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Streptozotocin-induced diabetes and glucocorticoid receptor regulation: tissue- and age-specific variation

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Abstract

Streptozotocin (STZ) -induced diabetic effects were analyzed for glucocorticoid receptor (GR) level and for in vitro activation of GR by specific binding analysis, using [^{3}H]dexamethasone, a synthetic glucocorticoid, and by DNA cellulose and nuclear binding assay, in the liver and kidney of 15- (immature) and 120-day-old (mature) male mice. Comparison of GR level (fmol/mg protein) among the control mice reveals decreased (22–33%) specific binding in the liver and kidney of mature mice compared with immature ones. Scatchard analyses, however, reveal no change in the affinity (K_d) of receptor at these two ages of mice. STZ-induced diabetes did not alter the level of GR in either of the tissues at both the ages studied. The GR from both the tissues underwent thermal activation, albeit the extent of activation was more pronounced in mature liver compared to immature, with no such difference of activation in the kidney. In diabetic mice, the activation of hepatic GR exhibits reduced DNA cellulose ($\sim$ 20–23%) and nuclear (24–30%) binding compared to control mice. In contrast, thermal activation of kidney GR does not show marked differences in diabetic mice at either of the ages studied. Cross-mixing experiments (i.e. binding of activated GR from diabetic mice to nuclei of control and vice-versa) performed on the mature liver, indicate receptor specificity. These findings reveal tissue- and age- specific variations in the level of GR that is not influenced under diabetic conditions. However, the activation of hepatic GR is reduced during STZ-induced diabetes that might play a role in controlling glucose homeostasis in diabetic animals.

Keywords: Streptozotocin; Diabetes; Mice; Postnatal ages; Glucocorticoid receptor

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1. Introduction

Glucocorticoids (GCs) are indispensable for the maintenance of homeostasis and for allowing the body to respond to external and internal alterations (Miller and Tyrrel, 1995). The whole range of actions of GCs is mediated through binding to high affinity glucocorticoid receptors (GRs), which are ubiquitously expressed ligand-inducible transcription factors that encompass members of the nuclear hormone receptor superfamily (Beato et al., 1995; Kellendonk et al., 1999). Upon entry into target cells, GCs bind to cytosolic GRs and the hormone-receptor complexes undergo activation and translocation into the nucleus. Activation of GR is a time-dependent, multifactorial process, involving dissociation of several chaperone proteins which maintain the GR complex in a conformation optimal for hormone binding. Subsequent apposition of the hormone-receptor complexes to regulatory DNA sequences of target genes modulate their expression (Tsai and O'Malley, 1994).

GCs are diabetogenic hormones since they decrease glucose uptake and increase hepatic glucose production under normal conditions (Delaunay et al., 1997). In liver, GCs are primarily gluconeogenic whereas, they influence renal glomerular filtration rates, ion transport and other metabolic functions. To evaluate the possible role of diabetes on the modulation of GR function, we induced diabetes in mice, with streptozotocin (STZ), a drug which selectively destroys insulin-producing β-cells of the pancreas, providing a model of type 1 diabetes. The level, affinity (K_d) and activation process of GR were studied in order to find out modulation of such functions during diabetic conditions. Several investigators have reported GR function modulation in diabetic animals. However, reports on age- and tissue- specific effects of diabetes on GR are scanty. A significant decrease in hepatic GR level of STZ-induced diabetic rats compared to control was earlier reported (Yourick and Beuving, 1985). However, a report indicates no change in the GR content and affinity (K_d) in the pancreas of STZ-induced diabetic rats (Svec, 1985). Studies of GR in the brain and liver in genetically diabetic (mdb/mdb) mice indicate decreased GR level and activation (Webb et al., 1986). In the present study, we have compared GR concentration and activation properties in diabetic and normal mice at the preweaning (immature) and postweaning (mature) ages in the liver and kidney. We report no change in receptor concentration both in the liver and kidney and a significant change in the activation of hepatic GR complexes in diabetic animals compared to control.

