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CORTICOSTERONE REGULATION OF BRAIN AND LYMPHOID CORTICOSTEROID RECEPTORS

MARTIN T. LOWY

Laboratory of Biological Psychiatry, Department of Psychiatry, School of Medicine, Case Western Reserve University, 2040 Abington Road, Cleveland, OH 44106, U.S.A.

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Summary—Circulating lymphocytes are often used as a model for brain corticosteroid receptor regulation in clinical disease states, although it is not known if lymphoid receptors are regulated in a similar manner as brain receptors. In the present study the regulation of brain (hippocampus, frontal cortex, hypothalamus and striatum), lymphoid (circulating lymphocytes, spleen and thymus) and pituitary glucocorticoid receptors in response to alterations in circulating corticosterone levels was examined. Seven days following adrenalectomy, type II corticosteroid receptors (i.e. glucocorticoid receptors) were significantly increased in the hippocampus, frontal cortex and hypothalamus, but not in any other tissues. Administration of corticosterone (10 mg/kg) for 7 days significantly decreased type II as well as type I (i.e. mineralocorticoid receptors) receptors in the hippocampus. Type II receptors in the frontal cortex, circulating lymphocytes and spleen were also significantly decreased by chronic corticosterone treatment. Immobilization stress (2 h a day for 5 days) failed to alter receptor density in any of the tissues. These results demonstrate that homologous regulation of corticosteroid receptors by corticosterone does not invariably occur in all tissues and emphasize the complex degree of regulation of these receptors. However, the simultaneous downregulation of both hippocampal and lymphocyte glucocorticoid receptors by corticosterone provides support for the hypothesis that circulating lymphocytes do reflect some aspects of brain glucocorticoid receptor regulation.

INTRODUCTION

Glucocorticoid receptors (GR) are found in almost all tissues of the body, including brain and lymphoid tissues. Circulating lymphocytes have frequently been used as a peripheral model to study GR regulation in clinical disease states associated with alterations in cortisol levels. In hypercortisolemic conditions a decreased number of lymphocyte GR has been found in patients with primary cortisol resistance [1], depression [2, 3] and anorexia nervosa [4], whereas patients with Cushing's disease have relatively normal levels of lymphocyte GR [5]. Adrenal insufficiency is associated with a decreased number of lymphocyte GR and an increase in binding affinity [6]. Post-traumatic stress disorder, a condition associated with low cortisol production, is accompanied by an increase in lymphocyte GR number [7]. If lymphocyte GR are regulated in a manner similar to brain or pituitary GR, then they may provide useful information concerning the status of GR located in tissues, such as the hippocampus, which are involved in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis, but which are inaccessible in clinical populations. The regulation of lymphoid GR is also important in regards to immune function, since glucocorticoids have pronounced inhibitory effects on most immune variables [8]. However, the degree to which GR density in lymphocytes is related to GR number in other tissues, especially the brain and pituitary, is not well established. Recent studies do indicate some correspondence between GR in brain and lymphoid tissues. Glucocorticoid receptor density, affinity and steroid specificity are similar in brain and lymphoid tissues [9]. Reserpine, a drug which depletes biogenic amines, decreases GR in both brain and lymphoid tissues [10].

Recently, corticosteroid receptors have been classified into two distinct subtypes, termed the type I and type II receptors [11, 12]. The type I receptors (i.e. mineralocorticoid receptors) are located predominantly, though not exclusively, in the hippocampus and are thought to mediate the tonic effects of corticosterone on brain function. In contrast, type II receptors (i.e. GR) have lesser affinity for corticosterone, are widely

distributed throughout the brain and body and mediate the effects of elevated corticosterone levels on brain function. Lymphoid tissues contain high levels of type II receptors and little or no detectable levels of type I receptors [9]. Type II receptors, especially those in the hippocampus, respond in an autoregulatory fashion to changes in glucocorticoid levels. Thus, increased levels of endogenous or exogenous corticosterone decreases type II receptors [13–15], whereas adrenalectomy increases type II receptors in the hippocampus as well as other tissues [14, 16]. Type I receptors tend to respond less to changes in circulating corticosterone levels as reported in some [14], but not all studies [17, 18]. From the above studies it is clear that although regulation of corticosteroid receptors by glucocorticoids occurs, it is not uniform in all tissues.

