Up-regulation of Insulin Receptors with Dexamethasone in Cultured Human Urinary Bladder Carcinoma Cells

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Abstract

Cultured human urinary bladder carcinoma cells (JTC-32) were used to investigate the regulation of insulin receptors by dexamethasone. When the cells were pre-incubated with dexamethasone at 37°C, insulin binding sites increased up to 24 h. A large increase in insulin binding sites took place for 14 h of preincubation with dexamethasone. At lower concentrations of dexamethasone (<1 nM), no significant increase in insulin binding sites was observed, but the maximal increase was observed at more than 10 nM. Scatchard plots showed that dexamethasone increased the number of high affinity insulin binding sites (2.8 fold) without any change in the apparent equilibrium constant in JTC-32 cells. In addition, this steroid hormone also increased the number of low affinity insulin binding sites (1.6 fold) with a small change in the apparent equilibrium constant. Although insulin and dexamethasone did not affect the number of cells or the amount of cellular proteins per dish, dexamethasone plus insulin slightly increased them.

We know “down-regulation”, in which the number of insulin receptors decreases with insulin treatment (Gavin et al., 1974; Blackard et al., 1978; Livingston et al., 1978; Petersen et al., 1978), and “up-regulation”, in which the number of insulin receptors increases with glucocorticoid treatment (Fantus et al., 1981; Knutson et al., 1982), to regulate insulin receptors. Recently, Stevens et al. (1983) showed that insulin increased its binding sites in cultured chondrosarcoma chondrocytes. In some experiments, however, glucocorticoids decreased insulin binding sites (Cigolini and Smith, 1972; Kahn et al., 1979; Olefsky et al., 1975). It was reported that fatty acids decreased insulin binding and insulin action (Grunfeld et al., 1981). We do not, however, clearly understand how those phenomena are involved in the physiological functions of insulin, even though other groups have pointed out that down-regulation contributes to the biological functions of insulin in cultured cells (Caro and Amatruda, 1982).

In this paper, we show that dexamethasone increased the number of high affinity insulin binding sites without changing the apparent equilibrium constant in cultured human urinary bladder carcinoma cells (JTC-32) (Kakuya et al., 1983).

Materials and Methods

Chemicals

Dexamethasone and insulin (24 I.U./mg) from bovine pancreas were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). ¹²⁵I-labeled insulin

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(〜100 μCi/μg) was purchased from New England Nuclear (Boston, Mass., U.S.A.).

Cell culture
JTC-32 cells were established from human urinary bladder carcinoma by Kakuya et al. (1983). The cells were maintained in glass culture flasks with a rubber stopper in Eagle's minimum essential medium containing 5% calf serum. For subsequent experiments, the cells were inoculated in plastic culture dishes with a 6-cm diameter or plastic culture plates with 24 wells (Corning, N.Y., U.S.A.), and cultured in an atmosphere of 5% CO₂ and 95% air. When the cells had almost reached confluence, the concentration of serum was reduced to 1% from 5%, and the preincubation of cells with dexamethasone was started. The amount of cellular proteins per dish was estimated by the method of Lowry et al. (1951), with bovine serum albumin as the standard.

Insulin binding
Insulin binding assay was performed according to the previously reported method for a monolayer cell culture (Sorimachi and Yasumura, 1981). The cells cultured in monolayer were incubated with ¹²⁵I-labeled insulin and unlabeled insulin at various concentrations (2 ml) at 25°C for the stated periods. After the cells were incubated with insulin, they were washed twice with ice-cold phosphate buffered saline, pH 7.4, and finally solubilized with 1% sodium dodecyl sulfate for radioactivity counting (Sorimachi and Yasumura, 1981).

Results
When JTC-32 cells were incubated with ¹²⁵I-labeled insulin with high specific activity at 25°C, the specific binding of insulin to the cells was evident, as shown in Fig. 1. The specific binding of insulin almost reached a plateau after 60 min, but the non-specific binding in the presence of a large excess of unlabeled insulin (20 μg/ml) reached a plateau even after 15 min. Since insulin binding to JTC-32 cells reached equilibrium after 60 min of incubation at 25°C, the incubation period for cells with ¹²⁵I-labeled insulin was 60 min for the Scatchard analysis.