2. Materials and methods

2.1. Animals and chemicals

Male Swiss albino mice (balb/c strain), maintained under standard colony conditions, of two different postnatal age groups (15- and 120-day old) were used. [1,2,4,6,7-^{3}H]dexamethasone (sp. act. 89 Ci/mmol) was purchased from Amersham,

England. Nonradioactive dexamethasone and other biochemicals were from Sigma Chemical Company USA. Routine chemicals used were of highest analytical grade.

2.2. Buffers

(A) 0.25 M sucrose/10 mM Tris–HCl, pH 7.5/1 mM EDTA/10 mM sodium molybdate/10% (v/v) glycerol/1 mM dithiothreitol/10 mM NaCl; (B) 0.25 M sucrose/10 mM Tris–HCl, pH 7.6; (C) 0.25 M sucrose/10 mM Tris–HCl, pH 7.6/0.5% (v/v) Triton X-100.

2.3. Preparation and assay of glucocorticoid receptors

The mice were killed by cervical dislocation at a fixed time of the day (10:00 h), their livers and kidneys were quickly removed, washed in chilled normal saline (0.9% NaCl) and blotted dry. A 20% homogenate of these tissues was prepared in chilled buffer A and centrifuged at 27 500 × *g* for 60 min at 2°C. Pilot experiments were done to find out the maximally saturable equilibrium binding and the time taken for [^{3}H]dexamethasone. Aliquots (100 μl) of clear, fat-free cytosol were incubated with 40 nM [^{3}H]dexamethasone alone or with 500-fold excess nonradioactive dexamethasone for 4 h at 0°C to get the maximally saturable binding. For Scatchard analysis, 1–120 nM [^{3}H]dexamethasone was used. Fifty microliter dextran-coated charcoal (4% activated charcoal + 0.4% dextran T-70) was added to remove any unbound steroid. Specific saturable binding was obtained by subtracting the radioactivity (CPM) bound in the presence of unlabeled dexamethasone from that bound in the presence of labeled dexamethasone alone. The number of specific binding sites and the dissociation constant (K_d) were calculated from Scatchard plot (Scatchard, 1949).

2.4. DNA cellulose and nuclear binding assay

Pooled tissues from 4–5 mice of each age group were homogenized in buffer B and centrifuged at 2000 × *g* for 10 min at 2°C to sediment nuclei. Supernatant thus obtained was further centrifuged at 27 500 × *g* for 60 min at 2°C. Finally, the fat-free cytosol was incubated with 40 nM [^{3}H]dexamethasone for 4 h at 0°C; bound hormone-receptor complexes were separated using dextran-coated charcoal (in buffer B). These complexes were then subjected to activation by heat (25°C) and salt (20 mM Ca^{2+} at 0°C) for 45 min (Schmidt et al., 1985; Sharma and Timiras, 1988). The magnitude of activation was determined by incubating the hormone-receptor complexes with pre-washed DNA cellulose pellet for 60 min at 0°C. DNA cellulose-bound hormone-receptor complexes were obtained by washing the pellets twice with buffer B. The final pellets were suspended in 4 ml of cocktail-T and bound radioactivity counted in a Beckman LS 1801 liquid scintillation counter with 65% efficiency for tritium. For the nuclear binding assay, crude nuclei obtained as above were further purified using buffer C and suspended finally in buffer B. Pellets containing 100–150 μg DNA were incubated with hormone-receptor complexes and processed in the same manner as describe for DNA cellulose.

2.5. Protein and DNA estimation

Protein concentration of the receptor preparation was estimated according to the dye-binding method of Bradford (1976), using BSA as standard. DNA content of purified nuclear suspension was determined by the method of Burton (1956).

2.6. Blood glucose estimation

Blood glucose level was routinely determined using a glucometer according to the user's guide with certain modifications. Blood was collected from the tail and a drop of blood was applied to the pad of the glucose stripes. After 20 s, the pad surface was carefully blotted dry and inserted into the test slot of the glucometer. The values of glucose levels, as indicated in the display screen, were recorded. The final blood glucose concentration was concurrently estimated with *O*-toluidine method (Sigma Technical Bulletin, 1980).

