Time Dependent Effects of Dexamethasone on Serum Insulin Level and Insulin Receptors in Rat Liver and Erythrocytes

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Abstract. The effects of glucocorticoid excess on regulation of insulin receptors were investigated in dexamethasone-treated rats. Glucocorticoid excess was produced by administration of dexamethasone (0.5 mg/100 g b.w.) 30 min, 4, 12, 18, 24, 42 or 70 h before experiments. This treatment caused time-dependent changes of glucose and insulin concentration in blood, as well as in amounts of specific insulin binding and insulin receptors of liver cells and erythrocytes. The time intervals in which dexamethasone produced the increase in insulin concentration were accompanied with decrease in insulin binding to receptors in membranes of liver cells, while significant changes in insulin binding to receptors of erythrocytes were not observed under the same experimental conditions. The effect is maximal 18 and 42 h after dexamethasone treatment that increase insulin blood level by about 85 % and 60 %, respectively. Receptor analysis revealed that changes in specific binding of insulin could be due to significant changes in amount of binding sites on cell surface rather than to mild alteration in receptor affinity. These findings suggest that besides the changes in insulin level, the alterations in insulin receptor number and affinity may play a major role in the states of altered insulin sensitivity which accompany glucocorticoid excess.

Key words: Insulin receptor — Insulin — Dexamethasone — Erythrocytes — Liver

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Introduction

The effects of a certain kind of hormone in appropriate target cell comprise also the regulation of binding of the other hormones to the same cell and, thereby, potentially modulates the biological response to the other hormones (Montiel et al. 1987). It has been documented that glucocorticoid hormones play the very significant role in the expression of insulin (INS) action on the synthesis of protein, glycogen and lipids (Folli et al. 1996). The clinical findings also indicate that the treatment of patients with large doses of glucocorticoids is often accompanied by a development of INS resistant state (Andrews and Walker 1999; Reynolds and Walker 2003). Accordingly, the elucidation of the effect of the glucocorticoid on insulin receptor (IR) and INS responsiveness is very important.

It has been documented that glucocorticoids have influence on INS binding and its action, both *in vivo* and *in vitro* conditions (for review, see Folli et al. 1996). The effect of these hormones on the IR depends on the experimental conditions, i.e. on the applied glucocorticoid and cell or tissue studied (Rouiller et al. 1988). Glucocorticoid added to adipocytes (Buren et al. 2002) and 3T3 fibroblasts (Grunfeld et al. 1981) decreased INS binding and on the other hand it increased IR binding in lymphocytes (Fantus et al. 1982) and human promonocytic U-937 cells (Leal et al. 1992), while receptor in hepatocytes has remained unchanged (Klein et al. 2002). The published results also suggest that an increase in the level of IR mRNA could be an early step in the regulation of the IR by glucocorticoids (Hines et al. 1994; Lee and Tsai 1994). Although there is no obvious explanation of these discrepancies, the cell, i.e. tissue-specific differences may be responsible for variety of glucocorticoid effects on regulation of IR.

Besides that, the IR on the plasma membrane are the subject of dynamic regulation, which is influenced by variety of factors. INS binding in most cells induces an internalization of the hormone-receptor complex. The intenalized receptor is at least partly degraded, but the rate of the *de novo* synthesis mainly determines the final number of receptors in the plasma membrane (Okabayashi et al. 1989). These processes are some of the steps in which glucocorticoids can influence the mechanism of INS action. The published results also indicate that glucocorticoids could be indirectly involved in INS secretion through their effect on the enhancement of the blood glucose levels (Delaunay et al. 1997).

In order to bring about to the elucidation of above observations, the relationship between glucose and INS level in blood and INS binding to purified erythrocytes as well as liver plasma membranes prepared from animals treated with dexamethasone (DEX) at various time intervals have been investigated in the present study. The obtained results indicate that DEX induces a time dependent changes in amount of glucose and INS in blood as well as IR in the plasma membranes of analyzed cells.

Materials and Methods

Materials

INS RIA kit as well as [^{125}I] INS (SA $\sim$ 200 μCi/μg) was a product of Department for Radioisotopes, "Vinča" Institute of Nuclear Sciences, Belgrade, Serbia and Montenegro. Porcine INS was purchased from Galenika, Zemun, Serbia and Montenegro. All other chemicals of p.a. grade were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Animals

All experiments were conducted with 2–3 months old male rats of Wistar strain, weighting 200–220 g. They were maintained under controlled environment (22–24 ℃), 12/12 h light-dark schedule, with standard rat chow and water, supplied *ad libitum*. Groups of 3 animals in each analyzed time point were injected intraperitonealy with DEX (0.5 mg DEX in 0.1 ml 0.14 mol/l NaCl/100 g b.w.) 30 min, 4, 12, 18, 24, 42 or 70 h before experiments. Animals of control groups (2 rats in each time point) were injected with the same volume of 0.14 mol/l NaCl at the same time intervals as treated rats. Experiments were repeated 7 times. All experiments were started between 8:00 and 10:00 h a.m. All possible consideration was given to the care of animals. Experimental protocols were approved by local ethical committee and they were in compliance with "Good laboratory animal practise".