Dexamethasone increased insulin binding to JTC-32 cells. The total cellular radioactivity represents not only insulin bound to the cell surface, but also insulin taken into cells. In order to know which part was increased by dexamethasone, after the cells were incubated with ¹²⁵I-labeled insulin, they were exposed to fresh medium containing a large excess of unlabeled insulin; the released radioactivity would mainly represent insulin bound to the cell surface, and the remaining radioactivity would represent insulin taken into the cells.

The treatment of cells with dexamethasone induced a 1.9-fold increase in insulin binding to the cell surface, and a 1.7-fold increase in insulin internalization (Table 1). The increase in the total cellular radioactivity with dexamethasone was almost equal to the increase in the released radioactivity from the cell surface. In other words, dexamethasone increased insulin binding in JTC-32 cells. No significant difference in the non-specific insulin binding was observed between the control and dexamethasone-treated cells.

It was reported by Ozaki and Kalant (1977) that the incubation of insulin with plasma membranes induced the degradation of insulin. We therefore measured the increase in the radioactivity of the trichloroacetic acid-soluble fraction of the medium used for insulin treatment with cells. When the fresh cells, untreated with dexamethasone, were incubated with ¹²⁵I-labeled
Vol. 30, No. 6

UP-REGULATION OF INSULIN RECEPTORS

Table 1. Effects of dexamethasone on insulin receptors in JTC-32 cells.

<table>
<thead>
<tr>
<th></th>
<th>Released radioactivity (cpm/dish)</th>
<th>Remaining radioactivity (cpm/dish)</th>
<th>Total cellular radioactivity (cpm/dish)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3,500±200 (80)</td>
<td>860±60 (20)</td>
<td>4,400±200</td>
</tr>
<tr>
<td>+ Unlabeled insulin</td>
<td>270±40 (68)</td>
<td>130±50 (33)</td>
<td>400±70</td>
</tr>
<tr>
<td>Dexamethasone treated cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>6,500±300 (82)</td>
<td>1,470±80 (18)</td>
<td>8,000±300</td>
</tr>
<tr>
<td>+ Unlabeled insulin</td>
<td>250±30 (71)</td>
<td>100±10 (29)</td>
<td>360±30</td>
</tr>
</tbody>
</table>

Cells (2.59×10⁶) were incubated with or without dexamethasone (10⁻⁷ M) for 24 h, and then treated with 125I-labeled insulin (49,700 cpm, 0.5 ng) for 30 min at 25°C in the presence or absence of unlabeled insulin (20 μg/ml). After washing the cells with phosphate buffered saline, the cells were incubated with a large excess of unlabeled insulin (20 μg/ml) for 30 min at 25°C. The value given is the mean±S.D. of the values for three culture dishes. The value in parentheses is the percentage of the total cellular radioactivity.

When the cells cultured in monolayer were preincubated with dexamethasone for 30 min, the radioactivity in the trichloroacetic acid-soluble fraction of the incubation medium very slightly increased (~1.4% of the total radioactivity). These results indicate that deiodination and insulin fragmentation did not take place apparently after 30 min of incubation of the cells with 125I-labeled insulin at 25°C, although a minor modification which was undetectable by the trichloroacetic acid precipitation might take place.

When the cells cultured in monolayer were preincubated with dexamethasone for the stated periods, insulin binding increased during the entire 24 h of incubation, as shown in Fig. 2. After the 24 h of incubation, insulin binding increased to 145% of the control cells which were not pretreated with dexamethasone. Therefore, for the subsequent experiments, in which the cells were pretreated with dexamethasone, the period of preincubation of the cells with the hormone was 24 h to get a high insulin binding factor.

