Adrenalectomy Enhances the Susceptibility of Pancreatic Islets to Interleukin-1β: Immunohistochemical Study

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Abstract. To determine the importance of adrenal steroid in the effects of interleukin-1, we investigated changes in the number of islet cells reactive toward antiserum to insulin (anti-Ins) by intraperitoneal administration of recombinant human interleukin-1β (IL-1) in intact and adrenalectomized (ADX) rats. IL-1 significantly reduced serum insulin levels in ADX rats only, while it similarly decreased plasma glucose levels. In intact rats, IL-1 did not affect the number of islet cells reactive to anti-Ins, although cytoplasmic immunostaining tended to be reduced by IL-1 treatment. Only adrenalectomy decreased the number of islet cells immunostained by anti-Ins. Furthermore, IL-1 treatment significantly reduced the number of islet cells reactive to anti-Ins in ADX rats. The present study immunohistochemically supported our working hypothesis that the withdrawal of adrenal steroids by adrenalectomy enhances the islet cell sensitivity to exogenous administration of IL-1.

Key words: Interleukin, Adrenalectomy, Insulin, Islet, Rat. (Endocrinol Japon 39: 485-490, 1992)

INTERLEUKIN, one of the cytokines, is released from inflammatory cells and involves inflammatory responses [1]. Since interleukin-1 reduces the insulin content of islet cells in a dose-related fashion and suppresses insulin release from the perfused islets, interleukin-1 is supposed to be involved in the development of insulin dependent diabetes mellitus [2, 3]. We have recently demonstrated that adrenalectomy potentiates the reduction of serum immunoreactive insulin (IRI) levels after intraperitoneal administration of recombinant human interleukin-1β (IL-1) [4]. These data suggest that the existence of adrenal steroid may protect against islet cell damage by endogenously released IL-1. In addition, our finding that glucocorticoid supplement blocked the effect of IL-1 on serum insulin levels demonstrated the importance of glucocorticoid in the appearance of the IL-1 effects on pancreatic islets [5]. However, histological change in the islets after exogenous IL-1 treatment remains to be established in adrenalectomized (ADX) animals.

In the present study, we investigated immunohistochemical changes in pancreatic islet cells after exogenous IL-1 treatment in both intact and ADX rats.

Materials and Methods

Animals

The male Wistar rats weighing about 200 g were obtained from Imai Animal Laboratories (Saitama, Japan). The animals were housed in a temperature-controlled (23±1°C) room with a 14 h light-10 h dark cycle. The rats were given free access to laboratory chow pellets and drinking water. The animals were bilaterally adrenalectomized through flank incisions under light ether anesthesia. After operation, the animals were maintained on 0.9%
Preparation of IL-1

The IL-1 was kindly provided by Dr. Y. Hirai (Otsuka Pharmaceuticals, Inc., Tokushima, Japan). The preparation had a specific activity of $2 \times 10^7$ half maximal units/mg protein, estimated by the mouse thymocyte $^3$H-thymidine incorporation assay. The material has been judged to exhibit a purity of at least 99% based on analysis by high performance liquid chromatography and polyacrylamide gel electrophoresis [6, 7]. The injection material was dissolved in physiological saline.

Treatment

IL-1 (1.0 µg/kg) or vehicle was intraperitoneally injected. Four hours later, all animals were decapitated and blood samples were collected in tubes. The whole pancreas was immediately dissected out in Bouin-Holland solution. On sacrifice, residual adrenal tissue was anatomically checked and serum corticosterone levels were measured by HPLC. The animals in which bilateral adrenal glands were completely removed were used for data analysis.

