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Decreased Binding of Insulin to Erythrocytes in Myotonic Dystrophy

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SAKAI, K., TAKASE, S., KUMAGAI, K. and SHIMIZU, F. Decreased Binding of Insulin to Erythrocytes in Myotonic Dystrophy. Tohoku J. exp. Med., 1985, 146 (2), 193-199 — Recent several studies have shown that the insulin resistance exists in patients with myotonic dystrophy. Using in vitro radioreceptor assay with 125I-labeled insulin, we evaluated the binding of insulin to erythrocytes in patients with myotonic dystrophy. It was found that there was a decreased binding of insulin to erythrocytes in the patients with myotonic dystrophy. On quantitative analysis, this decrease in binding was found to be the result of a decrease in receptor affinity rather than receptor concentration. There was no evidence that the alteration in insulin receptor affinity was due to hyperinsulinemia. All of 7 patients with myotonic dystrophy also were shown to have an excessive response of endogenous insulin to a glucose challenge. No circulating antibody to insulin was found. These data suggest that a decrease in affinity of insulin receptors may, in part, cause insulin resistance and a compensatory elevation of the plasma insulin concentration in patients with myotonic dystrophy.

— erythrocyte; insulin binding; insulin receptor; insulin resistance; myotonic dystrophy

Myotonic dystrophy is a multisystem disease including abnormalities not only in skeletal muscle (Reddy et al. 1977) but also in plasma membranes of many other cells such as monocytes (Banerjee et al. 1982), erythrocytes (Plishker et al. 1978), platelets (Bousser et al. 1975), and neutrophils (Seay et al. 1978). In recent several studies on myotonic dystrophy, hyperinsulinemia (Barbosa et al. 1974; Kobayashi et al. 1977; Festoff and Moore 1979), glucose intolerance (Barbosa et al. 1974; Festoff and Moore 1979) and insensitiveness to exogenous insulin (Tevaarwerk et al. 1979; Stuart et al. 1983) were reported. These findings are interpreted as a cause of insulin resistance. Since the initial action of insulin is exerted through the receptors for insulin, it seems likely that an abnormality in

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the insulin receptors exists in this disease. In the present experiments, we found that the binding of insulin to erythrocytes was decreased in both male patients with myotonic dystrophy compared with normal males (controls). This decrease in binding was found to be the result of a decrease in receptor affinity rather than receptor concentration.

**Materials and Methods**

**Patients**

Seven male patients and 7 sex- and weight-matched normal volunteers were studied. The diagnosis of myotonic dystrophy was made on the basis of physical findings, electromyography and family history (Table 1). All of them were unrelated each other. After informed consent, subjects were admitted to the Tohoku University Hospital in Sendai.

**Glucose tolerance test**

Patients were given a 75-g oral glucose load. Plasma glucose and serum insulin concentration were determined at the times indicated in Table 2. The normal values for an oral glucose tolerance test (OGTT) and plasma insulin are also given in Table 2. Glucose tolerance was considered to be normal when the fasting plasma glucose concentration was less than 100 mg/100 ml, the 2 hr sample was less than 140 mg/100 ml, and all intermediate samples were less than 160 mg/100 ml.

**Chemicals and buffer**

Crystalline porcine insulin was purchased from Novo Laboratories Inc. (Wilton, CT, U.S.A.). Bovine serum albumin (fraction v) was from Sigma Chemical Co. (St. Louis, MO U.S.A.). 125I-labeled porcine insulin (150-200 Cl/μg) was from Dinabot Co. (Tokyo). The assay buffer for insulin binding to erythrocytes was 50 mM Hepes (pH 8.0) containing 70 mM NaCl, 1.2 mM MgSO4, 2.5 mM NaC2H3O2, 10 mM glucose, 1 mM EDTA, 100 U/ml bacitracin and 1 mg/ml bovine serum albumin. This buffer has a calculated osmolarity of 265.5 mosmol.

**Preparation of cells**

For the preparation of erythrocytes, 10-15 ml of heparinized blood was obtained from each individual following an overnight fast. Blood was overlayed onto Ficoll-Hypaque gradients, and centrifuged at 450 x g for 20 min at 15°C (Bouyn 1968). The plasma lymphocyte layer and upper portion of the erythrocyte layer were then aspirated. The remaining erythrocytes pellet was suspended with an equal volume of phosphate saline buffer and centrifuged through Ficoll-Hypaque gradients. Erythrocytes were then washed twice with assay buffer, and resuspended to a final concentration of 5.25 x 10⁹ erythrocytes/ml in Hepes buffer (pH 8.0).