The effect of dexamethasone on insulin binding was investigated with various concentrations of dexamethasone (Table 2). A significant effect of dexamethasone was found at 10 nM. In our previous experiments

Table 2. Effect of dexamethasone at various concentrations on insulin binding to JTC-32 cells.

<table>
<thead>
<tr>
<th>Concentration of dexamethasone (M)</th>
<th>Insulin binding to cells (cpm)</th>
<th>Percent of control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4,150±250</td>
<td>100</td>
</tr>
<tr>
<td>10⁻¹</td>
<td>4,020±140</td>
<td>96.7</td>
</tr>
<tr>
<td>10⁻¹⁰</td>
<td>4,040±70</td>
<td>97.4</td>
</tr>
<tr>
<td>10⁻⁹</td>
<td>4,040±40</td>
<td>97.4</td>
</tr>
<tr>
<td>10⁻⁸</td>
<td>5,080±120</td>
<td>122</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>5,210±130</td>
<td>126</td>
</tr>
</tbody>
</table>

Cells were preincubated with dexamethasone at various concentrations at 37°C for 24 h. The number of cells was 1.6×10⁶ cells per dish. The radioactivity of insulin was 68,900 cpm. The value given is the mean±S.E. of the values for three culture dishes.
Fig. 3. Scatchard plots for the binding of insulin to JTC-32 cells. Cells were pretreated with (right panel), or without dexamethasone (10^{-7} M) at 37°C for 24 h. B/F represents the ratio of bound insulin to unbound insulin in the system. The number of cells was 3.2 \times 10^6 per dish. The non-specific binding was obtained in the presence of a large excess of unlabeled insulin (20 \mu g/ml).

(Sorimachi et al., 1981), at the same concentration, this hormone induced the maximal activity of tyrosine aminotransferase and phenylalanine hydroxylase in Reuber rat hepatoma cells (R-Y 121 B) (Niwa et al., 1980).

The cells were incubated with 125I-labeled and unlabeled insulin for the competitive binding assay in monolayer of cells, according to our previous method (Sorimachi and Yasumura, 1981). The pretreatment of cells with dexamethasone (0.1 \mu M) for 24 h prior to the binding assay of insulin, increased insulin binding to the cells, as shown in Fig. 3. Scatchard plots (Scatchard, 1949) were not linear. Therefore, these curves were divided into two lines according to the method of Rosenthal (1967) with a pdp 11/40 computer (Digital Equipment Corporation, Maynard, Mass., U.S.A.). The values for the apparent equilibrium constant and the number of insulin binding sites are shown in Table 3. When the cells were pretreated with dexamethasone at 0.1 \mu M for 24 h, a 2.8-fold increase in the number of high affinity insulin binding sites was found, compared with the fresh cells untreated with dexamethasone. The apparent equilibrium constants of high affinity insulin binding sites were almost the same in the fresh and dexamethasone-treated cells. Regarding low affinity insulin binding sites, the dexamethasone treatment induced a 1.6-fold increase in the number of binding sites, although there was a slight difference between apparent equilibrium constants in the two samples.

After JTC-32 cells were treated with insulin, dexamethasone or insulin plus dexamethasone, the number of cells and the amount of cellular proteins per dish were measured (Table 4). Neither insulin nor dexamethasone affected the number of cells or the amount of cellular proteins. However, when the cells were pretreated with dexamethasone and further treated with dexamethasone plus insulin, not only the number of cells but also the amount of cellular proteins increased.

Table 3. The effects of dexamethasone on insulin receptors in JTC-32 cells.

