Glucocorticoid-induced Recovery from Streptozotocin Diabetes in the Adult Rat

MICHEL ROUDIER, BERNARD PORTHA, AND LUC PICON

SUMMARY

The effect of a large dose of cortisol (5 mg/kg), injected 24h and 3h before or 3h and 24h after streptozotocin (SZ), on the course of SZ-induced diabetes has been studied in the rat. Glucose tolerance tests, performed 1 or 2, 8, and 30 days after the SZ injection (35 mg/kg), gave the same results in cortisol-treated rats regardless of the time of cortisol injection (before SZ (C.SZ) or after it (SZ.C)). During the first test, elevated plasma glucose, decreased glucose tolerance, and decreased glucose-induced insulin secretion were present in SZ, C.SZ, and SZ.C rats. During the second and the third tests, glucose tolerance and insulin secretion were significantly improved in both groups of cortisol-treated rats. On the contrary, rats receiving only SZ showed a worsening of the diabetic state. It should be noted that cortisol-induced recovery of diabetes was not obtained with larger doses of SZ (50 mg/kg).

These findings indicate that an improvement of diabetes can be obtained by cortisol treatment after the onset of chemically induced diabetes. Whether this effect is obtained through an effect on pancreatic regeneration and/or through the anti-inflammatory properties of glucocorticoids is unknown at the present time. DIABETES 29:201-205, March 1980.

It has recently been proposed that the diabetogenic effect of streptozotocin in the rat is reduced by pretreatment with cortisol. Unlike several agents known to modify the diabetogenic activity of streptozotocin, cortisol did not improve the diabetic state during the first day, but the diabetes became milder in cortisol-treated rats, with glycemia returning to normal values in the fed state after one week. It was important to know the long-term efficiency of this cortisol treatment and to determine the relationship between (1) the cortisol dose and the degree of recovery from diabetes and (2) the severity of diabetes and the degree of recovery.

MATERIAL AND METHODS

Female rats of the Sherman strain, weighing about 150 g, were fed ad libitum with commercial, pelleted food containing 47% carbohydrates, 8% lipids, and 20% proteins (U.A.R. France). Streptozotocin (SZ) (lot 60 140 U 9899) (Upjohn Laboratories) was dissolved in citrate buffer (pH 4.5) and immediately injected intravenously under slight ether anesthesia at the doses of 20, 35, and 50 mg per kg body weight. The concentration of SZ in the buffer varied according to the doses, and the volume of citrate buffer injected was always 100 μl/100 g body weight. Cortisol acetate (2.5, 5, 10, or 25 mg/kg), methylprednisolone acetate (5 mg/kg), and dexamethasone acetate (5 mg/kg), as a 100 μl/100 g b.w. solution, was injected subcutaneously 24h and 3h after the injection of SZ (C.SZ rats) or 3h and 24h after it (SZ.C rats).

Intravenous glucose tolerance tests (IVGTT) (0.5 g glucose/kg b.w.) were performed under pentobarbital anesthesia (4 mg/100 g b.w., i.p.) 1, 8, and 30 days after the SZ injection for the C.SZ rats or 2, 8, and 30 days for the SZ.C rats. Blood was withdrawn from the tail vein in the fed state. Blood samples (300 μl) were immediately centrifuged at 4°C; plasma was stored at −20°C until assayed. After dissection, the pancreas was weighed and homogenized for 1 min by ultrasonic disintegration at 4°C (sonifier Branson B12, Heat Systems-Ultrasonics, Plainview, N.Y.) in acid alcohol solution (75% ethanol, 1.5% v/v 12 mol/L, HCI, 23.5% distilled water). After one night at −20°C, the extracts were centrifuged and the supernatant was kept at −20°C until assayed.

Plasma glucose was determined using a glucose analyzer (Beckman Inc., Palo Alto, Calif.). Pancreatic and plasma immunoreactive insulin (IRI) was estimated using purified rat insulin as standard (R 171, Novo, Copenhagen, Denmark), antibody to human insulin, and porcine monoidinated 125I-insulin. The method allows the determination of 6 μU/ml (0.25 ng/ml) with a coefficient of variation within...
and between assays of 10%. Silicate was used to separate free from bound hormone. Results are given as mean ± SEM. Statistical analysis was performed using Student's unpaired t test.

RESULTS

Effect of the dose of streptozotocin on plasma glucose. A relationship exists between the dose of streptozotocin and plasma glucose values in fed rats (Figure 1). With 20 mg/kg, plasma glucose was not modified 1 day and 8 days after streptozotocin and was not different from the control values. With 35 mg/kg, plasma glucose was significantly increased on the first day, was still higher on the eighth day, and remained high on the 30th day (356 ± 88 mg/100 ml). With 50 mg/kg, plasma glucose values were higher than with 35 mg/kg. We checked (Table 1) that the citrate buffer injection per se had no effect on glucose tolerance. As a consequence, all the SZ-treated groups were compared with the nontreated group.