The heparinized blood of each analyzed groups of animals has been used for preparation of plasma and erythrocytes as previously described (Ribarac-Stepic et al. 1988).

INS binding assay

For preparation of liver cell membranes, the method of Hoffman et al. (1987) was used. Livers were homogenized in Potter-Elvejhem homogenizer in TES buffer, pH 7.4 (25 mmol/l Tris, 1.25 mmol/l EDTA, 0.25 mol/l sucrose, with protease inhibitors cocktail) 1 : 4 (m : V) on 4 ℃. Homogenate was centrifuged 30 min on 12,000 $\times$ *g*, NaCl and $MgSO_4$ were added to the supernatant in final concentration of 100 mmol/l and 0.2 mmol/l, respectively. Membranes were pelleted 40 min on 40,000 $\times$ *g*, washed two times in 50 mmol/l Tris (pH 7.4) and finally resuspended in the same buffer containing 0.1 % bovine serum albumin (BSA). This suspension (crude plasma membranes) was used for INS binding.

Erythrocytes were isolated according to method of Gambhir et al. (1977). Fresh rat blood was drawn in heparinized tubes and centrifuged 10 min at 2,500 rpm on 4 ℃ to remove plasma and white blood cells. Erythrocyte pellet was washed 3 times with saline and once with the buffer, pH 7.4 (in mmol/l: 50 HEPES, 50 Tris, 2 EDTA, 10 glucose, 10 $MgCl_2$, 10 $CaCl_2$, 5 KCl, 50 NaCl, 0.1 % BSA) and finally resuspended in the same buffer. After counting of erythrocytes, suspension was adjusted for binding assay to contain 1×10^9 cells/ml.

INS binding to liver membranes and erythrocytes was performed according to slightly modified method of Zorad et al. (2003) and Gambhir et al. (1977), respectively.

Liver cell membranes (50 μg of plasma membrane proteins) were incubated 18 h on 4 ℃ with 0.2 nmol/l [^{125}I] INS in presence of increasing concentration of unlabeled INS (1 pmol/l–1 μmol/l) in Tris buffer (pH 7.4), containing 0.1 % BSA. The reaction was stopped by addition of 16 % polyethylene glycol. After centrifugation, supernatant was removed and pellet of plasma membranes was counted in the gamma counter (Nuclear Enterprises, Ltd., Edinburgh, Scotland, UK)

To determine INS binding to erythrocytes, 400 μl of erythrocyte suspension was incubated with 0.1 ng of [^{125}I] INS and various amount of unlabeled INS (0–10^5 ng) in the buffer used for resuspension of erythrocytes, in total volume of 0.5 ml. After 18 h incubation at 4 ℃, 200 μl of cold buffer was added to 200 μl of suspension and layered over 200 μl of dibutyl phthalate. After centrifugation, the erythrocyte pellet was counted in the gamma counter (Nuclear Enterprises, Ltd.).

Specific binding (SB) was calculated from binding of radioactive labeled INS in absence and presence of unlabeled INS excess. Receptor concentration and dissociation constant were obtained by Ligand program (Munson and Rodbard 1980) according to Scatchard method (1949). From Scatchard equation, the ratio of bound to free INS, expressed as a function of bound INS, results in a curvilinear plot which indicates the presence of two or more classes of binding sites. Curvilinear plot could be resolved in two linear components whose reciprocal value of negative slope is equal to the dissociation constant and whose abscissa intercept is equal to the number of binding sites (Pollet and Levey 1980).

Protein concentration was determined as previously described (Lowry et al. 1951).

Determination of blood glucose

Blood glucose level was measured by Accutrend analyzer (Roche Diagnostics GmbH, Mannheim, Germany) in control and DEX-treated animals (30 min, 4, 12, 18, 24, 42 or 70 h after treatment). For measurement of glucose concentration, animals were exposed to overnight fast.

Determination of serum INS

For measurement of INS concentration, animals were fasted overnight before collecting blood samples. INS was measured by RIA method according to instruction of the producer (Department for Radioisotopes, "Vinča" Institute of Nuclear Sciences), using rat INS as a standard.

Statistics

All data points presented in this study are the means ± SD of at least seven individual experiments which were performed for each time point, with 3 treated and 2 control animals in each of them. The SPSS program for Windows (Chicago, IL, USA) was used for statistical analysis. The results were evaluated by parametric

statistics, and the significance of the differences between two groups was estimated by ANOVA test. The level of statistical significance was $p < 0.05$. Pearson's correlation analysis were performed to examine the relationship of glucose with INS as well as INS with [^{125}I] INS SB to liver membranes and erythrocytes.

Results

DEX significantly increased glucose blood level (Fig. 1) starting from 12 h after treatment ($p < 0.01$ for 12, 18, 24 and 42 h; $p < 0.001$ for 70 h).