2.7. Streptozotocin treatment

Overnight fasted mice were injected intraperitoneally with a single dose of STZ (20 mg/100 g body weight) in ice-cold 0.1 M sodium citrate buffer, (pH 4.5). Control mice received only the vehicle of STZ. Mice were allowed food and water ad libitum and the blood glucose levels were determined routinely. When the blood glucose level was increased to three fold or more compared to control values, the mice were killed on day 7 after STZ treatment. Tissues were removed and processed similarly as above.

2.8. Statistical analysis

Data obtained from different sets of experiments were analyzed statistically. The level of significance (*P*-value) between two sets of data was calculated according to Student's *t* -test.

3. Results and Discussion

Glucocorticoids exert a range of metabolic effects on a variety of animal tissues including liver and kidney. Development and aging may partly be characterized by changes in the responsiveness of tissues and cells to certain hormonal modulators (Roth, 1988; Singh and Sharma, 1995). These hormone-mediated responses are attributed by binding to specific high affinity intracellular receptors, by activation of hormone-receptor complexes and subsequent nuclear translocation of these complexes where they interact with specific acceptors' sites in the promoter regions of responsive genes. STZ has earlier been reported to induce diabetes in experimental animals (Yourick and Beuving, 1985). A single large dose of STZ is sufficient to induce hyperglycemia resulting from a loss of pancreatic β-cells. This alkylating

agent induces high levels of DNA strand breaks in β-cells and finally cell death (Pieper et al., 1999). In our study, blood glucose levels (mg/dl) of STZ-treated animals were found to be elevated by approximately 3.5 fold to that of controls and hence, ensured that animals had responded to STZ and were diabetic. The diabetic effect was age-independent (Fig. 1).

Our study of Scatchard analysis indicates a reduced (22–33%) level of GR in the liver and kidney of mature (120-day) mice compared to immature (15-day) in control groups (Table 1). Also slopes of the plots (not shown) exhibit no alteration in the affinity (K_d) of GR for its cognate ligand at these two ages. The higher level of GR in the liver and kidney of immature mice may be a contributory factor for the role of this hormone in early growth and development of mice (Munck et al., 1984). It has earlier been reported that the GR level is higher in the young liver and kidney of rats (Sharma and Timiras, 1987, 1988; Kalinyak et al., 1989). These results agree with earlier reports (Kalimi, 1984; Kalimi et al., 1988) that there is no apparent age-associated alteration in the binding affinity of GR in both these tissues. STZ-induced diabetes did not alter the level of GR in either of the tissues and ages studied. These findings reveal that the level of GR exhibits tissue- and age-specific correlation and is not influenced by diabetic conditions. Our findings of no change in the level and affinity of GR contrast with the report of reduced GR-specific binding sites by Yourick and Beuving (1985). However, it corroborates with the observation of Svec (1985).

