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STRESS-INDUCED CHANGES OF GLUCOCORTICOID RECEPTOR IN RAT LIVER

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Summary—The effect of corticosterone injection and of acute and repeated stress on rat liver cytosol glucocorticoid receptor was studied to ascertain whether corticosterone-induced glucocorticoid receptor (GR) regulation also takes place in intact animals as it does in adrenalectomized ones. Adult male rats were exposed to six different stressors (swimming, 10 mg/kg histamine i.p., 500 mU/kg vasopressin s.c., heat, immobilization and cold) acutely or three times daily for 18 days (repeated stress). Each of the stressors applied acutely provoked a pronounced increase of plasma corticosterone with subsequent induction of hepatic tyrosine aminotransferase activity. Depletion of cytosol receptor was however only noticed after swimming and histamine injection. On the other hand, sustained hypersecretion of corticosterone evoked by repeated stress significantly reduced the number of GR in rat liver cytosol without any change in K_d. It is concluded that in the presence of intact adrenal glands cytosol receptors are more resistant to corticosterone-induced depletion than in their absence. Further, repeated stress causes down-regulation of GR in the liver, most probably by sustained corticosterone secretion, yet the effect of other stress factors cannot be excluded.

INTRODUCTION

It is now well established that glucocorticoids down-regulate the number of their own receptors. The first studies documenting this process used cloned cell models *in vitro* [1–3]. Recently much data has accumulated on down-regulation of glucocorticoid receptor (GR) *in vivo*. Administration of exogenous glucocorticoids to both adrenalectomized and intact animals down-regulated GR in the liver [4–8] and in the brain [9–11]. Similar changes were described in human lymphocytes [12, 13]. However, conflicting data are found in the literature on the role of endogenous glucocorticoid secretion on GR [9, 14, 15]. The regulation of GR by glucocorticoids could be an important issue for the explanation of physiological and pathological changes of GR in human medicine. Therefore a great deal of attention should be paid in studying the significance of GR regulation in the intact organism.

In the present work we attempted to elucidate the significance of endogenous glucocorticoid secretion in the process of down-regulation. We studied the effect of a single corticosterone injection, of a brief increase of corticosterone secretion (acute stress), as well as the effect of sustained elevation of hormonal level (repeated stress) on GR concentration in the liver cytosol of intact rats.

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METHODS

Chemicals

[1,2,3-^{3}H]dexamethasone ([^{3}H]Dex, sp. act. 42 Ci/mmol) was purchased from Radiochemical Centre (Amersham). Dexamethasone was obtained from Sigma (St Louis, MO).

Animals

Male Sprague–Dawley specific pathogen-free rats (Velaz, Prague) weighing 250 g were kept at 24°C under a 12 h light–dark cycle, and fed a pelleted diet. Animals were exposed to six different stressors: swimming (3 times for 15 min in 37°C water with a 10 min interval between exposures), histamine injection (10 mg/kg i.p.), injection of lysine vasopressin (500 mU/kg s.c.), exposure to heat for 20 min (41°C), immobilization (150 min) and cold exposure (4°C for 2 h).

In the first set of experiments, the dose of 100 μg/100 g corticosterone was injected s.c. to intact and adrenalectomized (ADX) animals (7 days after surgery). The animals were killed 1 h later to measure GR concentration in liver cytosol. Moreover, subgroups of rats were sacrificed 3 h after injection to determine the

activity of the enzyme tyrosine aminotransferase (TAT).

In the second set animals were exposed acutely to a single stressor. Decapitation was performed either immediately or 30 min after histamine and vasopressin injection to measure GR content in liver cytosol and corticosterone level in blood plasma. TAT activity was measured 3 h after stress exposure.