The administration of DEX to normal rats has resulted in a time dependent changes of INS concentration in blood. The excess of DEX (1 mg/200 g b.w.) alters the INS level in serum during observed time intervals (Fig. 2). The tendency of increase in INS concentration in serum has been observed already 30 min after DEX administration. However, significant increase ($p < 0.001$) was detected 18 h after treatment, while 24 h after steroid injection, the concentration of INS in serum has returned to about the control level. 42 h after DEX administration, the amount of INS again significantly increased ($p < 0.001$), and the concentration of INS decreased 70 h after steroid treatment up to control value. The obtained results indicate that DEX induced biphasic increase in INS concentration in serum of treated rats during 70 h (Fig. 2). There was no sig-

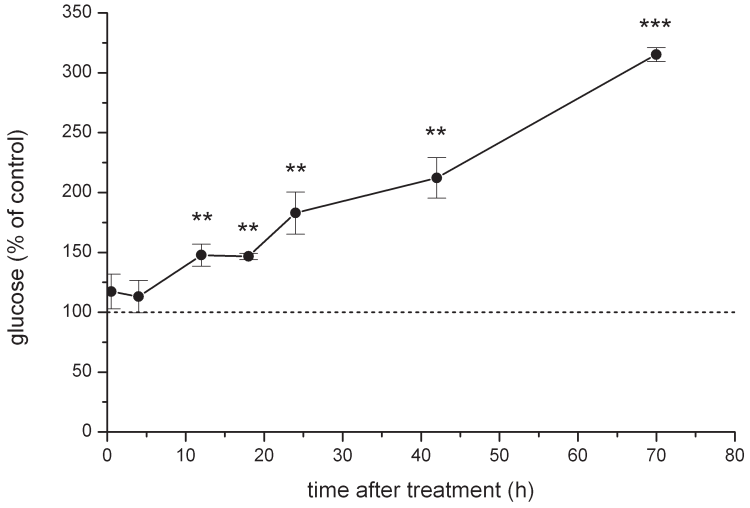

Figure 1. DEX effects on blood glucose concentration. The glucose concentration was measured in blood of control and DEX-treated rats (•) in different times after treatment as described in Materials and Methods. The values are expressed as the percent of time matched control animals. Each point represents mean value ± SD of 3 independent experiments included in this study. The groups for each time point consist of 3 animals (total $n = 9$). $^{**}p < 0.01$, $^{***}p < 0.001$.

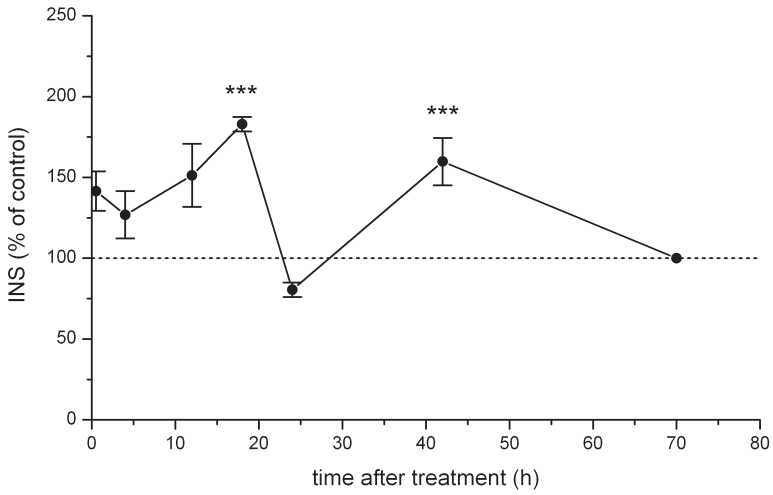

Figure 2. Time-dependent effects of DEX on INS level in serum. The concentration of INS was determined in serum of control or treated (●) animals as described in Materials and Methods. The values are expressed as the percent of time matched control animals. Each point with corresponding bars represents mean value ± SD of seven independent experiments included in this study. The groups for each time point consist of 3 treated (total $n = 21$) and 2 control animals (total $n = 14$). $^{***}p < 0.001$.

nificant correlation between blood glucose and serum INS level in DEX-treated group.

In order to explore the effect of DEX on INS binding to the receptors, the competition analysis between labeled and unlabeled INS was conducted in plasma membranes of liver and erythrocytes obtained from control and treated animals. It was detected that decrease in SB of INS to receptors in liver plasma membranes (Fig. 3) and increase in concentration of INS in serum (Fig. 2) were occurred at the same time intervals after DEX administration. The serum INS of the DEX group reciprocally correlated with SB of INS to liver plasma membranes ($r = -0.685$, $p < 0.05$). However, there were no significant changes in SB of INS to receptors of erythrocytes after glucocorticoid treatment (Fig. 4). In addition, SB of INS to receptors of erythrocytes did not show a significant correlation with serum INS after DEX treatment. These evidences indicate that glucocorticoids modulate concentration of INS in circulation as well as that they affect INS binding to its receptors depend on the kind of analyzed cells.