The present study was undertaken to directly compare the regulation of corticosteroid receptor levels in the brain (hippocampus, frontal cortex, hypothalamus and striatum), lymphoid (circulating lymphocytes, spleen and thymus) and pituitary tissues in response to exogenous corticosterone administration and long-term adrenalectomy. The effect of a 5 day immobilization procedure was also examined, since it elevates endogenous levels of corticosterone and has been proposed as an animal model of depression [19].

EXPERIMENTAL

Animal procedures

Male Sprague–Dawley rats, weighing 175–225 g upon delivery, were purchased from Zivic Miller Lab. (Hillson, PA). Animals were housed 4 to 6 per cage in a temperature controlled room (22–24°C) with a light–dark cycle of 12/12 h (lights on at 06:00 h). Food and tap water were available *ad lib*. Adrenalectomy (ADX) or sham ADX was performed under ether anesthesia using the dorsal approach between 08:00–11:00 h. The completeness of the ADX procedure was evaluated by the absence of corticosterone ($<0.2\ \mu g/dl$) in serum collected at the time of sacrifice. Serum corticosterone was measured using an RIA procedure [20]. For the sham ADX procedure the adrenal glands were manipulated with the hemostats, but were not removed. Following ADX, rats were maintained on 0.9% NaCl as their water source. In the chronic corticosterone and stress studies the excised adrenal glands were carefully trimmed of fat and weighed to the nearest 0.1 mg.

For the chronic ADX study rats were ADX ($n = 11$) or sham ADX ($n = 7$) as described above. Six days later the sham ADX group was ADX and the ADX group was subjected to the sham ADX procedure. Rats were sacrificed 24 h later by rapid decapitation and tissues collected as described below. In the chronic corticosterone study, rats ($n = 9$/group) were injected s.c. with vehicle (sesame oil) or 10 mg/kg corticosterone dissolved in oil for 7 consecutive days. The injection volume was 1 ml/kg. As reported by Sapolsky *et al.* [21] the 10 mg/kg dose of corticosterone produces circulating corticosterone levels of about 30 μg/dl for 12 h with a return to baseline levels after 24 h and also decreases hippocampal GR. 24 h Following the last injection rats were ADX and then sacrificed 24 h later (i.e. 48 h after the last injection). The 24 h time period between ADX and sacrifice is the standard time interval used, since it allows for corticosterone to be cleared from the body and precedes the ADX-induced upregulation of receptor levels [22]. For the stress study ($n = 9$/group) rats were immobilized in conical plastic restraining devices for 2 h a day (09:00–11:00 h) for 5 consecutive days. Control rats were handled daily, but were not subjected to the immobilization procedure. 24 h After the last stress session rats were ADX and then sacrificed 24 h later.

Following decapitation the brain was rapidly removed and dissected on a chilled glass plate. The hippocampus, frontal cortex, hypothalamus, striatum, pituitary, spleen and thymus were removed, frozen on dry ice and stored at −80°C until the time of assay. In the chronic corticosterone and stress studies the excised thymus was trimmed of excess tissue and weighed to the nearest 1 mg. Heparinized blood was collected following decapitation and circulating lymphocytes were isolated as described previously [9]. Briefly, the heparinized blood (5–7 ml) was diluted up to 40 ml with room temperature Hank's balanced salt solution, layered over 10 ml of a 70% Percoll solution and centrifuged at 400 *g* for 45 min at room temperature. The buffy coat containing lymphocytes and other mononuclear cells was carefully removed, washed twice with cold Hank's balanced salt solution, pelleted in ultracentrifuge tubes and frozen at −80°C until the time of assay.