In the third set of experiments with repeatedly stressed animals, each stressor was applied three times per day (8 a.m., 1 and 6 p.m.). To avoid adaptation the six stressors were alternated in a given sequence which was repeated three times (total exposure 18 days). The animals were sacrificed 24 h after the last exposure. After decapitation blood was collected and the adrenal glands, thymus and spleen were removed, dried and weighed. Blood lymphocyte counts were determined by the chamber method on calculating cells in 50 squares. Livers were perfused *in situ* through the portal vein with ice-cold saline, homogenized in 6 vol of TED buffer (10 mM Tris–HCl, pH 7.5 containing 1 mM EDTA, 2 mM dithiotreitol, 10 mM sodium molybdate and 10% glycerol) and centrifuged at 105,000 *g* for 1 h. The top layer of lipids was sucked off. Aliquots of supernatant (cytosol) containing approx. 2 mg of protein per tube were incubated with varying concentrations (1.8–37.0 nM) of [^{3}H]Dex in the presence or absence of 2.5 μM Dex in a final volume of 0.4 ml at 0°C for 20 h. Under these conditions, no loss of receptor activity occurs and the exchange is over 90% [16]. After the incubation period, unbound steroids were removed by treatment with dextran-coated charcoal. The radioactivity was counted in a liquid scintillation counter with an efficiency of 32% as determined by external standardization. Maximum number of binding sites (B_{max}) and K_d were assessed by the method of Scatchard [17].

Protein concentration was determined according to Lowry *et al.* [18] with the use of bovine serum albumin as standard.

TAT activity was assayed in the 15,000 *g* supernatant of 10% liver homogenate in 0.14 M KCl [19].

The concentration of corticosterone in plasma was measured by the method of Murphy [20] with slight modification [21].

Student's *t*-test was used for statistical analysis.

RESULTS

In the first set of experiments the effect of exogenous corticosterone on both cytosol receptor depletion and hepatic TAT activity was compared in intact and ADX animals. The finding of a nearly 2-fold increase in basal receptor concentration is a known effect of adrenalectomy [22]. One hour after corticosterone injection (100 μg/100 g s.c.) to ADX animals significant depletion of cytosol GR was recorded ($P < 0.05$) [Fig. 1(a)]. On the other

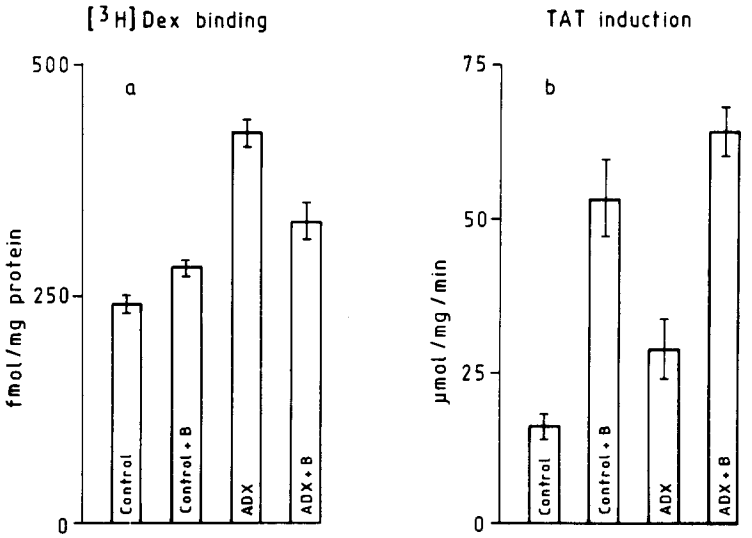

Fig. 1. Effect of corticosterone on GR concentration (a) and TAT activity (b) in the liver of intact and ADX rats. Intact and ADX rats (7 days after ADX) were injected with corticosterone (100 μg/100 g, s.c.), [^{3}H]Dex receptor binding and TAT activity in the liver cytosol was measured as described in Methods. Values are the mean ± SE of 6 animals.

hand, no cytosol receptor depletion was noticed in intact animals. In spite of this fact, however, hepatic TAT activity was induced significantly in both intact ($P < 0.05$) and ADX ($P < 0.001$) animals [Fig. 1(b)].

In the second set, acute exposure of intact rats to each single stress resulted in a prompt elevation of circulating corticosterone. The most effective stimulus was swimming (Fig. 2) which led to an almost 20-fold increase in plasma corticosterone as compared to control values (74.0 ± 2.7 vs 4.4 ± 0.3 μg/100 ml plasma). At the same time a significant depletion of GR in the cytosol was observed after swimming ($P < 0.001$) (Fig. 3). A significant, but less pronounced depletion of cytosol GR was induced by histamine injection ($P < 0.05$). Liver cytosol receptor concentration was not affected by the other stressors used. On the other hand, the brief pulse of corticosterone secretion, induced by any of the transient external stimuli, increased hepatic TAT activity (Fig. 4).