The results indicating that changes in SB of INS are the most significant ($p < 0.001$) after 42 h of DEX administration, allow the presumption that glucocorticoid can also affect the concentration of IR in plasma membranes of liver cells. In order to test this presumption, the displace of SB of INS to liver cell membranes has been analyzed as a function of increasing INS concentration. The results represented by

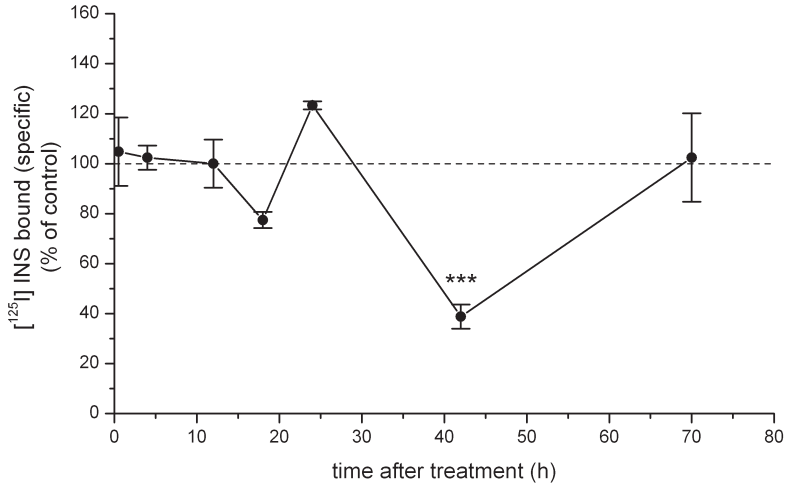

Figure 3. Time course of DEX effects on SB of INS in liver plasma membranes. The liver membranes were prepared from control or DEX-injected (●) rats at indicated time intervals. The purification of liver membranes, as well as [^{125}I] INS binding assay were carried out as described in the Materials and Methods. The SB to liver membranes of treated animals is expressed as percentage of SB to the control samples. Each data point represents mean value ± SD of 7 individual experiments performed with 2 control and 3 treated animals in each time interval. $^{***}p < 0.001$.

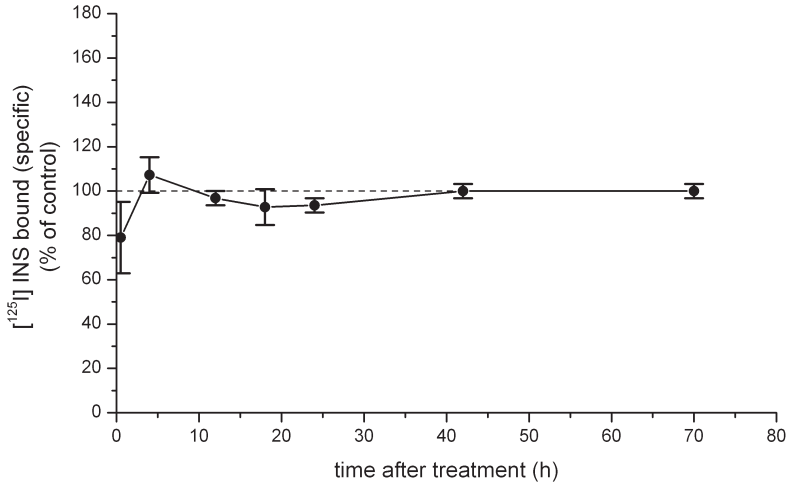

Figure 4. Time course of DEX effects on SB of INS in erythrocytes. The erythrocytes were prepared from control or treated (●) rats, injected at indicated time intervals by saline or DEX, respectively. The preparation of erythrocytes, as well as [^{125}I] INS binding assay were done as described in the Materials and Methods. The SB to erythrocytes of treated animals is expressed as percentage of specifically bound INS to the erythrocytes of control animals. Each data point represents mean value ± SD of 7 determinations using 3 treated and 2 control animals *per* individual time interval.

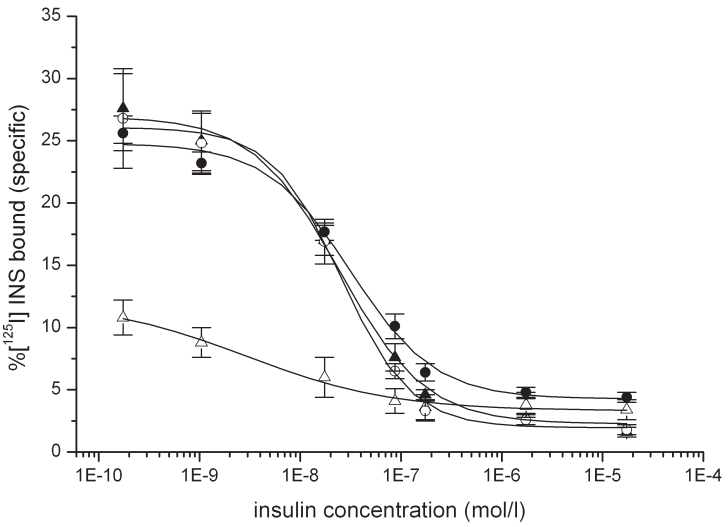

Figure 5. Effect of DEX on SB of INS to membranes of liver cells. Displacement of [^{125}I] INS binding by increasing concentration of unlabeled INS was determined in liver plasma membranes prepared from control rats (●) or animals treated by DEX 30 min (▲), 42 h (△) or 70 h (○) before experiments. All other procedures were conducted as described in Materials and Methods. Each point with corresponding bars represents mean value ± SD of 7 experiments included in this study.