Receptor binding assays

Corticosteroid receptor assays were performed as described previously [9, 10]. All procedures were performed at 4°C. The assay buffer for the receptor assay was TEDGM (10 mM Tris, 1 mM EDTA, 2.5 mM dithiothreitol, 10% glycerol, 20 mM Na_2MoO_4; pH = 7.4 at 4°C). Tissues were homogenized in cold TEDGM buffer (0.6–2.0 ml depending on the tissue). For the lymphocyte receptor measurements, TEDGM buffer (0.65 ml) was added to the frozen pellet and vortexed vigorously until the pellet was dissolved. Extraction was continued for another 20 min with frequent vortexing. The tissue homogenates were transferred to ultracentrifuge tubes and spun at 250,000 *g* for 30 min. The resulting supernatant is termed cytosol. 200 μl Aliquots of cytosol were added to microcentrifuge tubes containing labeled or unlabeled steroids. For type II receptor measurements a saturating 10 nM concentration of [^{3}H]-RU 28362 was used. Nonspecific binding was assessed using a 500-fold excess of unlabeled RU 28362 in a parallel set of tubes. The total incubation volume was 250 μl. For type I receptor measurements, a 3 nM saturating concentration of [^{3}H]aldosterone was used in conjunction with 0.5 μM unlabeled RU 28362 to block [^{3}H]aldosterone binding to type II receptors. 2.5 μM Corticosterone was used to define nonspecific binding. Type I receptors were only measured in the hippocampus, since a previous study demonstrated that it is the only one of the target tissues which contains appreciable amounts of type I receptors [9]. Following 20–24 h incubation at 4°C, duplicate 100 μl aliquots of cytosol were applied to Sephadex LH-20 minicolumns equilibrated with TEDGM buffer. The sample was washed in with 100 μl of TEDGM buffer and eluted into scintillation vials 30 min later with 400 μl TEDGM. 10 ml Of scintillation fluor was added and samples were counted at approx. 40% efficiency. Protein content in the cytosol fraction was estimated with the Lowry protein assay using bovine serum albumin as the standard [23]. Receptor concentrations are expressed as fmol [^{3}H]steroid/mg protein.

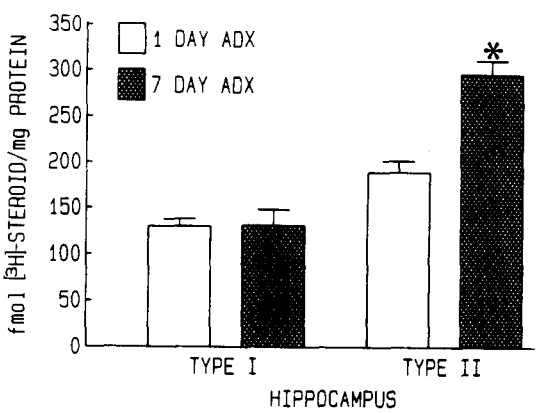

Fig. 1. Effect of a 7 day ADX period ($n = 11$) on hippocampal type I and II corticosteroid receptors. The 1 day ADX group ($n = 7$) represents basal receptor values. Type I and II receptor values were measured as described in Methods. *$P < 0.01$ vs 1 day ADX group.

Materials

[^{3}H]-RU 28362 (SA = 77 Ci/mmol), [^{3}H]-aldosterone (SA = 80 Ci/mmol, [^{3}H]corticosterone (SA = 87 Ci/mmol) and unlabeled RU 28362 were purchased from New England Nuclear (Boston, MA). Percoll and Sephadex LH-20 were purchased from Pharmacia (Piscataway, NJ). Hank's balanced salt solution was from Gibco (Grand Island, NY). The corticosterone antibody for the RIA was purchased from Radioassay Systems Labs, Inc. (Carson, CA). Corticosterone and other chemicals were purchased from Sigma (St Louis, MO).

Statistics

Results are expressed as the mean ± SE. Student's 2 tailed *t*-tests were used to assess statistical difference ($P < 0.05$) between 2 groups. Correlational coefficients were determined according to the method of Spearman.

