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Glucocorticoid Receptor Regulation During Streptozotocin Induced Type1 Diabetes in Mice

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Summary

Glucocorticoid receptor (GR) is an ubiquitously expressed transcription factor involved in the regulation of many different physiological processes. Activated by endogenous glueocorticoids, the receptor regulates gene expression positively or negatively by direct binding to glucocorticoid responsive elements (GREs) or by protein-protein interaction. Streptozotocin (STZ) selectively destroys insulin producing β cells of the pancreas, providing a model of Typel diabetes. STZ induced diabetic effects were analyzed for GR level and for in vitro activation of GR by maximum saturable binding analysis, using [3H]dexamethasone, and by DNA-cellulose binding characteristic respectively, in the liver and kidney of IS- (immature) and 120-day-old (mature) male mice. Comparison of GR level (fmoilmg protein) among the control mice reveals decreased (25-30%) maximum saturable binding in the liver and kidney of mature mice than immature ones. However, Scatchard analyses reveal no change in the affinity of receptor at these two ages. STZ-induced diabetes does not alter the level of GR in either of the tissues at both the ages of mice. The GR from both the tissues underwent thermal activation, albeit the extent of activation (cpm/10Ogg DNA) was more pronounced in mature liver compared to immature, with no such difference of activation in the kidney of mice. In diabetic mice, the activation of hepatic GR exhibits reduced (-20-23%) DNA-cellulose binding compared to control mice. In contrast, thermal activation of kidney GR does not show marked difference in diabetic mice at either of the ages studied. These findings reveal that the level of GR exhibits tissue- and age-specific correlation and is not influenced under diabetic conditions. However, the activation of hepatic GR is reduced during diabetes that might play an important role in controlling glucose homeostasis in diabetic animals.

Introduction

Glucocorticoids (GCs) are an absolute necessity for the maintenance of haemostasis and for allowing the body to respond to external as well as internal alteration's(1). GC levels are precisely controlled by an endocrine cascade, the hypothalamus-pituitary-adrenal axis(HPA). The entire ranges of actions of GCs are transmitted through binding to high affinity intracellular cytoplasmic glucocorticoid receptors (GRs), which are ubiquitously expressed ligand- inducible transcription factors that encompass members of the nuclear hormone receptor superfamily[2,3]. Upon entry into the target cells, G0s bind to cytosolic receptors and the hormone-receptor complexes undergo activation and translation into the nucleus Activation involves dissociation of several chaperone proteins (heat shock proteins immunophilins, cyclophilins etc.) which maintain the GR complex in an inactive form, yet in a

confirmation optimal for hormone binding. Subsequent interactions of the hormone-receptor complexes with regulatory DNA sequences of target genes modulate their expression[4]. GCs play an important role in glucose metabolism and that they are diabetogenic hormones since they decrease glucose uptake and increase hepatic glucose production in normal conditions[5]. In liver, GCs primarily induce gluconeogenic effects whereas, in kidney, they regulate glomerular filtration rate, ion transport and other metabolic functions. To evaluate the possible effect of diabetes on the modulation of GR function, we experimentally induced diabetes in mice, using streptozotocin (STZ), a drug that selectively destroys insulin producing β-cells of the pancreas, providing a rnodel of type-1 diabetes. The concentration affinity (Kd) and activation processes of GR were studied in order to find out modulation of such functions during diabetic conditions. Several workers 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[6]. However, a report indicates no change in the GR content and affinity (Kd) in the pancreas of STZ-induced diabetic rats[7] Analysis of GR in the brain and liver in genetically diabetic (mdblmdb) mice indicate reduced
GR level and activation[8]. 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 decrease in the activation of hepatic GR complexes, in diabetic animals compared to control.

Materials and methods

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. [1,2,4,6,7-3H]dexamethasone (sp. act. 89 Cilmmol) was purchased from Amersham, England. Nonradioactive dexamethasone and other biochemicals were from Sigma Chemical Company, USA. Routine chemicals used were of highest analytical grade.