<table>
<thead>
<tr>
<th></th>
<th>High affinity</th>
<th>Low affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kd a ( \times 10^4 \text{cell} )</td>
<td>N b ( \times 10^4 \text{cell} )</td>
</tr>
<tr>
<td>Fresh cells</td>
<td>0.18</td>
<td>0.24</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.20</td>
<td>0.74</td>
</tr>
<tr>
<td>treated cells</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a: The apparent equilibrium constant.
b: The number of insulin binding sites.
Table 4. Effect of dexamethasone and insulin on JTC-32 cells.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Experiment I</th>
<th></th>
<th>Experiment II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell number</td>
<td>Protein</td>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td></td>
<td>( \times 10^6/\text{dish} )</td>
<td>(mg/dish)</td>
<td>( \times 10^6/\text{well} )</td>
<td>( \times 10^6/\text{well} )</td>
</tr>
<tr>
<td>None</td>
<td>1.15±0.05</td>
<td>0.467±0.050</td>
<td>0.112±0.003</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>1.11</td>
<td>0.430</td>
<td>0.115±0.007</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1.11±0.15</td>
<td>0.460±0.020</td>
<td>0.115±0.004</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone + insulin</td>
<td>1.32(*)**</td>
<td>0.500</td>
<td>0.130±0.007(\dagger)</td>
<td></td>
</tr>
</tbody>
</table>

In Experiment I, the cells were cultured in plastic culture dishes with 35-mm diameter, and in Experiment II, the cells were cultured in plastic culture plates with 24 wells. The conditions used were: none, cells not treated with dexamethasone or insulin; insulin, cells treated with insulin (1 mU/ml) for 24 h; dexamethasone, cells treated with dexamethasone (10^{-7} M) for 48 h; dexamethasone + insulin, cells treated with dexamethasone (10^{-7} M) for 24 h, and then with dexamethasone plus insulin (1 mU/ml) for 24 h. The value given is the mean±S.D. of the values for three culture dishes or the mean of the values for two culture dishes in Experiment I, and the mean±S.D. of the values for four to six wells in Experiment II. * The p value was larger than 0.05, but smaller than 0.1, when compared with dexamethasone treated cells. ** The p value was less than 0.005, compared with control cells or insulin treated cells. \(\dagger\) The p value was smaller than 0.005, compared with every case.

slightly, even though these increases were very small.

**Discussion**

In this study, the number of high affinity insulin binding sites increased without changing the apparent equilibrium constant with the pretreatment of the cells with dexamethasone. Similar results were reported by Fantus et al. (1981) and Knutson et al. (1982). The latter (Knutson et al., 1982) showed that an increase in the number of insulin binding sites with dexamethasone was due to a decrease in the degradation rate of insulin receptors. Furthermore, dexamethasone changed preadipocytes to adipocytes, accompanied by an increase in the number of insulin receptors while changing the affinity for insulin (Rubin et al., 1978). In the latter case, however, it appeared that the changes in insulin receptors were due to cell differentiation with dexamethasone rather than changes in insulin receptors themselves; the differentiation of cells eventually caused the changes in insulin receptors on the cells.

Among the various cell lines examined in our study, dexamethasone increased the number of insulin receptors (up-regulation) only in HTC cells, derived from rat hepatoma, and JTC-32 cells. In HTC cells, the cellular morphology was altered by dexamethasone, and the preincubation of the cells with this hormone was necessary for the induction of tyrosine aminotransferase activity with insulin (Sorimachi et al., 1983).

Although dexamethasone did not alter the cellular morphology in JTC-32 cells (unpublished data), this hormone increased the number of high affinity insulin binding sites. In addition, although neither insulin nor dexamethasone increased the number of cells or the amount of cellular proteins per dish, the addition of insulin to the incubation medium, in which the cells were preincubated with dexamethasone, increased them. Judging from these results, the increase in the number of insulin receptors with dexamethasone might contribute to the appearance of insulin effects. It is known that insulin stimulates the incorporation of thymidine into fibroblasts, even though this effect is very weak (Perdue and Raizada, 1976).

In our previous results related to the induction of tyrosine aminotransferase activity with insulin in HTC cells (Sorimachi et al., 1983) and the present results, dexamethasone plays an important role in yielding insulin effects in the cultured cells. It
seems, therefore, that up-regulation of insulin receptors with dexamethasone would be involved in some physiological functions of insulin in certain cell lines.

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References