RESULTS

Effect of 7 day adrenalectomy on brain and lymphoid corticosteroid receptors

A 7 day time interval was used to evaluate the effect of total removal of circulating corticosterone on corticosteroid receptor levels. A 1 day ADX group was used to determine basal receptor levels. Seven days post-ADX there was a significant ($P < 0.01$) 56% increase in type II receptors in the hippocampus, whereas the hippocampal type I receptor number was not altered (Fig. 1). In the other brain areas examined the 7 day ADX period increased type II receptors in the frontal cortex (+41%) and hypothalamus (+20%), whereas there was no change in striatal type II receptors [Fig. 2(A)]. The 7 day ADX period also produced no change in type II receptors in the thymus, spleen, circulating lymphocytes or pituitary [Fig. 2(B)].

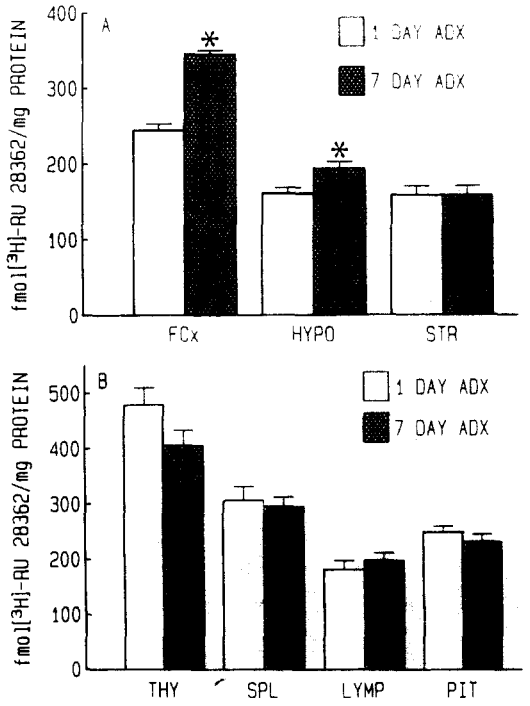

Fig. 2. Effect of a 7 day ADX period ($n = 11$) on (A) brain and (B) lymphoid and pituitary type II corticosteroid receptors. The 1 day ADX group ($n = 7$) represents basal receptor values. Type II receptors were measured as described in Methods. FCx, frontal cortex; HYPO, hypothalamus; STR, striatum; THY, thymus; SPL, spleen; LYMP, lymphocytes; PIT, pituitary. *$P < 0.05$ vs 1 day ADX group.

Effect of chronic corticosterone administration on brain and lymphoid corticosteroid receptors

The effect of excess corticosterone levels on corticosteroid receptor levels was initially examined by injecting high doses of corticosterone (10 mg/kg) in a sesame oil base. As reported by Sapolsky *et al.* [21] this dosing regimen produces prolonged (12 h) elevations of circulating corticosterone levels in the range (30 μg/dl) which occurs following major stressors. The effectiveness of this regimen in producing glucocorticoid-mediated effects was examined by measuring body wt, thymus wt and adrenal wt. The chronic administration of 10 mg/kg corticosterone produced significant ($P < 0.01$) decreases in body wt gain (vehicle = 79.1 ± 2.8 vs corticosterone = 51.7 ± 4.4 g), thymus wt (vehicle = 680 ± 37 vs corticosterone = 354 ± 35 mg) and adrenal gland wt (vehicle = 29.3 ± 3.3 vs corticosterone = 16.1 ± 0.9 mg. The decrease in thymus and adrenal wt was significant whether it was expressed in absolute wt or relative to body wt (not shown).

Corticosterone administration produced significant decreases (−24%) in both hippocampal type I and II receptor subtypes (Fig. 3). Type II receptors in the frontal cortex were also significantly decreased (−22%) by corticosterone, but there was no effect on type II receptors in the hypothalamus or striatum [Fig. 4(A)]. With regards to the lymphoid tissues, corticosterone decreased type II receptors in circulating lymphocytes (−27%) and spleen (−30%), but had no effect on receptor levels in the thymus [Fig. 4(B)]. Type II receptors in the pituitary were not affected by corticosterone. In order to