Buffers

(A) 0.25 M sucrose/ 1 0 mM Tris-HCl, pH 7.51 1 mM EDTA/ 1 0 mM sodium molybdatel 1 0% (vlv) glycerol/ 1 mM dithiothreitoll 1 0 mM NACI ; (B) 0.25 M sucrose/ 1 0 mM Tris-HCl, pH 7.6

Glucocorticoid receptor preparation and assay

The mice were killed by cervical dislocation at a fixed time of the day (10:00h), their livers and kidneys were quickly removed, washed in chilled normal saline (0.9% NACI) and blotted dry. A 20% homogenate of these tissue was prepared in chilled

buffer A and centrifuged at 27,500 x g for 60 min at 2"C. Pilot experiments were done to find out the maximum saturable equilibrium binding and the time taken for [3H]dexamethasone. Aliquots (1 00 gi) of clear, fat free cytosol were incubated with 40 nM [3H]dexamethasone alone or with 500-fold excess nonradioactive dexamethasone for 4h at OIIC to get the maximum saturable binding. For Scatchard analysis, 1-120 nM [3H]dexamethasone was used. 50 gi 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 presence of uniabeled dexamethasone from that bound in presence of labelled dexamethasone alone. The number of specific binding sites and the dissociation constant (Kd) were calculated from Scatchard plot[9].

DNA-cellulose binding assay

Pooled tissues from 4-5 mice of each age group were homogenized in buffer B and centrifuged at 2,000 x g for 10 min at 2"C to sediment nuclei and other cellular debris Supernatant thus obtained was further centrifuged at 27,500 x g for 60 min at 20C. Finally, the fat free cytosol was incubated with 40 nM [3H]dexamethasone for 4 h at 0"O ; bound hormone-receptor complexes were separated using dextran coated charcoal (in buffer B).

These complexes were then subjected to activation by heat (25110) and salt (20 mM Ca2+ at O-C) for 45 min[10,11]. 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 mi of cocktail-T and bound radioactivity counted in a Beckman LS 1801 liquid scintillation counter with 65% efficiency for tritium.

Blood glucose estimation

Blood glucose level was routinely determined using glucometer according to the user's guide with certain modifications. Blood was collected from the tail of the mice and a drop of blood was applied to the pad of the glucose stripes. After 20 seconds, the pad surface was carefully blotted dry and inserted into the test slot of the glucometer. The. values of glucose level, as indicated in the display screen, were recorded. The final blood glucose concentration was concurrently estimated with 0-toluidine method[12].

Streptozotocin treatment

Two groups of overnight starved mice of 15- and 120- day old were injected with STZ intraperitoneally as a single dose of 20 mgll 009 body weight in ice-cold 0. 1 M

sodium citrate buffer, (pH 4.5). A set of control mice received only the vehicle. Mice were allowed food and water ad libitum and the blood glucose level determined routinely. Animals whose blood glucose level was increased to 3 fold or more compared to control were killed on 7th day of STZ treatment. Tissues were removed and processed similarly as above,

Protein estimation and statistical analysis

Protein concentration of the receptor preparation was estimated according to the dye- binding method of Bradford[13], using BSA as standard. The level of significance (p -value) between two sets of data was calculated according to Student's t -test

Results and Discussion

Glueocorticoids serve a variety of important functions in the body including liver and kidney. They are not only involved in controlling adult physiology but also influence development and ageing processes. Development and ageing may partly be characterized by changes in the responsiveness of tissues and cells to certain hormonal modulators[14,15] The mechanisms of action of GCs are mediated by binding to specific high affinity intracellular receptors, by activation of hormone-receptor complexes and by subsequent nuclear transiocation and apposition of these complexes with specific acceptors' sites in the promoter regions of responsive genes, culminating in upregulation or downregulation of target gene expression[4]. STZ has earlier been reported to induce diabetes in experimental animals[6] A single 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[16]. In our study, blood glucose levels (rng per 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. Both these ages responded to an almost similar extent as evident in fig 1
Scatchard analysis of the binding data indicates a reduced (22-33%) concentration (fmoilmg protein) of GR in the liver and kidney of mature (120-day) mice compared to immature (15-day) in control groups (Table 1). However, slopes of the plots (not shown) exhibit no significant change in the affinity (Kd) of GR for [3H]dexamethasone at these two ages. The increased 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 [17]. It has earlier been reported that the GR concentration is higher in the young liver and kidney of rats [11,18,19]. These results agree with earlier reports[20,21] that there is no apparent age- associated alteration in the binding affinity of GR in both the 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 alterations and is not influenced under diabetic conditions. Our findings of no change in the level and affinity of GR corroborate the observation of Svec[7] and contrast the report of reduced GR specific binding sites by Yourick and Beuving[6].