Table 1. Time dependent effects of DEX on IR in rat liver. The experimental results were obtained as described in the Materials and Methods and analyzed by Scatchard plot using two-site model for INS-receptor interaction. Data are presented as mean ± SD

	Kd_1 (nmol/l)	Kd_2 (nmol/l)	N1 (pmol/mg prot.)	N2 (pmol/mg prot.)
Control	9.10 ± 2.00	62.50 ± 6.05	0.38 ± 0.05	7.12 ± 0.97
30 min	8.00 ± 2.05	61.50 ± 5.50	0.40 ± 0.07	7.60 ± 1.80
42 h	7.30 ± 0.25*	73.00 ± 7.00*	0.11 ± 0.01**	2.09 ± 0.28**
70 h	9.09 ± 3.05	62.50 ± 6.00	0.25 ± 0.06	4.75 ± 1.01

Kd_1, dissociation constant of high affinity binding sites; Kd_2, dissociation constant of low affinity binding sites; N1, amount of receptor high affinity binding sites; N2, amount of receptor low affinity binding sites; $^{*}p < 0.02$; $^{**}p < 0.001$.

displacement curves (Fig. 5) indicate that 30 min after DEX treatment there is no significant change in INS binding. DEX markedly reduced INS binding to its receptor 42 h after administration. The higher decrease has been observed at low INS concentration while at high concentration of INS, the curves have had tendency

