this increase was due to a rise in both the receptor concentration and affinity. The number of receptors was comparable in the two- and eight-week-streptozotocin rats while the increase in the affinity was more pronounced in the latter group. Insulin binding to the erythrocytes was also increased in both two- (5.0±0.7% v.s. 4.2±0.6%) and eight-week- (4.3±0.6% v.s. 2.7±1.2%) streptozotocin rats. This increase was due to a rise in the receptor concentration rather than the affinity. However, compared to hepatocytes, these changes were inconsistent and statistically not significant. Furthermore, no correlation was obtained between the binding and plasma insulin concentration.

These results indicate that insulin receptors on rat erythrocytes are less sensitive to a change in the plasma insulin concentration and do not always reflect accurately the receptor state on hepatocytes.

Recently Gambhir et al. (1978) have demonstrated that circulating erythrocytes, which are not target cells for insulin action, have specific insulin receptors. If erythrocyte insulin receptors behave similarly to those on insulin target cells under various conditions, they may provide a convenient tool for evaluating the insulin receptor state in human subjects as well as experimental animals. The present experiments were therefore designed to study the relationship between insulin receptors on erythrocytes and insulin responsive cells. A previous study has shown that circulating insulin concentrations inversely regulate insulin receptors on insulin responsive cells (Soman and DeFronzo, 1980). Thus a hyperinsulinemic state decreases while hypoinsulinemic state increases insulin receptor concentrations. In this study we measured insulin receptors on both hepatocytes and erythrocytes simultaneously in streptozotocin diabetic rats to see if they change in a parallel way.

Materials and Methods

Animals
Male Wistar rats weighing 160-200 g were used for all experiments. Hypoinsulinemic diabetic rats
were produced by injecting 50 mg/kg of streptozotocin dissolved in citrate buffer (pH 4.5) through the tail vein of rats that had been fasted overnight. They were used for the experiments two or eight weeks after the injection. All animals were fed ad libitum until the experiments.

**Hepatocytes and Erythrocytes**

Blood was collected from the inferior vena cava (IVC) and portal vein for determination of blood glucose, plasma insulin and insulin receptors on erythrocytes. Hepatocytes were isolated using a modification of the method of Berry and Friend (Berry and Friend, 1969). The cell pellets were washed three times in ice-cold Krebs-Ringer-bicarbonate buffer containing 0.25% bovine serum albumin and resuspended in the minimal essential medium, pH 7.4, containing 1 mM pyruvate, 1 mM glutamine, 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 10 mM N-tris (hydroxymethyl) methylglycine, 1% bovine serum albumin, and 0.5% glucose. The hepatocytes were then preincubated for 30 minutes at 30°C under the gas phase of 95% O₂-5% CO₂. More than 90% of the hepatocytes were found intact as judged from their ability to exclude trypan blue. The erythrocytes were prepared with a modification of the method of Gambhir et al. (Gambhir et al., 1978). Buffer used for the incubation of erythrocytes was 50 mM Hepes-Tris buffer, pH 8.0, containing 10 mM MgCl₂, 2 mM EDTA, 10 mM dextrose, 2 mM CaCl₂, 50 mM NaCl, 5 mM KCl, and 1% bovine serum albumin.

**Binding study**

The hepatocytes were suspended to give a final concentration of 0.5×10⁹/ml in the minimal essential medium, mixed with ¹²⁵I-insulin and unlabeled porcine insulin over a range of concentrations from 0.1 to 1000 ng/ml and incubated for 150 minutes at 15°C in a final volume of 0.5 ml. After the incubation, duplicate 200 µl aliquots of the incubation mixture were transferred into chilled plastic microfuge tubes containing 100 µl dibutyl phthalate and 100 µl assay buffer. The microfuge tubes were then centrifuged for 150 seconds in a Beckman Microfuge and radioactivity in the cell pellets was determined. Nonspecific binding defined as radioactivity in the cell pellet in the presence of 2×10⁵ ng/ml of unlabeled insulin was approximately 0.4% of the total radioactivity. Degradation of ¹²⁵I-insulin during the binding assay determined with the TCA precipitation method was less than 13% of total radioactivity. The erythrocytes were suspended to give a final concentration of 5.0×10⁹/ml in the buffer, mixed with ¹²⁵I-insulin and unlabeled porcine insulin over a range of concentration from 0.2 to 1000 ng/ml and incubated for 150 minutes at 15°C in a final volume of 0.5 ml. After the incubation, duplicate 200 µl aliquots of the incubation mixture were transferred into chilled plastic microfuge tubes containing 100 µl dibutyl phthalate and 100 µl assay buffer. The microfuge tubes were then centrifuged for 150 seconds in a Beckman Microfuge and radioactivity in the cell pellets was determined. Nonspecific binding was approximately 1% of the total radioactivity. Degradation of ¹²⁵I-insulin was less than 5%. The receptor number and the “empty site” affinity constant (Ke) were calculated from Scatchard plots by the method of DeMeyts (DeMeyts et al., 1975).