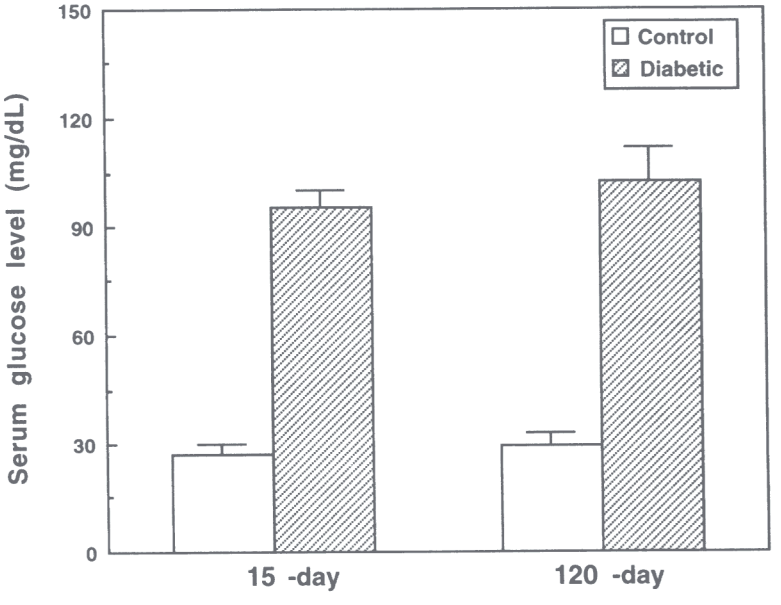

Fig. 1. Serum glucose level in streptozotocin- treated diabetic and control mice of 15- and 120-day old. Values are mean of 4–5 mice in each age group. Bars represent standard deviation. The observed differences are statistically significant ($P < 0.001$) as compared to control.

Table 1
Concentration and affinity of [^{3}H]dexamethasone-receptors in the liver and kidney of immature (15-) and mature (120-day) control and diabetic mice[a]

Condition	Tissue	Age (Days)	Number of specific binding sites (fmol/mg protein)	K_d (nM)
Control	Liver	15	192.00 ± 7.10	3.10 ± 0.25
		120	148.80 ± 6.63[b]	2.78 ± 0.30
	Kidney	15	144.00 ± 5.93	3.80 ± 0.36
		120	97.00 ± 4.89[b]	3.70 ± 0.33
Diabetic[NS]	Liver	15	189.70 ± 6.79	3.20 ± 0.28
		120	147.30 ± 5.69	2.78 ± 0.30
	Kidney	15	141.00 ± 6.62	3.70 ± 0.35
		120	95.10 ± 5.08	3.80 ± 0.35

[a] The data were collected from 4–5 mice of each age group and analyzed using Scatchard plot as given in the Section 2. The results are mean ± standard deviation of four separate experiments for each age group.

[b] Statistically significant ($P < 0.05$) with respect to day 15; NS, not signaificant as compared to control.

Our study of the activation process of the GR complexes at two ages in the liver and kidney of control and diabetic mice using DNA cellulose and nuclear binding assays shows that both temperature and salt significantly increase the DNA cellulose binding of the hormone-receptor complexes in both the tissues and ages in diabetic and control animals. The GR from both the tissues underwent thermal activation, albeit the extent of activation was more pronounced in mature liver compared to immature, with no such difference of activation in the kidney. A higher level of activation of hepatic GR has earlier been reported in male Long-Evans rats (Sharma and Timiras, 1987). Greater thermal activation of GR in mature animals may compensate for the low receptor level and support for the role of this hormone at such phase of lifespan. The observed difference in activation of GR may be due to the differences in the endogenous modulators of GR functions. In diabetic mice, the thermal activation of hepatic GR exhibits reduced (~ 20–23%) DNA cellulose binding compared to control (Fig. 2A). In contrast, thermal activation of kidney GR does not show marked differences in diabetic mice at either of the ages studied (Fig. 2B). Salt-dependent activation of GR complexes does not exhibit any change in either tissues and ages of mice (Fig. 2A and B). Since DNA cellulose being a non-specific binding system could not unequivocally implicate differences in the activation of GRs in control and diabetic mice, purified nuclei were used to provide a more physiological assay system than the DNA-cellulose binding assays. Results indicate that nuclear binding of thermally activated hepatic GR complexes is also significantly reduced (24–30%) in diabetic mice compared to control at both the ages (Fig. 3A). Again, the nuclear binding of thermally activated kidney GR does not show marked difference in diabetic mice at either of the ages (Fig. 3B). Data from cross-mixing experiments (thermally activated GR of diabetic and nuclei of control and vice-versa) performed only on