Anatomical evidence of sustained hypersecretion of corticosterone during 18 days of stress exposure in the third set of experiments was provided by findings of hypertrophied adrenal glands, involuted thymus, and decreased spleen and body weight (Table 1). In addition, significantly reduced lymphocyte counts were obtained. One day after the last stress exposure the hepatic TAT activity and plasma corticosterone returned to control levels. As determined by Scatchard analysis, repeated stress led to significant reduction of the number of cytosolic GR in the liver (Table 1, Fig. 5). The concentration of [^{3}H]Dex receptor binding sites decreased by 33% as compared to the control group, no change of receptor affinity for [^{3}H]Dex was found (K_d 1.7 ± 0.09 nM).

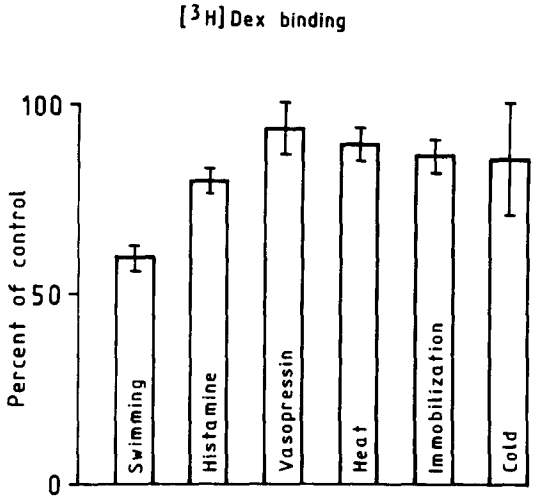

Fig. 3. Effect of various stressors on GR concentration in liver cytosol. Animals were killed 30 min after single stress exposure. Liver cytosol was incubated with saturating concentraton of [^{3}H]Dex (37 nM) in the presence or absence of 2.5 μM Dex. Results are expressed as percentage of those of the control group (100% = 472.4 ± 28.5 fmol/mg protein). Values are the mean ± SE of 4–11 animals.

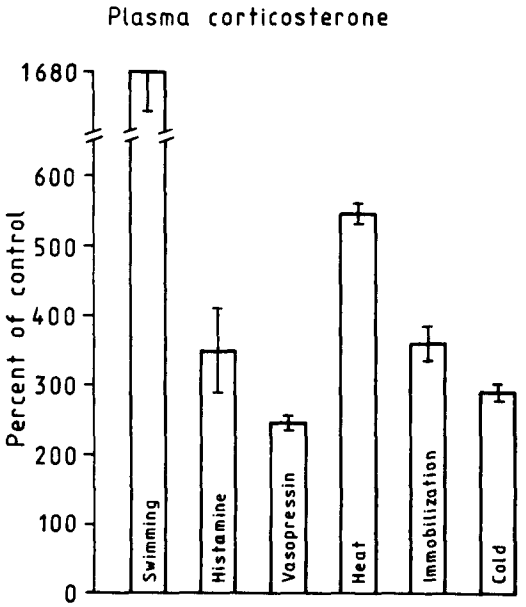

Fig. 2. Effect of various stressors on corticosterone level in rat plasma. Animals were killed 30 min after single stress exposure. Results were compared to levels of untreated controls (100% = 7.93 ± 1.1 μg/100 ml plasma). Values are the mean ± SE of 4–11 animals.

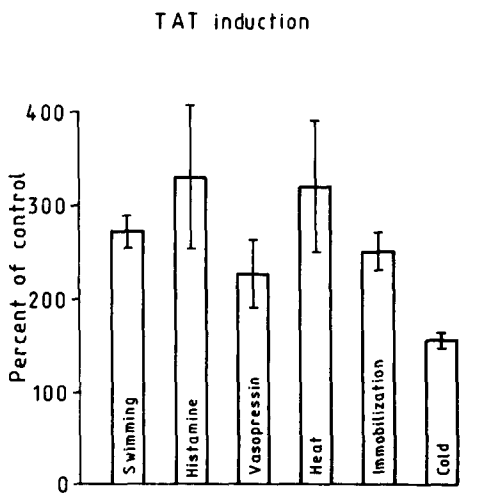

Fig. 4. Effect of various stressors on hepatic TAT activity. Animals were killed 3 h after single stress exposure. Enzyme activity was compared to values of untreated animals (100% = 27.2 μmol/mg/min). Values are the mean ± SE of 4–11 animals.