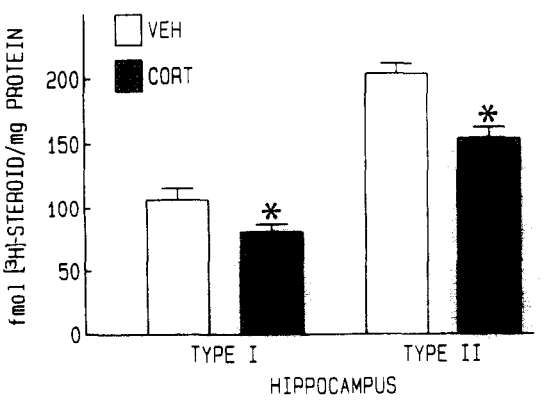

Fig. 3. Effect of 7 day corticosterone (CORT; 10 mg/kg) or vehicle (VEH) administration on hippocampal type I and II corticosteroid receptors. $n = 9$/group. Type I and II receptors were measured as described in Methods. *$P < 0.05$ vs VEH group.

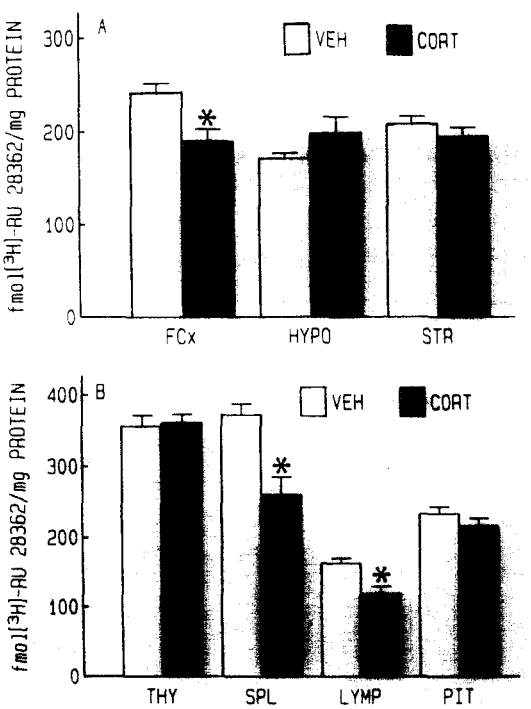

Fig. 4. Effect of 7 day corticosterone (CORT; 10 mg/kg) or vehicle (VEH) administration on (A) brain and (B) lymphoid and pituitary type II corticosteroid receptors. $n = 9$/group. Type II receptors were measured as described in Methods. For abbreviations see the legend for Fig. 2. *$P < 0.05$ vs VEH group.

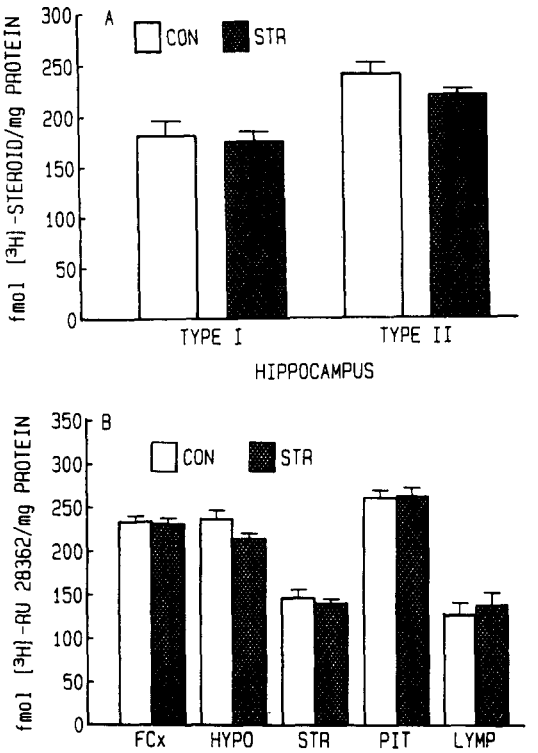

Fig. 5. Effect of a 5 day immobilization stress procedure (2 h/day) on corticosteroid receptors in (A) hippocampus and (B) other target tissues. Type I receptors were measured only in the hippocampus. Type II receptors were measured in the hippocampus and the other target tissues. CON, nonstressed group; STR, immobilization stress group.