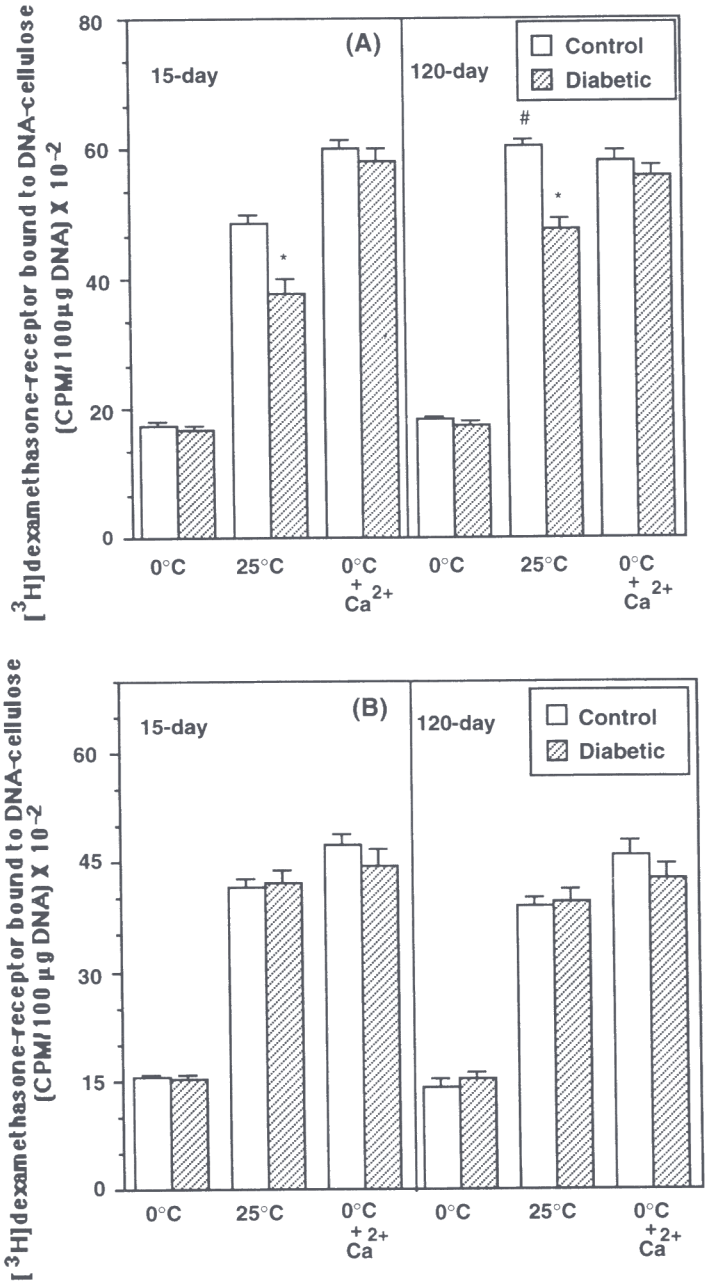

Fig. 2. Binding of hepatic (A) and kidney (B) [^{3}H]dexamethasone-receptor complexes to DNA-cellulose in 15- and 120-day old control and diabetic mice. Cytosol from these tissues were prepared in buffer without molybdate and the hormone-receptor complexes obtained by incubating with 40 nM [^{3}H]dexamethasone for 4 h at 0°C. Activation, DNA-cellulose binding and further processing of the pellets were performed as described in Section 2. The results are mean ± standard deviation of four experiments with 4–5 mice of each age group. *Statistically significant ($P < 0.05$) compared to control. # Significantly ($P < 0.05$) higher thermal activation as compared to 15-day control.