Effect of a pretreatment with cortisol (two injections of 25 mg/kg) on the course of diabetes induced by 50 or 35 mg/kg of streptozotocin. With the higher dose of streptozotocin, no protective effect of the cortisol was observed; plasma glucose values were the same in C.SZ and SZ rats on the first day (496 ± 10 versus 480 ± 11 mg/100 ml) and on the eighth day (595 ± 16 versus 598 ± 64 mg/100 ml).

With the lower dose of streptozotocin (35 mg/kg), pretreatment with cortisol improved the glucose tolerance on the eighth day (Table 1). However, this treatment resulted in a worsening of the diabetic state on the first day. So, in an effort to obtain an improvement of the glucose tolerance on the eighth day without increased hyperglycemia on the first day, we checked the effect of smaller doses of cortisol.

Effect of different doses of cortisol in rats injected with 35 mg/kg of streptozotocin. Table 1 indicates that, after a pretreatment with 5 mg/kg of cortisol, the glucose tolerance was better than with any other dose of cortisol both on the first and on the eighth day after streptozotocin injection. So, this dose of cortisol was chosen for further experiments. Methylprednisolone (2 × 5 mg/kg) had an effect similar to that of cortisol (2 × 10 mg/kg); with dexamethasone (2 × 5 mg/kg), the glucose tolerance test was normal on the eighth day, but hyperglycemia was important on the first day.

Comparison of the effect of cortisol (5 mg/kg) given before or after streptozotocin on the course of the diabetes. On the eighth day after streptozotocin treatment (Figure 2), diabetes was improved in SZ.C rats and C.SZ rats when compared with the SZ rats. During the IVGTT, plasma glucose values in C.SZ or SZ.C rats were not significantly different from each other and were significantly different from values of SZ rats. Plasma IRI was lower in SZ rats (15 ± 3 μU/ml) than in C.SZ rats (39 ± 9 μU/ml, P < 0.05) and in C.SZ rats (35 ± 9 μU/ml, P < 0.05).

On the 30th day (Figure 2) as compared with the 8th day, there was no improvement in SZ rats: the basal plasma glucose value was high, glucose tolerance was similar, and insulin secretion was not significantly increased by i.v. glucose. In SZ.C and C.SZ rats, the basal plasma glucose value and the glucose tolerance did not differ from those in

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Minutes after glucose load</th>
<th>Minutes after glucose load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>None</td>
<td>142 ± 4</td>
<td>263 ± 10</td>
</tr>
<tr>
<td>Citrate buffer</td>
<td>135 ± 3</td>
<td>246 ± 9</td>
</tr>
<tr>
<td>SZ</td>
<td>276 ± 26</td>
<td>399 ± 26</td>
</tr>
<tr>
<td>Cortisol + SZ 25 mg/kg</td>
<td>373 ± 60</td>
<td>495 ± 58</td>
</tr>
<tr>
<td>10</td>
<td>264 ± 50</td>
<td>382 ± 41</td>
</tr>
<tr>
<td>5</td>
<td>201 ± 28</td>
<td>324 ± 33</td>
</tr>
<tr>
<td>2.5</td>
<td>228 ± 42</td>
<td>342 ± 37</td>
</tr>
<tr>
<td>Dexamethasone + SZ 5 mg/kg</td>
<td>444 ± 41</td>
<td>501 ± 40</td>
</tr>
<tr>
<td>Methylprednisolone + SZ 5 mg/kg</td>
<td>328 ± 43</td>
<td>416 ± 35</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. Number of rats was between 8 and 10. On the first day, all the PG values of pretreated rats are significantly higher than values in controls (P < 0.05). Only dexamethasone-pretreated rats have PG values higher than values in SZ rats (P < 0.05). On the eighth day, all glucocorticoid-pretreated rats have lower PG values as compared with SZ rats (P < 0.05).
FIGURE 2. Normalization of glucose tolerance after cortisol pre (C.SZ) or post (SZ.C) treatment (2 × 5 mg/kg) of streptozotocin-diabetic rats (SZ). Glucose tolerance (0.5 g/kg, i.v.) and plasma insulin response were determined 1 (or 2), 8, and 30 days after streptozotocin injection in SZ rats (○), in C.SZ rats (△), and in SZ.C rats (▲). Each point is the mean ± SEM of 5 to 10 animals in each group. The same animals were studied sequentially; the dotted shadow refers to five control rats.