more rigorously assess the relationship between corticosterone-induced changes in lymphocyte type II receptors and type II receptors in other tissues, correlational analyses were performed. There were significant ($P < 0.05$) positive correlations between lymphocyte type II receptors and type II receptors in the hippocampus ($\rho = +0.54$), frontal cortex ($\rho = +0.50$) and spleen ($\rho = +0.68$) as well as with hippocampal type I receptors ($\rho = +0.51$). No significant correlations were observed between lymphocyte type II receptors and type II receptors in the hypothalamus, pituitary, striatum or thymus.

Effect of a 5 day immobilization stress period on brain and lymphoid corticosteroid receptors

In order to assess whether elevations in endogenous levels of corticosterone can decrease corticosteroid receptor levels, rats were subjected to a 2 h a day immobilization stress for 5 consecutive days. This procedure had no effect on hippocampal corticosteroid receptors or on type II receptors in other brain regions, circulating lymphocytes or the pituitary (Fig. 5). The 5 day stress protocol produced a significant decrease in body weight gain (control = 62 ± 2 vs stress = 36 ± 3 g; $P < 0.01$). Thymus wt was not decreased by the stress procedure (control = 818 ± 52 vs stress = 772 ± 58 mg). Absolute adrenal gland wt was not altered by the stress procedure (control = 24.6 ± 0.9 vs stress = 27.6 ± 1.3 mg). However, a modest increase (+19%; $P < 0.01$) in adrenal wt was observed when expressed relative to body wt (control = 7.67 ± 0.09 vs stress = 9.12 ± 0.39 mg/100 g body wt).

DISCUSSION

The present results provide further evidence that corticosteroid receptors are regulated by marked alterations in circulating levels of corticosterone. Many previous studies examining brain corticosteroid receptor regulation have focused on the hippocampus. The corticosterone-induced decrease in hippocampal type II receptors observed in this study agrees with several other reports [13–15]. The ability of corticosterone to decrease type I receptors in the present study was not observed by Reul *et al.* [14] who used adrenalectomized rats implanted with corticosterone pellets which were removed 24 h prior to sacrifice. There is however a difference in the response of the 2 hippocampal corticosteroid receptors to adrenalectomy as the present results and other studies [14, 24] indicate that hippocampal type II, but not type I receptors increase following adrenalectomy. The regulation of corticosteroid receptors in other brain regions and the pituitary has been less well studied. As seen in this study and in another report [25], type II receptors in the frontal cortex respond in an autoregulatory fashion to changes in circulating corticosterone levels in a manner similar to hippocampal type II receptors. In contrast, hypothalamic type II receptors increase following adrenalectomy, but are not downregulated following corticosterone. Pituitary type II receptors do not vary under either conditions. The lack of downregulation of hypothalamic and pituitary type II receptors is in accord with previous reports [13, 15]. The striatum also contains high levels of type II receptors, but the regulation of these receptors has not been extensively studied. Drugs of abuse, such as 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine, decrease type II receptors in the striatum, an effect which is independent of changes in corticosterone [26, 27]. Of the

4 brain regions examined in the present study, the striatum was the only area that was completely insensitive to both adrenalectomy and corticosterone administration. It appears that factors other than corticosterone, such as neurotransmitters, may play an important role in regulating striatal GR.