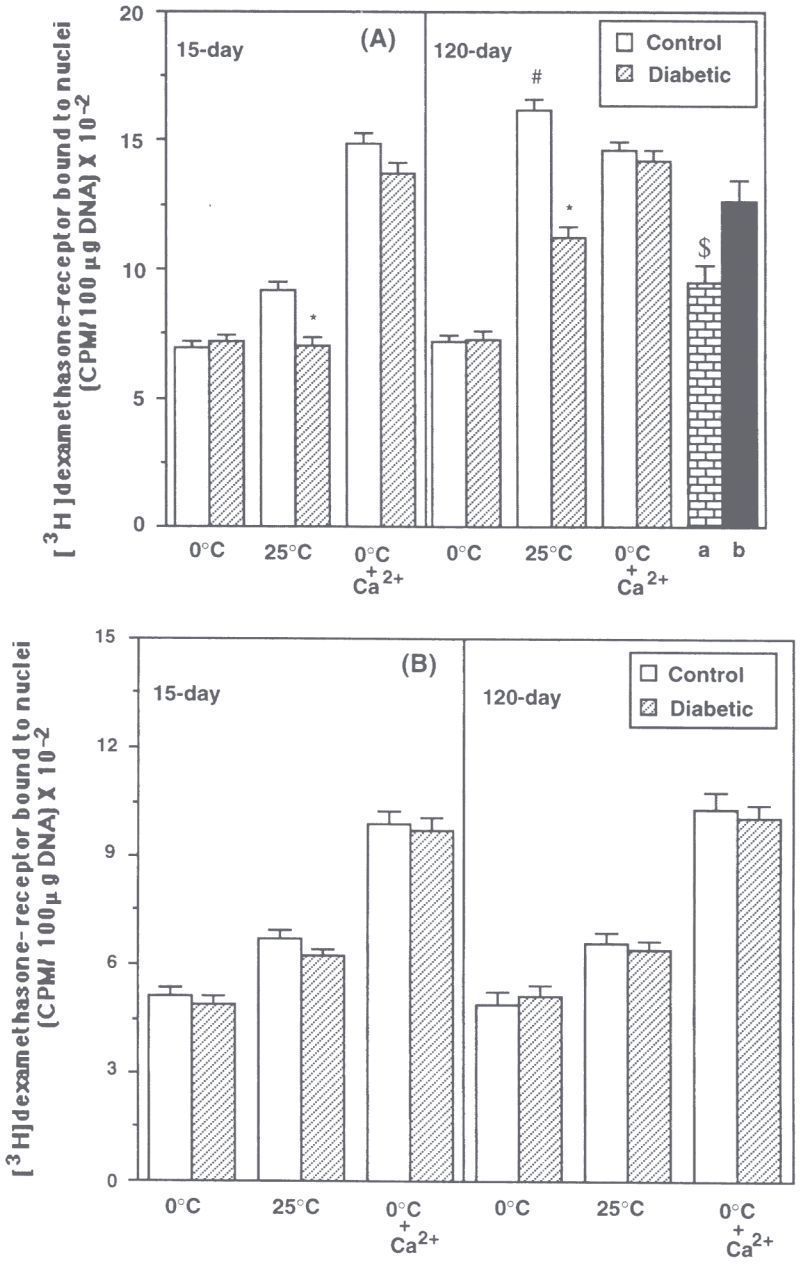

Fig. 3. Binding of hepatic (A) and kidney (B) [^{3}H]dexamethasone-receptor complexes to purified nuclei in 15- and 120-day old control and diabetic mice. Purified nuclei of these tissues were used instead of DNA-cellulose for activation studies. Other experimental procedures are same as for Fig. 2. *Statistically significant ($P < 0.05$) compared to control. The (a) and (b) barograms in (A) represent cross-mixing experiments in which 25°C activated GR complex from 120-day old diabetic mice incubated with the nuclei of 120-day old control (a) and 25°C activated GR complex from 120-day old control mice with the nuclei of 120-day old diabetic (b). $^{\$}$Statistically significant ($P < 0.05$) compared to (b). $^{\#}$ Significantly ($P < 0.05$) higher thermal activation as compared to 15-day control.

120-day-old mice's liver showed significantly lower (25%) nuclear binding of diabetic GR, indicating receptor specificity (Fig. 3A). Thus, a STZ-induced diabetic decrease in the thermal activation of hepatic GR is found to be tissue specific.

During diabetes several metabolic adjustments take place to tailor high circulating blood glucose levels. Glucocorticoid and GR might play a critical role in such metabolic conditions. Our findings indicate that STZ-induced diabetes reduces activation of hepatic GR that might play an influencing role in controlling glucose homeostasis in diabetic animals. The precise mechanism of GR regulation during STZ-induced diabetes is, however, unclear. Moreover, it will be of much clinical significance, in future, to unravel the correlation between STZ-induced diabetes and the regulation of GR function.

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