Immunohistochemical method

The pancreas was removed and immediately fixed with Bouin-Holland-sublimate for 2 days. After hydration and embedding in Paraplast, 4 µm-thick sections were cut and mounted on gelatin-coated slides. The deparaffined sections were treated with iodine alcohol and sodium pyrosulfite to eliminate the sublimate. They were then incubated in a solution of 0.3% H$_2$O$_2$ in methanol to inhibit the endogenous peroxidase activity. After they were rinsed with distilled water (DW) and PBS, the sections were immunostained by an indirect enzyme-labeled antibody method. The sections were incubated sequentially with 1% bovine serum albumin normal for 2 h, goat anti-human insulin serum (anti-Ins; 1:5,000) and peroxidase-labeled rabbit anti-goat IgG serum (1:200) for 2 h. After these steps, the sections were washed three times with PBS, stained with 10 mg of 3,3'-diaminobenzidine tetrahydrochloride and 0.005% H$_2$O$_2$ in 100 ml of 0.05 M Tris-HCl buffer, pH 7.6 to detect the location of the peroxidase activity, rinsed with DW, stained with 1% methyl green, dehydrated through a graded ethanol series, and mounted in Entellan.

For data analysis, the number of Ins-Ab positive cells in the islets was calculated.

Assay

Serum immunoreactive insulin (IRI) levels were measured by radioimmunoassay with rat insulin standard. Plasma glucose levels were measured in an automatic glucose analyzer.

Statistical analysis

All data were expressed as the mean±SEM. The statistical differences were determined by analysis of variance (ANOVA), followed by Duncan's multiple range test.

Table 1. Changes in serum immunoreactive insulin (IRI) and plasma glucose levels after intraperitoneal administration of recombinant human interleukin-1 β (IL-1: 1.0 µg/kg) or vehicle in intact and adrenalectomized (ADX) rats

<table>
<thead>
<tr>
<th></th>
<th>Intact vehicle</th>
<th>Intact IL-1</th>
<th>ADX vehicle</th>
<th>ADX IL-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRI (ng/ml)</td>
<td>2.68±0.65</td>
<td>3.69±0.65</td>
<td>2.61±0.36</td>
<td>1.07±0.15*</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>128±22.2</td>
<td>114.8±3.0*</td>
<td>117.8±4.3</td>
<td>93.8±5.4*</td>
</tr>
<tr>
<td>number</td>
<td>5</td>
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All data represent the mean±SEM. a, P<0.01 compared with the value for vehicle-treated rats.
Results

Table 1 shows changes in serum IRI and plasma glucose levels at 4 h after IL-1 injection. In intact rats, IL-1 increased serum IRI and plasma glucose was significantly decreased. In contrast, IL-1 significantly reduced serum IRI in ADX rats and plasma glucose was significantly reduced.

Changes in the islet cells immunostaining with anti-Ins are shown in Figs. 1 and 2. In intact rats, IL-1 reduced the islet cell cytoplasmic immunostaining against anti-Ins. However, there was no difference between vehicle- and IL-1-treated groups in the percentage of the population of the islet cells reacting against anti-Ins (Fig. 1). On the other hand, each islet size was decreased in ADX rats, and IL-1 obviously reduced the percentage of the population of the islet cells reacting against anti-Ins in ADX animals (Fig. 2b). We calculated the average number of cells reacting against anti-Ins in each islet. Adrenalectomy reduced the number of cells reacting against anti-Ins (Table 2). IL-1 failed to decrease the number of cells reacting against anti-Ins in adrenal-intact animals. In con-

Fig. 1. Light micrograph of pancreatic islet in vehicle (a)- or recombinant human interleukin-1β (IL-1: b)-treated intact rats. magnification: × 40.
Fig. 2. Light micrograph of pancreatic islet in vehicle (a)- or recombinant human interleukin-1β (IL-1: b)-treated adrenalectomized rats. Magnification: × 40.

Table 2. Number of anti-human insulin serum (anti-Ins) stained cells in the islets of interleukin-1β (IL-1)- or vehicle-treated intact and adrenalectomized (ADX) rats

<table>
<thead>
<tr>
<th></th>
<th>vehicle-treated intact</th>
<th>IL-1-treated intact</th>
<th>vehicle-treated ADX</th>
<th>IL-1-treated ADX</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>17</td>
<td>17</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>number of anti-Ins reactive cells/islet</td>
<td>48.8±6.4</td>
<td>38.2±4.9</td>
<td>25.8±3.3*</td>
<td>13.2±1.2*</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±SEM. N, number of islets examined. a, P<0.01 compared with intact rats; b, P<0.05 compared with vehicle-treated group.