Blood glucose was measured with an Autoanalyzer. The serum immunoreactive insulin concentration was determined by the polyethylene glycol method (Desbuquois and Aurbach, 1971) using rat insulin as a standard. Results were expressed as mean±SEM. Statistical analysis was performed by Student’s t-test.

**Results**

Body weight, blood glucose and plasma insulin levels in the control and streptozotocin-treated rats are shown in Table 1.

| Table 1. Characteristics of Experimental Animals |
|-----------------|-----------------|-----------------|
| **2 Weeks** | **STZ Rats** | **Control Rats** |
| **Body Weight** | 231±12 | 176±13 ** |
| **Blood Glucose (mg/dl)** | 107±9 | 274±44 ** |
| **Plasma Insulin (ng/ml)** | 8.0±1.5 | 1.2±0.3 ** |
| **Portal Vein IVC** | 3.1±0.8 | 0.5±0.1 ** |
| **8 Weeks** | | |
| **Body Weight** | 400±30 | 203±18 ** |
| **Blood Glucose (mg/dl)** | 131±6 | 330±9 ** |
| **Plasma Insulin (ng/ml)** | 7.7±1.1 | 0.6±0.1 ** |
| **Portal Vein IVC** | 3.4±0.4 | 0.3±0.1 ** |

*Values are given as Means±S. E. M.*

** P<0.01

*** IVC stands for inferior vena cava.
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Fig. 1-a. Specific $^{125}$I-insulin binding (%) to hepatocytes from streptozotocin treated and control rats. Rats were used 2 weeks after streptozotocin (50 mg/kg) injection. (**P<0.025, *P<0.05)

Fig. 1-b. Scatchard plots of the insulin binding to hepatocytes from streptozotocin treated (2 weeks) and control rats. The data were derived from Fig. 1-a. Receptor numbers and affinity constants are shown in Table 2.

Fig. 2-a. Specific $^{125}$I-insulin binding (%) to erythrocytes from streptozotocin treated (2 weeks) and control rats.

Fig. 2-b. Scatchard plots of the binding data from streptozotocin treated (2 weeks) and control rats. The data were derived from Fig. 2-a. Receptor numbers and affinity constants are shown in Table 2.

Streptozotocin treated rats are summarised in Table 1. The blood glucose levels were markedly increased while the insulin concentrations were decreased in both two- and eight-week-streptozotocin rats. The weight gain was attenuated in the diabetic rats. As shown in Figure 1-a, the binding of insulin to hepatocytes from 2-week-streptozotocin rats was significantly higher than that of controls at all insulin concentrations. The specific binding at the tracer concentration of insulin (0.1 ng/ml) was 14.0±2.5% in the streptozotocin rats and 7.7±0.7% in the controls. The receptor concentration and affinity constant obtained from the

Scatchard plot (Scatchard, 1949) (Fig. 1-b) were $60 \times 10^4$ per cell and $3.3 \times 10^8 M^{-1}$ respectively, in the streptozotocin-diabetic rat hepatocytes and $38 \times 10^4$ per cell and $2.6 \times 10^8 M^{-1}$ in controls (Table 2). Thus the increase in the binding found in the 2-week-streptozotocin rat hepatocytes was accounted for by both the increased receptor concentration and affinity. On the other hand, erythrocytes from the 2-week-streptozotocin rats bound more insulin than the controls at each concentration of insulin, but the differences were small and statistically not significant (Fig. 2-a). The binding to erythrocytes at the tracer concent-
Table 2. Receptor Numbers and Affinity Constants.