A major focus of this study was to compare the regulation of both brain and lymphoid GR. This is relevant in terms of clinical studies which use circulating lymphocytes as a peripheral model of brain GR and also in terms of the role of glucocorticoids in regulating immune function. The results indicate only a partial correspondence between brain and lymphoid GR in their response to alterations in corticosterone levels. None of the three tissues examined (lymphocytes, spleen and thymus) showed an increase in GR following a 7 day adrenalectomy period. The effect of adrenalectomy on lymphoid GR may be time-dependent, since GR in lymphocytes, and to a lesser extent the spleen, do increase following a shorter (3 day) adrenalectomy period [10]. However, in response to high levels of corticosterone there is a concomitant decrease in both brain (i.e. frontal cortex and hippocampus) and lymphoid (lymphocyte and spleen) GR. Interestingly, GR in these same 4 tissues are also consistently decreased by reserpine, a drug which depletes biogenic amines [10]. Not all lymphoid tissues respond to the elevations in corticosterone with a decrease in receptor levels, since the thymus was not affected by corticosterone administration. However, it is possible that the thymus and other tissues may show a downregulation of receptors following higher doses of corticosterone or in response to synthetic glucocorticoids, such as dexamethasone. Other factors may also be involved in the tissue specific effects of corticosterone on receptor levels. Binding of corticosterone by corticosteroid binding globulin can influence the local concentration of biologically active corticosterone. Peripheral tissues tend to have higher concentrations of corticosteroid binding globulin than the brain [12]. In addition, local metabolism of corticosterone and differential rates of receptor recovery or synthesis could also be important factors in the tissue specific effects of corticosterone and ADX on receptor levels.

In order to determine if elevations in endogenous corticosterone levels can decrease corticosteroid receptors, a 5 day immobilization stress procedure was used. This paradigm has been suggested to be a useful animal model of depression and also produces marked elevations in serum corticosterone levels [19]. This paradigm however failed to alter corticosteroid receptor levels in any of the tissues examined. It is possible that this stress procedure is not intense enough or of long enough duration to result in the prolonged elevations of circulating corticosterone which may be necessary for downregulation of corticosteroid receptors. Although this regimen produced a modest degree of adrenal hypertrophy, it had no significant effect on thymus weight. This contrasts with the marked reduction in thymus weight which was observed following corticosterone injections. Three other studies have examined the effect of stress on GR regulation. Sapolsky *et al.* [13] utilized a 3 week regimen consisting of 3 major stressors per day. This regimen produced thymic involution, adrenal hypertrophy and decreased GR levels in the hippocampus and amygdala, but not in the hypothalamus or pituitary. Kitayama *et al.* [28] used a 22 h/day immobilization procedure for 14 days with a 7 day recovery period. Significant increases in nuclear GR immunoreactivity were observed in monoaminergic cell bodies, but no change was detected in discrete areas of the hippocampus, hypothalamus or cortex. Young *et al.* [29] employed an 8 day course of electrically induced seizures. This procedure increased adrenal weight and elevated basal corticosterone levels. No decrease in type II receptors in the hippocampus or cortex was observed. However, a significant increase in the number of type I receptors in the hippocampus was detected. Thus, it is unclear to what extent fluctuations in physiological or stress-induced changes in endogenous corticosteroid levels influence GR levels.

The basis for the differential regulation of GR in various tissues by glucocorticoids is not well-established. This differential regulation that has been reported using radioligand binding techniques has also been observed when GR messenger RNA (mRNA) is used as a measure of receptor expression [30–32]. There is also evidence based on *in situ* hybridization histochemistry of MRNAs for the type I and II receptor that discrete subfields of the hippocampus display differences in their sensitivity to regulation by glucocorticoids [33]. In one study where both mRNA measurements and radioligand binding techniques were employed to study hippocampal type I and II receptors, there was no

correspondence between changes in binding and mRNA levels [24]. *In vitro* studies with hepatoma cells indicates that dexamethasone decreases both GR mRNA as well as the half-life of the receptor protein suggesting that both transcriptional and posttranslational processes may be involved in homologous regulation of GR [34].

In conclusion, the present results document a considerable degree of differential regulation of GR in various tissues in response to both decreases and increases in circulating corticosterone levels. Since corticosterone- and ADX-induced changes in brain GR is region specific, it is apparent that lymphocyte GR will reflect only some aspects of brain GR regulation and that caution is needed in generalizing among tissues. However, the ability of corticosterone to downregulate GR in both the hippocampus and circulating lymphocytes suggests that receptors in these 2 tissues do share some common regulatory mechanisms.

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