The pancreatic IRI was measured in control, C.SZ, and SZ.C rats killed on day 1 or 2, 8, and 30 after SZ injections (Table 2). In the SZ rats, the pancreatic IRI was 14% of the control value on day 8, and it was 20% of the control value on day 30. In C.SZ and SZ.C rats, pancreatic IRI was also decreased (24% and 28% of the control values on day 8, respectively) but was significantly higher than in SZ rats. Moreover there was an increase of the pancreatic IRI between the 8th and the 30th day by 94% in C.SZ and by 42% in SZ.C rats.

DISCUSSION
In rats, a relationship between the dose of streptozotocin and the level of plasma glucose has been observed. Ganda et al. obtained the maximum diabetogenic effect in their rats given 60 mg/kg. Our results confirm the lack of spontaneous recovery from the streptozotocin-induced diabetes in adult rats in contrast to the findings in neonatal rats. A pharmacologic dose of cortisol has been found to have a protective effect only against a moderate dose of streptozotocin (35 mg/kg). Although we did not find any improvement of diabetes induced with a dose of 50 mg/kg, Katsada and Ui have reported such an improvement. However, in their study, diabetes induced with higher doses of streptozotocin did not improve with the cortisol pretreatment; thus, differences in diabetogenic activity of various batches of streptozotocin could explain this discrepancy. Their contents of α or β anomers are not always the same, and α anomer has been proved to be more diabetogenic.
munn and Volk\textsuperscript{14} found lower blood glucose values and a persistence of identifiable, but poorly granulated, \(\beta\) cells on the seventh day after streptozotocin (65 mg/kg) after 6 wk of pretreatment with cortisol, but the mortality in cortisol-pretreated rats was greater than in rats receiving streptozotocin alone. Pretreatment with cortisol has also been found to improve alloxan diabetes in rabbit\textsuperscript{16} and guinea pig.\textsuperscript{17} The ineffectiveness of cortisol on diabetes induced by a high dose of streptozotocin indicates that the mechanism of the protective effect of corticosteroids against streptozotocin differs from that of protective agents like glucose and its analogues,\textsuperscript{7,18,19} which have an effect even against high doses of streptozotocin and are said to compete with the glucose moiety of the streptozotocin molecule, thus preventing its interaction with the \(\beta\) cells. These agents must be injected before streptozotocin. The protective effect of cortisol injected before streptozotocin is not caused by the hyperglycemia, since at the time of the injection of streptozotocin (3 h after the last injection of cortisol), we observed decreased plasma glucose values (86 \(\pm\) 5 mg/100 ml in cortisol-treated as compared with 142 \(\pm\) 4 mg/100 ml in controls, \(n = 10, P < 0.001\)), probably because of insulin secretion induced by cortisol. Houssay et al.\textsuperscript{18} found that 6 mo of cortisol treatment in subtotally pancreatectomized rats resulted in a diminution of the percentage of rats becoming diabetic; the insulin secretion and the glucose tolerance were, however, not studied and the course of diabetes after interruption of cortisol treatment was not checked. In our study, we find that basal plasma glucose and glucose tolerance are much improved in cortisol-treated rats, the pancreatic insulin content is increased on the 30th day, however, we do not know whether the number of \(\beta\) cells is larger in C.SZ or SZ.C than in SZ rats.

It has been found, in vivo, that corticoid treatment results in an increased \(\beta\) cell number in rodents and subhuman primates;\textsuperscript{19} on the contrary, in vitro, corticosteroids decrease the replication of \(\beta\) cells in pancreatic monolayer cultures.\textsuperscript{20} Cortisol could act by another mechanism; Rossini et al.\textsuperscript{21} have reported that, in some strains of mice injected with multiple subdiabetogenic doses of streptozotocin, there is insulitis, progressive \(\beta\) cell destruction, and diabetes. Immunosuppressive treatment has been used successfully in this model.\textsuperscript{22} In streptozotocin-injected rats, one may speculate that cortisol could also be effective, owing to its anti-inflammatory or immunosuppressive properties. As a matter of fact, this possibility is presently without supporting data in the rat, since the SZ-induced insulitis model has been observed only in mice. Nevertheless, whatever the mechanism of action, the ability of cortisol, injected after the action of a diabetogenic agent, to improve the course of the subsequent diabetes deserves further analysis in view of the possible clinical implications in recent human diabetes.

**ACKNOWLEDGMENTS**

Streptozotocin was kindly provided by Dr. J. P. Paturaud (Upjohn France, Paris). We wish to thank Dr. G. Rosselin (INSERM-U55) for the gift of iodinated insulin.

The technical assistance of Jean-Claude Cros is gratefully acknowledged.

This study was supported in part by the Université Paris VII and the Institut National de la Santé et de la Recherche Médicale (INSERM) (contrat de recherche libre no. 76.1.022.4).

**REFERENCES**