<table>
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<tr>
<th></th>
<th>Control Rats</th>
<th>STZ Rats</th>
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<tr>
<td><strong>Hepatocytes</strong></td>
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<tr>
<td>Receptor Number (×10⁴/cell)</td>
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<tr>
<td>Ke (×10⁸ M⁻¹)</td>
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<td><strong>Erythrocytes</strong></td>
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<tr>
<td>Ke (×10⁸ M⁻¹)</td>
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**8 Weeks**

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<th>STZ Rats</th>
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<tr>
<td>Receptor Number (×10⁴/cell)</td>
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<td>Ke (×10⁸ M⁻¹)</td>
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<tr>
<td>Ke (×10⁸ M⁻¹)</td>
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Fig. 3-a. Specific ¹²⁵I-insulin binding (%) to hepatocytes from streptozotocin treated (8 weeks) and control rats. (***P<0.01, **P<0.025, *P<0.05)

Fig. 3-b. Scatchard plots of the binding data from hepatocytes from streptozotocin treated (8 weeks) and control rats. The data are derived from Fig. 3-a. Receptor numbers and affinity constants are shown in Table 2.

Fig. 4-a. Specific ¹²⁵I-insulin binding (%) to erythrocytes from streptozotocin treated (8 weeks) and control rats.

Fig. 4-b. Scatchard plots of the binding data from streptozotocin treated (8 weeks) and control rats. The data are derived from Fig. 4-a. Receptor numbers and affinity constants are shown in Table 2.
ration of insulin (0.2 ng/ml) was 5.0 ± 0.7% in the streptozotocin diabetic rats and 4.2 ± 0.6% in the controls. Scatchard plot (Fig. 2-b) revealed that the receptor concentration and affinity were 26 per cell and $3.9 \times 10^8 M^{-1}$ respectively, in the streptozotocin-rat erythrocytes and 19 per cell and $4.2 \times 10^8 M^{-1}$ in controls (Table 2).

Since changes in the receptor on erythrocytes were less obvious than those of hepatocytes in rats 2 weeks after the streptozotocin injection, we studied the effects of a more prolonged (8 weeks) hypoinsulinemic state on the receptors. Like the two-week-streptozotocin group, hepatocytes from rats 8 weeks after the streptozotocin administration bound more insulin than controls at all insulin concentrations (Fig. 3-a). The specific binding to hepatocytes at the tracer concentration of insulin was $15.9 \pm 1.9\%$ in the diabetic rats and $6.6 \pm 1.1\%$ in controls. As demonstrated in Fig. 3-b, an initial slope of the Scatchard plot was increased in this group and both the receptor concentration ($60 \times 10^4$ v.s. $40 \times 10^4$ per cell) and affinity ($3.8 \times 10^8 M^{-1}$ v.s. $2.1 \times 10^8 M^{-1}$) were markedly increased compared to those in controls (Table 2). The insulin binding to erythrocytes was higher in the 8-week-streptozotocin rats than controls (Fig. 4-a). However, the difference was again not significant at each concentration of insulin. Scatchard analysis revealed a tendency to increased receptor concentration (14 v.s. 8 per cell) in the streptozotocin rat erythrocytes (Fig. 4-b) (Table 2). Unlike the hepatocytes, the binding affinity was similar in the streptozotocin and control rat erythrocytes (Table 2).

Insulin binding to hepatocytes and erythrocytes at the tracer concentration of insulin was plotted against plasma insulin levels in each rat. An inverse correlation was found between the binding to hepatocytes and the insulin concentration in the portal vein (Fig. 5-a). In contrast, there was no significant correlation between the binding to erythrocytes and the peripheral insulin concentration (Fig. 5-b).

![Fig. 5-a. Correlation between specific $^{125}$I-insulin binding (%) to hepatocytes and serum insulin concentrations in the portal vein. All animals were fed ad libitum. (○ 2-week-control rats; △ 8-week-control rats; ● 2-week-streptozotocin rats; ▲ 8-week-streptozotocin rats).](image)

![Fig. 5-b. Correlation between specific $^{125}$I-insulin binding (%) to erythrocytes and serum insulin concentrations in the inferior vena cava. All animals were fed ad libitum. (○ 2-week-control rats; △ 8-week-control rats; ● 2-week-streptozotocin rats; ▲ 8-week-streptozotocin rats).](image)
Discussion