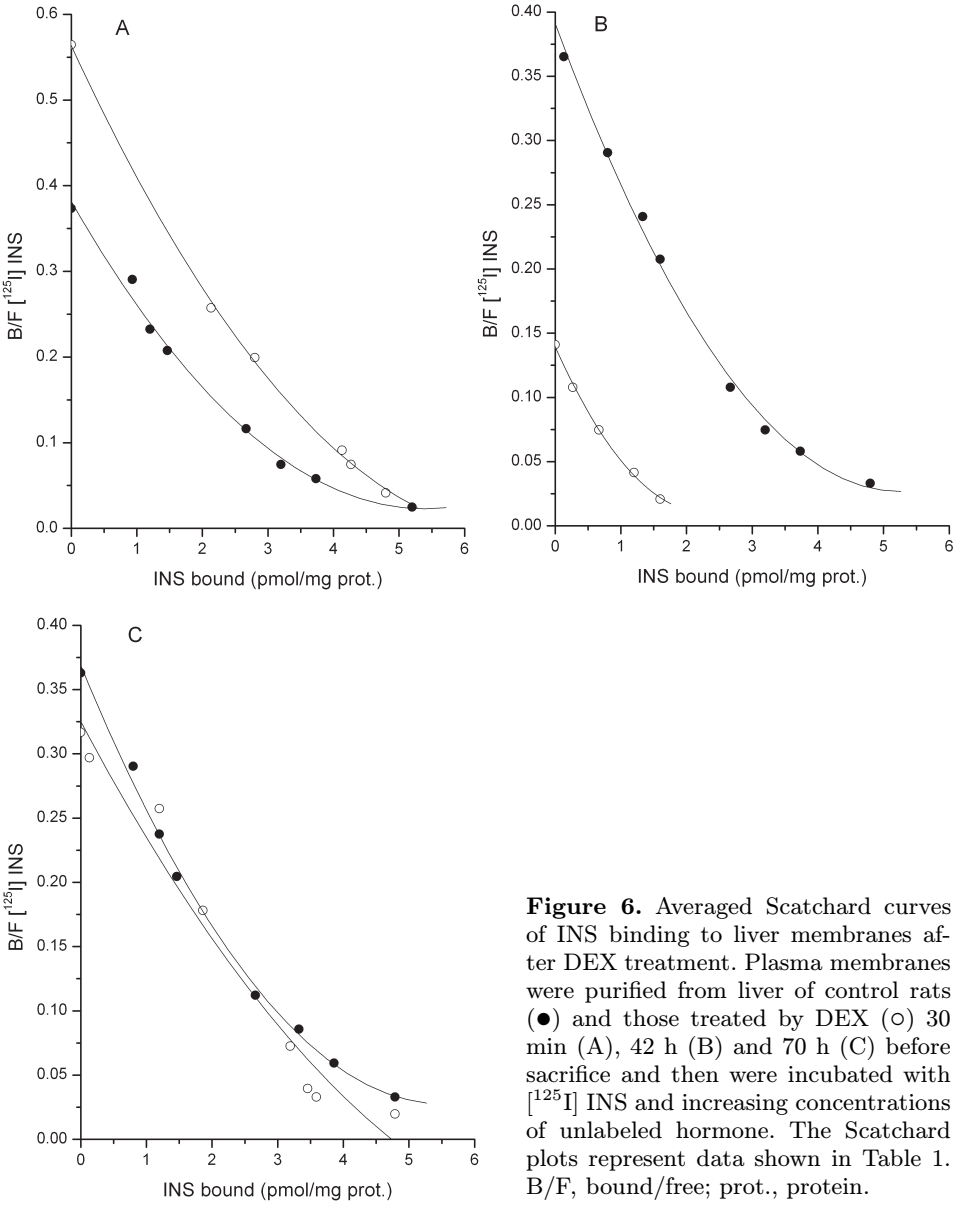

Figure 6. Averaged Scatchard curves of INS binding to liver membranes after DEX treatment. Plasma membranes were purified from liver of control rats (●) and those treated by DEX (○) 30 min (A), 42 h (B) and 70 h (C) before sacrifice and then were incubated with [^{125}I] INS and increasing concentrations of unlabeled hormone. The Scatchard plots represent data shown in Table 1. B/F, bound/free; prot., protein.

to approach one another, and 42 h after DEX treatment SB of INS has been also returned to the control level at the higher concentration of unlabelled INS (Fig. 5).

In order to differentiate whether the decrease in INS binding represents changes in the content of receptors or their affinity for the hormone, the Scatchard plot anal-

ysis were performed in fractions of liver plasma membrane prepared from control and DEX-treated rats (Table 1 and Fig. 6). The results obtained by two sites model revealed that after 42 h of DEX treatment, the content of both populations of binding sites of rats significantly decreased ($p < 0.001$) from a control level (Table 1). The dissociation constant for low affinity binding sites (Kd_2) in the liver plasma membranes of rats 42 h after hormone treatment was mildly increased in comparison to control animals ($p < 0.02$), while dissociation constant for high affinity binding sites (Kd_1) was decreased from 9.1 ± 2 nmol/l in the control to 7.3 ± 0.25 nmol/l ($p < 0.02$) in the same group of treated animals (Table 1). The Kd_1 and Kd_2 as well as the content of INS binding sites in other treated groups were not significantly changed in comparison to values obtained from control specimen.

Discussion

The different metabolic effects of glucocorticoids contributing to development of a sustained INS resistance, manifested by hyperglycemia, and decreased response to exogenous INS, have demonstrated in earlier studies (Andrews and Walker 1999; Reynolds and Walker 2003). It has been also shown that chronic administration of glucocorticoids increases INS concentration in blood, as well as induces changes in the INS binding to its receptor (Folli et al. 1996). Although the effects of chronic glucocorticoid administration as well as the direct, immediate effects of these hormones on INS secretion and IR have been subject of many studies (Grunfeld et al. 1981; Fantus et al. 1982; Leal et al. 1992; Buren et al. 2002; Klein et al. 2002), the published results appears to be contradictory, and the mechanism by which glucocorticoids influence INS action has remained unclarified so far. In spite of that, the available information on mechanism of glucocorticoids influence on INS action have been in the main obtained by studies of direct immediate effects of these hormones on INS secretion, as well as on IR.

The blood glucose has shown almost linear increase after DEX treatment to the end of analyzed period (Fig. 1) and the increase was significant since 12 h after hormone treatment. This effect of glucocorticoids on the raise of glycemia has already been published and represents the resultant of complex effect of the hormone on carbohydrate metabolism and transport of glucose into the cells (Coderre et al. 1996; Barthel et al. 2003). Although INS is very important regulator of blood glucose level, there is no significant correlation between blood plasma and serum INS (Fig. 2) in DEX-treated animals. High level of blood glucose 42 h after treatment (Fig. 1) is accompanied with the decrease in number of physiologically relevant hepatic high affinity binding sites for INS (Table 1). However, in other analyzed time intervals, the changes in IR binding parameters are not responsible for the increase in glucose level. These data can point out to the complexity of regulation of blood glucose level (Opherk et al. 2004).

On the basis of the presented results in this study (Fig. 2), indicating that significant increase in INS level in blood plasma has occurred 18 h after DEX injection, could be assumed that this change is a primary an early response to