Discussion

The present study demonstrated that IL-1 administration significantly reduces the population of islet cells reacting against anti-Ins only in ADX rats, although IL-1 tended to only decrease cytoplasmic immunostaining in intact rats. These data immunohistochemically suggest that withdrawal of adrenal steroids after adrenalectomy enhances the effect of IL-1 on insulin synthesis and/or secretion from pancreas islets.

Each islet size was decreased in ADX rats contrast, IL-1 significantly decreased the number of cells reacting against anti-Ins in ADX rats.
compared with that in intact rats. Since glucocorticoid has an insulinotropic action on the islets, it is possible that the withdrawal of glucocorticoid after bilateral adrenalectomy caused a reduction in the size of each islet.

The present study reconfirmed that serum IRI levels were significantly reduced by IL-1 treatment in ADX animals, but slightly increased in intact rats. In contrast, IL-1 similarly reduced blood glucose levels in both intact and ADX rats. The reduction of blood glucose levels has been explained by decreased hepatic glycogenolysis by IL-1, and adrenalectomy may not affect this step. We have recently demonstrated that serum IRI levels were decreased at 2 h after IL-1 injection by 48% of pre-injection levels in ADX animals, while intact rats did not show a significant reduction in serum IRI levels [4]. Glucose-stimulated insulin response tended to be increased in IL-1-treated intact rats. In contrast, insulin response of IL-1-treated ADX rats was significantly reduced and blood glucose levels were increased after intravenous glucose load [5]. The present results are consistent with these of our previous observations.

In vitro study demonstrated that short-term exposure to IL-1 stimulated insulin secretion and long-term exposure to this peptide inhibited insulin secretion, finally destroying islets [7]. Their results indicate that IL-1 may stimulate insulin secretion from the islets, but reduce de novo insulin biosynthesis in the islets. Furthermore, islet insulin biosynthesis has been reported to be inhibited by IL-1 [8]. These observations can explain the present data showing that IL-1 tended to reduce the cytoplasmic immunostaining against insulin in intact rats while serum IRI levels were increased. Withdrawal of adrenal steroid may shorten the appearance time of the IL-1 effects.

Electron microscopy of the pancreata perfused with IL-1 revealed significant β-cell lysis accompanied by peripheral degranulation and rearrangement of rough endoplasmic reticulum [9]. In their experiment, adrenal steroid supplement was not introduced into the perfusion system. The finding that the number of β-cells reactive with anti-Ins was markedly reduced in IL-1-treated ADX rats leads to the suggestion that defect in adrenal steroid may enhance the islet sensitivity to IL-1, resulting in the induction of the β-cell lysis in living animals. We believe that the lack of adrenal steroid in the perfusion medium can explain the difference in islet susceptibility to IL-1 in in vitro and in vivo experiments on intact rats. However, further studies including other immunocytochemical techniques such as chromogranin A or B staining may be necessary to confirm actual β-cell destruction following IL-1 injection.

In general, glucocorticoid has an immunosuppressive action. It has been recently reported that repeated administration of IL-1 causes an irreversible reduction in islet insulin content, suggesting permanent pancreatic damage due to IL-1 [11]. Nerup et al. suggested that IL-1 may be involved in the induction of islet β-cell damage at the onset of insulin dependent diabetes [12]. Taking into consideration that IL-1 may be involved in the development of pancreatic damage in diabetes, the present data raised the possibility that pancreatic damage due to IL-1 may be accelerated under adrenal insufficient condition.

The present study immunohistochemically confirmed that the withdrawal of adrenal steroid by adrenalectomy enhances the effects of exogenously administered IL-1 on pancreatic β-cell function.

Acknowledgments

The authors wish to thank Dr. Y. Hirai for his generous supply of recombinant human interleukin-1 beta.

References