A marked hypoinsulinemic and hyperglycemic state was produced in rats 2 weeks after 50 mg/kg streptozotocin injection. The metabolic derangement became more pronounced 8 weeks after the injection. Insulin binding to hepatocytes was elevated in the streptozotocin induced diabetic animals. These results confirmed previous observations made on adipocytes (Kobayashi and Olefsky, 1979), and liver plasma membrane (Davidson and Kaplan, 1977; Le Marchand et al. 1976). A negative correlation was found between the binding to hepatocytes and plasma insulin concentrations in the portal vein. Scatchard analysis (Scatchard, 1949) revealed that in both two- and eight-week streptozotocin rats the receptor number per hepatocyte was increased by approximately 1.5 times that of each control. The affinity was also increased in hepatocytes from the streptozotocin treated rats, and the increase was especially pronounced in the eight-week group. Thus, compared to the two-week group the prolonged and severe hypoinsulinemic state resulted in a further increase in affinity with no major changes in the receptor concentration. At present, the reasons for the rise in the affinity remain obscure. However similar findings have been reported by Kobayashi et al. in adipocytes from streptozotocin induced diabetic rat (Kobayashi and Olefsky, 1979). They observed that in mild diabetic rats induced by low dose (40 mg/kg) streptozotocin administration a rise in the receptor concentration was found while in severe diabetes developed by high dose streptozotocin (55 mg/kg) both the receptor concentration and affinity were increased. These observations suggest that severe hypoinsulinemia itself or a catabolic state resulting from hypoinsulinemia causes changes in the binding affinity through a mechanism which remains to be determined.

The present study also demonstrated that insulin binding to erythrocytes was increased in both the two- and eight-week streptozotocin diabetic rats. However this increase was smaller than that of hepatocytes and statistically not significant. Furthermore, in contrast to hepatocytes, no correlation was observed between the binding to erythrocytes and plasma insulin concentrations. The receptor concentration tended to be augmented in erythrocytes from the streptozotocin rat, but changes in affinity were not found. Thus it appears that changes in the receptor on erythrocytes are less clear and do not always accurately reflect those of insulin target cells in rats.

Circulating erythrocytes, which are the most easily obtainable cells, have recently been used in the evaluation of insulin receptor status in human subjects (Robinson et al., 1979). And in order to validate employing erythrocytes, several studies (Wachslicht-Rodbard et al., 1979; Depirro et al., 1980; Insel et al., 1980; Pederson et al., 1980) have compared the erythrocyte binding data with those of monocytes in the same individuals since the latter has been shown to reflect receptor activity in insulin responsive cells (Bar et al., 1976). Wachslicht-Rodbard et al. (1979) found an increased number of receptors on both circulating erythrocytes and monocytes form patients with anorexia nervosa. Changes in the affinity of the receptors were the same in both cell types in response to infusion of glucose and insulin (Insel et al., 1980) as well as exercise (Pederson et al., 1980). Furthermore, we found an inverse correlation between insulin binding to erythrocytes and the plasma insulin concentration in maturity onset types diabetes mellitus (Okamoto et al., 1981) suggesting that insulin receptors on human erythrocytes are also regulated by insulin as demonstrated in insulin responsive cells.
These results therefore seem to indicate that circulating erythrocytes provide a useful tool for studying insulin receptor activity. However, it should be noted that there are also some reports which demonstrate discrepancies between the binding data obtained with erythrocytes and monocytes (Dons et al., 1981; DePirro et al., 1980). Dons et al. (1981) found that insulin binding to both erythrocytes and monocytes from poorly controlled insulin-dependent diabetic patients was normal or elevated, while improved control decreased insulin binding in monocytes but not in erythrocytes. Our present data were obtained with rat erythrocyte and there may be some difference in the regulation in erythrocytes receptors between rat and human. Much more important, however, are the unique features of erythrocytes. For example, unlike insulin receptors on monocytes as well as other type of cells, erythrocyte insulin receptors are age dependent (Thomopoulos et al., 1978; Eng et al., 1980). Erythrocytes lose their receptors as they age. In addition, it has been suggested that regulation of erythrocyte insulin receptor concentrations takes place only when the cells are at a premature stage (Dons et al., 1981). These unique features of erythrocyte insulin receptors probably explain the discrepancies in the receptors on rat hepatocytes and erythrocytes in the present study, and may limit to some extent the usefulness of erythrocytes in evaluating the status of the receptors on insulin target cells.

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