**Insulin binding assay**

For 125I-labeled insulin binding to erythrocytes, we used the method introduced by Gambhir et al. (1977) with slight modifications as described previously (Shimizu and Kahn 1982). Erythrocytes were mixed with 125I-labeled insulin in the presence or absence of unlabeled insulin over a range of concentrations from 32 pM to 1.8 μM. These mixtures were incubated for 240 min at 15°C in a final volume of 0.5 ml in Hepes buffer with intermittent gentle shaking. After incubation, duplicate 200 μl aliquots of the suspension were layered onto 200 μl of chilled Ficoll-Hypaque solution in 500 μl polyethylene microfuge tube. The tubes were then centrifuged at 10,000 x g for 2.5 min. The supernatant was aspirated leaving approximately 50 μl and the radioactivity in the cell pellet was
counted in a gamma counter. Total binding is the radioactivity in the cell pellet; the nonspecific binding represents the radioactivity in the cell pellet in the presence of 1.8 $\mu$M. Specific binding is the difference between total binding and nonspecific binding. No difference was observed in nonspecific binding between the patients and control subjects.

**Data analysis**

The numbers of binding sites per cell were obtained by Scatchard analysis of $^{125}$I-labeled insulin binding data (Scatchard 1949). The affinity constants were obtained assuming a cooperative model, as described by De Meyts and Roth (1975).

**Table 1. Characterization of the patients with myotonic dystrophy**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>EMG*</th>
<th>Antibody to insulin †</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>M</td>
<td>68.5</td>
<td>165.3</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>M</td>
<td>58.0</td>
<td>157.0</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>M</td>
<td>60.0</td>
<td>167.0</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>M</td>
<td>51.0</td>
<td>157.6</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>M</td>
<td>70.0</td>
<td>169.0</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>M</td>
<td>58.5</td>
<td>155.8</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>M</td>
<td>65.0</td>
<td>165.6</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

* Electromyographic finding in myotonic dystrophy.
† Determined by a radioimmunoassay polyethylenglycol (RAPEG) method using commercial kits (Dainabot radioisotope Lab., Ltd., Tokyo).

**Table 2. Glucose tolerance tests of patients with myotonic dystrophy**

<table>
<thead>
<tr>
<th>Glucose (mg/100 ml)</th>
<th>Insulin (μU/ml)</th>
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<tr>
<td>0 30 60 90 120 180</td>
<td>0 30 60 90 120 180</td>
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</table>

<table>
<thead>
<tr>
<th>Patients</th>
<th>Glucose (mg/100 ml)</th>
<th>Insulin (μU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66 104 119 140 122 100</td>
<td>22 340 190 62 59 N.T.</td>
</tr>
<tr>
<td>2</td>
<td>93 153 116 105 99 N.T.</td>
<td>8.2 150 150 110 71 N.T.</td>
</tr>
<tr>
<td>3</td>
<td>73 132 108 78 90 N.T.</td>
<td>28 500 320 120 110 N.T.</td>
</tr>
<tr>
<td>4</td>
<td>91 147 127 101 88 N.T.</td>
<td>6.1 92 120 87 44 21</td>
</tr>
<tr>
<td>5</td>
<td>73 126 111 92 90 N.T.</td>
<td>7.8 110 230 83 150 32</td>
</tr>
<tr>
<td>6</td>
<td>74 120 138 110 108 N.T.</td>
<td>11 140 32 77 180 49</td>
</tr>
<tr>
<td>7</td>
<td>76 79 89 121 99 61</td>
<td>13 62 51 190 400 26</td>
</tr>
</tbody>
</table>

Controls mean* | Glucose (mg/100 ml) | Insulin (μU/ml) |
<table>
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<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>91 120 96 90 84 N.T.</td>
<td>12 72 48 48 34 N.T.</td>
<td></td>
</tr>
</tbody>
</table>

s.d. | Glucose (mg/100 ml) | Insulin (μU/ml) |
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<tbody>
<tr>
<td>4   16 27 16 13 N.T.</td>
<td>5 34 37 30 29 N.T.</td>
<td></td>
</tr>
</tbody>
</table>

* Fifteen adult controls.
RESULTS

Characterization of the patients with myotonic dystrophy is shown in Table 1. No circulating antibody to insulin was found in these patients. Table 2 shows the results of the glucose tolerance test and plasma insulin response to a 75-g oral glucose load. All of 7 patients had normal fasting glucose concentrations (73.7 ± 9.3 mg/100 ml, mean ± s.d.). These patients also had normal concentrations of blood glucose up to 180 min after glucose administration. These data suggested that all of patients were not glucose intolerant. In evaluation of a fasting insulin level, only one patient (#3) had an excessive insulin concentration, the other had a normal insulin concentration. All of patients, however, had an excessive insulin responses to a 75-g oral glucose load.