steroid treatment. The other increase in INS level occurring 42 h after injection indicates that glucocorticoids can take part in INS secretion indirectly, probably through enhancement of the blood glucose (Rouiller et al. 1988; Delaunay et al. 1997). The published results indicate that glucocorticoids have dual effects on INS action. One of them is concerned the increasing INS concentration in blood plasma, that has a major effect on down-regulation of cell surface receptors through increase in INS binding to receptors, that bring about to internalization and degradation of receptor complexes (Rouiller and Gorden 1987; Okabayashi et al. 1989; Rohilla et al. 1991); the another one is stimulation comprised on the stimulation of the INS proreceptor synthesis rate (Hines et al. 1994; Lee and Tsai 1994). The obtained evidences (Figs. 2 and 3) and significant reciprocal correlation between SB of INS to liver membranes with serum INS level confirm these presumptions indicating that a significant decrease in INS binding to its receptors in liver plasma membranes occurs parallel with increase in blood INS level (Figs. 2 and 3) after DEX treatment. The data indicating that the SB of INS to the plasma membranes of rat liver turns back to the control level 70 h after the DEX treatment (Fig. 3) are in agreement with results of Shibasaki et al. (1988) which suggest that glucocorticoids have time-dependent effects on gene expression of IR. These observations are also supported by presented results obtained in experiments with erythrocytes (Fig. 4) which are not glucocorticoid-responsive cells, and there were neither significant changes in the INS binding after DEX treatment, nor significant correlation of SB of INS with serum INS.

The evidences presented by displacement curves indicate that specific INS binding at low INS concentration is markedly higher to the membranes of control liver cells than to those prepared from liver of animals 42 h after DEX treatment (Fig. 5). However, INS binding turns back to the control level 70 h after DEX administration. The results obtained by Scatchard plot analysis are in agreement with these evidences, indicating that decrease in SB of INS to its receptors observed 42 h after DEX treatment (Figs. 3 and 5) could be a consequence of the significant decrease in the receptor number (Table 1, Fig. 6B), while the level of both populations of binding sites of the liver cells returns to the control level after 70 h of DEX treatment (Table 1, Fig. 6C). Furthermore, the observed various effects of DEX on the level of IR are in agreement with well documented evidences indicating that glucocorticoids raise INS concentration in blood (Rouiller et al. 1988; Delaunay et al. 1997), which then consequently down-regulate IR, and simultaneously they stimulate INS proreceptor synthesis (Hines et al. 1994; Lee and Tsai 1994). It can therefore be concluded that the net effects of glucocorticoids on INS binding *in vivo* will be the result of a variety of direct and indirect influences on IR. Our results are in agreement with published evidences indicating that DEX increases the INS secretion (Rouiller et al. 1988; Giorgino et al. 1993), as well as that increase in INS concentration raises the internalization of the INS-receptor complexes (Rouiller and Gorden 1987; Okabayashi et al. 1989; Rohilla et al. 1991), and then internalized receptor is, at least partly, degraded in the liver cells. The observed relation between decrease in receptor level in membranes of liver cell

(Fig. 3) and increase in INS concentration in DEX-treated blood plasma (Fig. 2) in the same time intervals is supported by this assumption and could be explained by internalization and degradation of IR. Namely, presented results indicate that the effects of DEX on IR may result from a time-dependent glucocorticoid action on INS secretion and/or synthesis. Consequently, the changes in INS concentration in blood may affect its binding to own receptors in plasma membranes of target cells. The modulation of IR number by DEX injection could be also due to its effect on receptor synthesis (Hines et al. 1994; Lee and Tsai 1994). The presented results are supported by preposition that the number of IR on the liver plasma membrane is subject of dynamic regulation. The balance between the rate of degradation of INS-receptor complexes and *de novo* synthesis of receptor protein, determines the final number of receptors on the plasma membrane (Okabayashi et al. 1989).

The findings presented in this study clearly indicate that the glucocorticoid produces time dependent changes both in INS binding and amount of IR on the surface of the liver cells, although this mechanism is not clear at the present. The observed differences between time-dependent effects of DEX on IR and INS binding to plasma membranes of liver cells and erythrocytes are just another evidence on existence of more than one mechanism of IR regulation of target cells. Further theoretical elaboration and experimental substantiation of the basic concepts on the role of glucocorticoids on regulation of IR and INS action are now in progress in our laboratory.

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References

Andrews R. C., Walker B. R. (1999): Glucocorticoids and insulin resistance: old hormones, new targets. Clin. Sci. (Lond.) **96**, 513—523

Barthel A., Scherbaum W. A., Bornstein S. R. (2003): Novel aspects in the mechanisms of steroid diabetes and the regulation of hepatic glucose production by insulin and steroids. Med. Klin. (Munich) **98**, 283—286 (in German)

Buren J., Liu H. X., Jensen J., Eriksson J. W. (2002): Dexamethasone impairs insulin signalling and glucose transport by depletion of insulin receptor substrate-1, phosphatidylinositol 3-kinase and protein kinase B in primary cultured rat adipocytes. Eur. J. Endocrinol. **146**, 419—429

Coderre L., Vallega G. A., Pilch P. F., Chipkin S. R. (1996): *In vivo* effects of dexamethasone and sucrose on glucose transport (GLUT-4) protein tissue distribution. Am. J. Physiol. **271**, E643—648

Delaunay F., Khan A., Cintra A., Davani B., Ling Z. C., Andersson A., Ostenson C. G., Gustafsson J., Efendic S., Okret S. (1997): Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. J. Clin. Invest. **100**, 2094—2098