Our study on the activation process of the GR complexes at two ages in the liver and kidney of control and diabetic mice shows that both temperature and salt significantly enhance the DNA-cellulose binding of the hormone-receptor complexes in both the tissues and ages in diabetic and control mice. 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 degree of activation of hepatic GR has earlier been reported in male Long-Evans rats[18]. Higher thermal activation of GR in mature mice may compensate for the low receptor level and support the role of this hormone at such phase of life span. 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. 2 A). In contrast, thermal activation of kidney GR does not show marked difference in diabetic mice at either of the ages studied (Fig. 2 B). Salt- dependent activation of GR complexes does not exhibit any alteration in either tissues and ages of mice (Fig. 2 A&B). Thus, STZ-induced diabetic decrease in the thermal activation of hepatic GR is found to be tissue specific.

Diabetes causes several metabolic adjustments in animal's body to tailor hyperglycemia. Glucocorticoid and its receptor might play an important regulatory role in such metabolic interventions. Our findings indicate that STZ-induced diabetes reduces activation of hepatic GR that might play an influencing role in regulating glucose homoeostasis in diabetic animals. The precise mechanism of GR regulation during STZ-induced diabetes is, however, not understood. Moreover, it will, be of much significance, in future, to reveal the correlation between STZ-induced diabetes and GR function regulation.

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Table

Number of specific binding sites and affinity of [3 H]dexamethasone-receptors in the liver and kidney of immature (15-) and mature (120-day) control and diabetic mice.

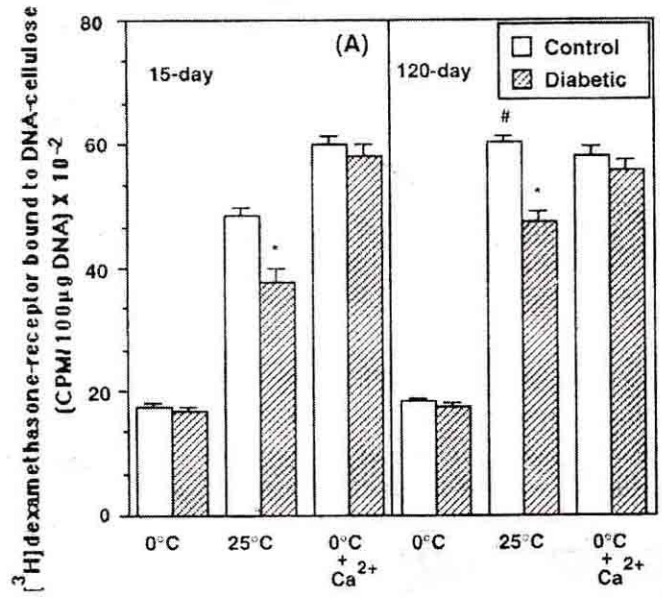
Conditions	Tissue	Age (Days)	Number of specific binding sites (fmol/mg protein)	Kd (nM)
Control	Liver	15	192.00+/- 7.10	3.10+/-0.25
		120	148.80+/-6.63*	2.78+/-0.30
	Kidney	15	144.00±5.93	3.80±0.36
		120	97.00±4.89*	3.70±0.33
Diabetic [NS]	Liver	15	189.70±6.79	3.20±0.28
		120	147.30±5.69	2.78±0.30
	Kidney	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 materials and methods. The results are mean± standard deviation of four separate experiments for each age group. * Statistically significant ($p<0.05$) with respect to day 15.
NS, not significant as compared to control.

Figure legends

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.

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 B and the hormone-receptor complexes obtained by incubating with 40 nM [3H]dexamethasone for 4 hr at OIIC. Activation conditions, DNA-cellulose binding and



other procedures were performed as described in materials and methods. 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.