Table 1. Effects of repeated stress in rats

	n	Body weight (g)	Adrenal gland (mg)	Spleen (mg)	Thymus (mg)	Lymphocytes (%)	TAT (μmol/mg/min)	B (μg/100ml)	B_{max} (fmol/mg)
Control	6	382.1 ± 8.2	33.6 ± 0.8	677.7 ± 6.5	572.8 ± 43.0	76.1 ± 2.1	44.5 ± 5.0	7.4 ± 1.7	351.0 ± 13.6
Stress	6	286.4 ± 19.6[c]	42.4 ± 1.2[a]	403.0 ± 32.4[b]	254.0 ± 19.0[b]	56.0 ± 5.1[c]	61.1 ± 8.3	11.8 ± 3.3	236.8 ± 21.5[c]

Intact animals were exposed to various stressors for 18 days (see Methods) and killed 24h after the last exposure. Peripheral WBC counts were obtained and percentage of lymphocytes were calculated. B_{max} was calculated according to the Scatchard analysis. Means ± SE. Levels of significance vs control: [a]$P < 0.02$; [b]$P < 0.001$; [c]$P < 0.005$. B, corticosterone.

DISCUSSION

Our previous results [23] and studies of other authors [5, 6, 24] have provided ample evidence that a single injection of glucocorticoids to ADX animals results in a rapid depletion of liver cytosol receptors. Both extent and duration of receptor depletion correlates well with the biological response as measured by hepatic TAT induction.

The present study shows that in the presence of intact adrenal glands, the response to corticosterone (exogenous and/or endogenous) may be different. Corticosterone administered in the same dose to ADX and intact animals, depleted liver cytosol receptors in the former yet not in the latter, though hepatic TAT activity was increased in both groups (Fig. 1). A burst of corticosterone secretion provoked by exposure of intact animals to acute stress resulted in a subsequent induction of hepatic TAT (Figs 2 and 4). However, cytosol GR depletion was found only on exposure to two of the stressors applied, i.e. swimming (also described by Omrani *et al.* [25]) and histamine injection (Fig. 3). Swimming, the most effective stressor used, caused a 20-fold increase of plasma corticosterone and cytosol GR depletion to 60% of the control level. Our results demonstrate that in the presence of intact adrenal glands cytosol receptors are more resistant to corticosterone-induced depletion than in their absence. Some facts have to be taken into consideration to evaluate this phenomenon. In ADX animals all receptors should be present in the cytosol. Immediately after agonist injection a hormone-receptor complex is formed, activated, translocated to the nucleus and these biochemical events are reflected in depletion of the cytosol receptor. In the presence of endogenous corticosterone, however, receptors are both in the cytosol and nucleus. As suggested, receptor function need not necessarily terminate after nuclear binding, and some replenishment of cytoplasmic receptor may occur via recycling [26–28]. In the intact organism a sudden increase of endogenous corticosterone secretion increases the rate of both activation and migration of the hormone-receptor complex to the nucleus [9, 25] and might also facilitate the recycling process. Substantial receptor depletion could probably be recorded only in the presence of a very high plasma corticosterone level (e.g. in swimming stress). It could be possible that after both corticosterone injection and acute stress, quite different intervals are needed to measure receptor depletion in intact animals. This is not the case, however, as following intervals were tested without any changes registered in our experiments at 0, 15 and 30 min, 1, 2 and 3 h (not shown).