Fantus I. G., Saviolakis G. A., Hedo J. A., Gorden P. (1982): Mechanism of glucocorticoid-induced increase in insulin receptors of cultured human lymphocytes. J. Biol. Chem. **257**, 8277—8283

Folli F., Saad M. J., Kahn C. R. (1996): Insulin receptor/IRS-1/PI 3-kinase signaling system in corticosteroid-induced insulin resistance. Acta Diabetol. **33**, 185—192

Gambhir K. K., Archer J. A., Carter L. (1977): Insulin radioreceptor assay for human erythrocytes. Clin. Chem. **23**, 1590—1595

Giorgino F., Almahfouz A., Goodyear L. J., Smith R. J. (1993): Glucocorticoid regulation of insulin receptor and substrate IRS-1 tyrosine phosphorylation in rat skeletal muscle *in vivo*. J. Clin. Invest. **91**, 2020—2030

Grunfeld C., Baird K., Van Obberghen E., Kahn C. R. (1981): Glucocorticoid-induced insulin resistance *in vitro*: evidence for both receptor and postreceptor defects. Endocrinology **109**, 1723—1730

Hines D., Hug V., Levy J. R. (1994): Dexamethasone mediated stabilization of insulin receptor mRNA. J. Recept. Res. **14**, 297—305

Hoffman K., Romovacek H., Titus G., Ridge K., Raffensperger J. A., Finn F. M. (1987): The rat liver insulin receptor. Biochemistry **26**, 7384—7390

Klein H. H., Ullmann S., Drenckhan M., Grimmsmann T., Unthan-Fechner K., Probst I. (2002): Differential modulation of insulin actions by dexamethasone: studies in primary cultures of adult rat hepatocytes. J Hepatol. **37**, 432—440

Leal M. A., Cabanas C., Rius C., Aller P., Calle C. (1992): Modulation by dexamethasone of insulin binding and insulin receptor mRNA levels in U-937 human promonocytic cells. Biochimie **74**, 545—549

Lee J. K., Tsai S. Y. (1994): Multiple hormone response elements can confer glucocorticoid regulation on the human insulin receptor gene. Mol. Endocrinol. **8**, 625—634

Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265—275

Montiel F., Ortiz-Caro J., Villa A., Pascual A., Aranda A. (1987): Glucocorticoids regulate insulin binding in a rat glial cell line. Endocrinology **121**, 258—265

Munson P. J., Rodbard D. (1980): Ligand: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. **107**, 220—239

Okabayashi Y., Maddux B. A., McDonald A. R., Logsdon C. D., Williams J. A., Goldfine I. D. (1989): Mechanisms of insulin-induced insulin-receptor downregulation. Decrease of receptor biosynthesis and mRNA levels. Diabetes **38**, 182—187

Opherk C., Tronche F., Kellendonk C., Kohlmuller D., Schulze A., Schmid W., Schutz G. (2004): Inactivation of the glucocorticoid receptor in hepatocytes leads to fasting hypoglycemia and ameliorates hyperglycemia in streptozotocin-induced diabetes mellitus. Mol. Endocrinol. **18**, 1346—1353

Pollet R. J., Levey G. S. (1980): Principles of membrane receptor physiology and their application to clinical medicine. Ann. Inter. Med. **92**, 663—680

Reynolds R. M., Walker B. R. (2003): Human insulin resistance: the role of glucocorticoids. Diabetes Obes. Metab. **5**, 5—12

Ribarac-Stepic N., Kovacina K., Zamaklar M., Devecerski M. (1988): Detection of compounds in serum inhibiting insulin binding to erythrocyte receptors. Endocrinol. Exp. **22**, 203—209

Rohilla A. M., Anderson C., Wood W. M., Berhanu P. (1991): Insulin downregulates the steady-state level of its receptor's messenger ribonucleic acid. Biochem. Biophys. Res. Commun. **175**, 520—526

Rouiller D. G., Gorden P. (1987): Homologous down-regulation of the insulin receptor is associated with increased receptor biosynthesis in cultured human lymphocytes (IM-9 line). Proc. Natl. Acad. Sci. U.S.A. **84**, 126—130

Rouiller D. G., McKeon C., Taylor S. I., Gorden P. (1988): Hormonal regulation of insulin receptor gene expression. Hydrocortisone and insulin act by different mechanisms. J. Biol. Chem. **263**, 13185—13190

Scatchard G. (1949): The attractions of proteins for small molecules and ions. Ann. N. Y. Acad. Sci. **51**, 660—672

Shibasaki Y., Sakura H., Odawara M., Shibuya M., Kanazawa Y., Akanuma Y., Takaku F., Kasuga M. (1988): Glucocorticoids increase insulin binding and the amount of insulin-receptor mRNA in human cultured lymphocytes. Biochem. J. **249**, 715—719

Zorad S., Jezova D., Szabova L., Macho L., Tybitanclova K. (2003): Low number of insulin receptors but high receptor protein content in adipose tissue of rats with monosodium glutamate-induced obesity. Gen. Physiol. Biophys. **22**, 557—560

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