In insulin binding assay on erythrocytes significant differences were found between the control curve and the curve obtained by averaging the individual binding curves of the patients (Fig. 1). The mean (± s.d.) amount of $^{125}$I-labeled insulin binding to erythrocytes in the patients was 3.14 ± 0.56% per $4.2 \times 10^9$ erythrocytes/ml. This value was significantly lower ($p < 0.05$) than the 3.77 ± 0.49% per $4.2 \times 10^9$ erythrocytes/ml observed in control subjects. The concentration of unlabeled insulin that produced 50% inhibition of tracer binding for erythrocytes was 6 ng/ml in patients and 10 ng/ml in controls, suggesting that in patients there was a decrease in receptor affinity. This was confirmed when the data were analysed by the method of Scatchard. By Scatchard analysis a curvilinear plot with an upward concavity was obtained (Fig. 2). Based on a

![Fig. 1. Insulin binding to erythrocytes in myotonic dystrophy. Erythrocytes (4.2 x 10^9 cells/ml) and $^{125}$I-labeled insulin were incubated for 240 min at 15°C with increasing concentrations of unlabeled insulin. Each point represents the mean ± one s.d. of binding to erythrocytes. Data are corrected for nonspecific binding. Symbols: ●, patients with myotonic dystrophy; ○, controls.](image-url)
negative cooperative model, the $K_e$ in patients was $4.47 \times 10^8$ M$^{-1}$, and the $K_f$ was $1.13 \times 10^7$ M$^{-1}$ with 95 binding sites per cell. On the other hand, the $K_e$ in control was $7.65 \times 10^8$ M$^{-1}$ and the $K_f$ was $2.62 \times 10^7$ M$^{-1}$ with 68 binding sites per cell.

These data showed that in myotonic dystrophy receptor activity in erythrocytes was impaired and that the specific insulin binding was reduced due to the decrease in affinity of insulin receptors rather than the concentration of insulin receptors.

**DISCUSSION**

When the binding of insulin to erythrocytes was studied in both male patients with myotonic dystrophy and male control subjects, it was found that there was a decreased binding of insulin to erythrocytes in the patients with myotonic dystrophy as compared with control subjects. The decrease in insulin binding to erythrocytes was due to the reduction in the affinity of receptors rather than the concentration of receptors. The alteration in binding did not directly correlate with hyperinsulinemia.

Recently, several studies have shown that an abnormality in glucose homeostasis exists in patients with myotonic dystrophy. In fact, patients with myotonic dystrophy have a decreased sensitivity to infusion of insulin at low doses (Stuart et al. 1983) and have an excessive response of endogenous insulin to glucose challenge (Huff et al. 1963; Kobayashi et al. 1977; Festoff and Moore 1979; Stuart et al. 1983).

There are several pieces of evidence that in the myotonic dystrophy the plasma membrane-associated abnormalities exist in many tissues such as monocytes (Banerjee et al. 1982), erythrocytes (Plishker et al. 1978), platelets...
(Bousser et al. 1975), and neutrophils (Seay et al. 1978). For instance, monocytes in patients with myotonic dystrophy expressed significantly greater number of Fc receptor than did monocytes of healthy subjects (Banerjee et al. 1982). Thus, it seems likely that structural abnormalities of the plasma membrane may cause the alteration in insulin receptors. Indeed, several lines of evidence in monocyte receptor studies suggest that the abnormality in glucose homeostasis in patients with myotonic dystrophy may be due to the altered insulin receptor (Festoff and Moore 1979; Fratino et al. 1982; Stuart et al. 1983), although there is one report that normal insulin binding to monocytes was found (Kobayashi et al. 1977).

In the present experiments, the erythrocytes were employed to characterize the insulin receptor status in myotonic dystrophy from 2 reasons. First, only 20-30 ml of whole blood is enough to study the insulin binding to erythrocytes, while 150-500 ml of blood must be removed to have receptors on monocytes studied in detail. Second, our previous data obtained in mouse model system indicated that insulin binding to leukocyte preparation can be easily altered during viral or bacterial infections, suggesting that other tissues in addition to leukocytes would be characterized to know the generalized status of insulin receptors (Shimizu et al. 1983). As far as we know, our report is the first in which the insulin receptors on erythrocytes in patients with myotonic dystrophy was quantitatively characterized.

In conclusion, a decreased binding of insulin to erythrocytes in myotonic dystrophy observed is a result from the alteration in receptor affinity rather than receptor concentration. The decreased affinity of insulin receptors may, in part, cause insulin resistance and an excessive response of endogenous insulin to a glucose challenge in patients with myotonic dystrophy. These data also support the evidence that patients with myotonic dystrophy have a systemic abnormality in plasma membranes.

References