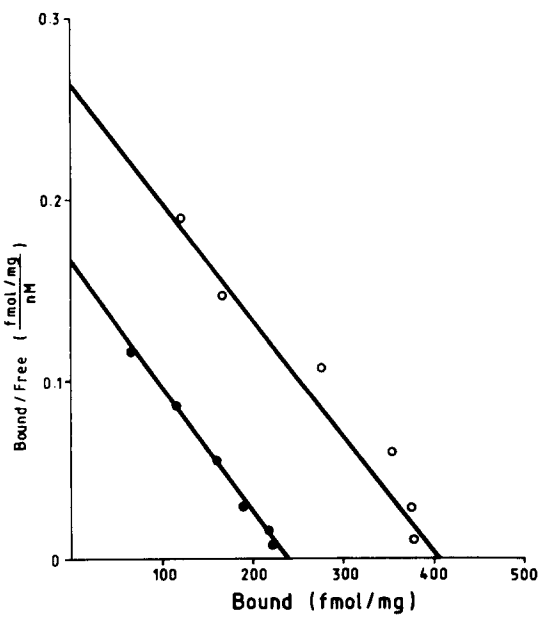

Fig. 5. Representative Scatchard plots of [^{3}H]Dex binding to liver cytosol of intact (○) and repeatedly stressed (●) animals. The values of B_{max} are indicated in Table 1. For details, see Methods.

The main finding of this study is that repeated stress results in a clear-cut down-regulation of GR in the rat liver. This is evidence for autoregulation of GR in intact organisms under relatively physiological circumstances.

As suggested by the changes of anatomical parameters (Table 1) the process of down-regulation is very probably caused by sustained

hypersecretion of endogenous corticosterone: individual stressors significantly increased corticosterone secretion and application of the stressors trice per day maintained high corticosterone levels in plasma.

The reduction of cytosolic receptor number after repeated stress cannot be due to residual corticosterone present in the cytosol of stressed animals for two reasons. First, previous studies indicated that 24 h after corticosterone administration the liver is cleared of the hormone [5]. Accordingly, our results showed a decrease of stress-induced corticosterone levels in plasma 24 h after the last stress exposure (Table 1). Second, the effect of endogenous corticosterone on receptor occupancy was eliminated by an exchange method, which measures total number of binding sites (occupied and unoccupied) [16].

Our results are in good agreement with the report of Sapolsky *et al.* [9]. These authors, however, used brain as target tissue. Anatomically specific down-regulation of GR in both the hippocampus and amygdala of intact rats was induced in repeated stress. A sustained high plasma corticosterone level was documented during the experimental period. This requirement was not fulfilled in the experimental model used by Svec *et al.* [14], in which repeated stress was not effective in causing down-regulation of GR in the liver of intact male mice. The animals of these authors were exposed to stress for three days, but the stressors were applied only once per day. The increase of corticosterone in plasma evoked in this way was not given, but according to similar experiments could be of short duration only [29–31] and hence unable to modulate GR to an extent attained by exogenous corticosterone administered to ADX animals in high doses [5] or in such a model of repeated stress as used in our study. Moreover, our acute stress experiments showed that a short pulse of endogenous corticosterone provoked by exposure of normal rats to physical stress, except swimming and histamine injection, failed to result in depletion of cytosol receptors.

Studies concerning GR regulation in human beings are in keeping with the experimental results. Chronic cortisol hypersecretion in patients with different forms of hypercortisolism was not sufficient to modulate GR in lymphocytes [32, 33], while administration of exogenous glucocorticoids to normal subjects caused a 33% decrease in GR concentration [12]. The degree of cortisol elevation in patients with hypercortisolemia may not reach hormone levels of normal subjects after glucocorticoid administration. It should however be noted that several additional factors may modulate GR. Thus in patients with adrenal insufficiency [15, 34] and in ADX rodents, after partial hepatectomy [35] a reduced number of GR was observed. Even our finding of receptor changes after repeated stress need not be the consequence of glucocorticoid-induced down-regulation only. Stress is a non-specific and rather complex stimulus. Besides activation of the hypothalamo–pituitary–adrenal axis a number of further endocrine, nervous and metabolic factors are involved. Further studies are needed to solve the problem of stress-induced down-regulation as well as that of the importance of changes of GR level for TAT activity regulation in intact organism—both questions are far from being solved.

In conclusion, we have shown that in the presence of intact adrenal glands the activity of TAT may be induced by corticosterone injection or by a burst of corticosterone secretion without noticing any depletion of GR from the liver cytosol. Further, repeated stress was found to result in down-regulation of GR in the rat liver without changing receptor affinity.

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