



Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies

Michael A. Cole, Paul J. Kim, Brian A. Kalman,
Robert L. Spencer *

Department of Psychology, University of Colorado, Campus Box 345, Boulder, CO 80309, USA

Received 9 September 1998; accepted 17 June 1999

Abstract

A dose of dexamethasone was determined in rats (50 µg/kg SC) that suppressed the corticosterone response to restraint stress by 80%. Corticosteroid receptor occupancy estimates found that the 50 µg/kg SC dose of dexamethasone had no significant effect on available glucocorticoid receptor (GR) or mineralocorticoid receptor (MR) binding in brain regions (hypothalamus, hippocampus and cortex); on the other hand dexamethasone produced a selective and significant decrease in available GR in peripheral tissues (pituitary and spleen). Functional studies showed that the 50 µg/kg SC dose of dexamethasone completely blocked the effects of corticotropin-releasing hormone (CRH; 0.3–3.0 µg/kg IP) on corticosterone secretion, but did not inhibit the corticosterone response to an adrenocorticotropin hormone (ACTH; 2.5 I.U./kg IP) challenge. These studies indicate that this dose of dexamethasone exerts its inhibitory effects on the HPA axis primarily by acting at GR in the pituitary. The plasma dexamethasone levels produced by this dose of dexamethasone are similar to those present in humans the afternoon after an oral dexamethasone suppression test (DST), a time at which many depressed patients escape from dexamethasone suppression. These results support and extend other studies which suggest that the DST provides a direct test of the effects of increased GR activation in the pituitary on ACTH and cortisol secretion. © 2000 Published by Elsevier Science Ltd. All rights reserved.

Keywords: HPA axis; Rats; Corticosterone; Pituitary; Hippocampus; Corticosteroid receptors

* Corresponding author. Tel.: +1-303-492-0854; fax: +1-303-492-2967.

E-mail address: spencer@clipr.colorado.edu (R.L. Spencer)

1. Introduction

Dexamethasone is a synthetic glucocorticoid that has been widely used for *in vitro* and *in vivo* studies of the glucocorticoid effects on a number of different cellular and physiological responses. In biological psychiatry dexamethasone has been extensively used to probe hypothalamic–pituitary–adrenal (HPA) axis negative feedback sensitivity to glucocorticoids (APA Task Force, 1987; Ribeiro et al., 1993). A decreased responsiveness to dexamethasone within the context of a dexamethasone suppression test (DST) has been repeatedly found in nearly 50% of depressed individuals (Murphy et al., 1991; Ribeiro et al., 1993). In addition, an impaired DST result has been observed in a similar proportion of Alzheimer's patients and in a smaller proportion of individuals diagnosed with schizophrenia (Carroll, 1982; Holsboer, 1983; APA Task Force, 1987; Sapolsky and Plotsky, 1990).

In spite of the widespread use of dexamethasone in biological psychiatry, there remains some debate as to the primary site of action for dexamethasone's suppressant effects on ACTH and corticosteroid secretion. Based primarily on animal studies, some investigators have made a case for a central site of action for dexamethasone (Feldman and Conforti, 1980a,b; Weidenfeld and Feldman, 1993), whereas others have provided evidence for primarily a pituitary site of action (de Wied 1964; de Kloet et al., 1974, 1975; Miller et al., 1992). This distinction has important implications for interpretation of the results of studies using systemic dexamethasone treatment. Determining the primary site of action for a DST relevant dose of dexamethasone in animal studies may be important for understanding the basis of impaired dexamethasone sensitivity in many depressed patients. Although it has become fairly well accepted that dexamethasone acts primarily at the level of the pituitary, a number of clinical and basic science reports assume or suggest that dexamethasone directly probes central glucocorticoid sensitivity (e.g. ; Carroll, 1982; Haracz et al., 1988; Calogero et al., 1990; Guillaume-Gentil et al., 1990; Sapolsky and Plotsky, 1990; Rupprecht et al., 1991; Pepin et al., 1992a,b; Bradbury et al., 1994; Maes et al., 1995).

Support for a centrally mediated effect of dexamethasone on HPA axis activity has been provided by Feldman and colleagues who found that lesions of various brain regions, such as the hippocampus, blunted the suppressive impact of dexamethasone treatment on stress-induced corticosterone levels (Feldman et al., 1973, 1983; Feldman and Weidenfeld 1991, 1993). De Kloet and colleagues, however, noted that there appears to be a limited ability of peripherally administered dexamethasone to be taken up by cells in the CNS (de Kloet et al., 1974, 1975). In addition, Miller et al. (1992) examined the dose-response relationship between dexamethasone and activation of corticosteroid receptors in the pituitary and the brain. The effects of endogenous glucocorticoids are mediated by two closely related intracellular receptors, the mineralocorticoid receptor (MR or type I receptor) and the glucocorticoid receptor (GR or type II receptor) (de Kloet et al., 1993). Low doses of dexamethasone were found to produce selective activation of GR in the pituitary, whereas MR in the pituitary or MR and GR in brain tissue were unaffected (Miller et al., 1992).

On the other hand, high doses of dexamethasone do lead to evidence of GR activation in the brain (Miller et al., 1992). Are these higher doses of dexamethasone necessary to produce effective suppression of corticosterone secretion? Lacking from previous animal studies is a systematic evaluation of both the pattern of corticosteroid receptor activation after a systemic dose of dexamethasone and a simultaneous functional evaluation of dexamethasone's effectiveness at suppressing corticosterone secretion. Consequently, in this study we examined the ability of a range of SC doses of dexamethasone to effectively suppress the HPA axis response to acute stress. Then, selecting a dose of dexamethasone that produced near maximal suppression, we examined whether that dose produced activation of MR or GR in peripheral tissues (pituitary and spleen) and in brain tissue (hypothalamus, hippocampus and cortex). Finally, we tested whether this dose of dexamethasone functionally produced suppression of corticosterone at the level of the pituitary or adrenal by combining dexamethasone treatment with a corticotropin releasing hormone (CRH) or adrenocorticotropin hormone (ACTH) challenge. Although a direct effect of dexamethasone on the adrenal seems unlikely, the adrenal cortex does express GR, and evidence supporting a direct suppressive effect of dexamethasone on adrenal corticosteroid production has been provided (Loose et al., 1980).

2. Methods

2.1. Subjects

For all experiments the subjects were young adult, male Sprague Dawley rats supplied from Harlan Sprague Dawley, Inc. (Indianapolis, IN) with weights ranging from 250 to 350 g. Rats were housed in hanging wire mesh cages (two or three per cage) and were maintained on a 12 h light–dark cycle (lights on at 0700 h). After arrival at the University of Colorado at Boulder animal care facilities, the animals were given a 2–4 week acclimation period before onset of experimentation. Rat chow and water were provided ad libitum. All experiments took place during the early portion of the light period. All experiments were approved by the University of Colorado Institutional Animal Care and Use Committee.

2.2. Experiment 1: dexamethasone dose response

Four groups of rats were treated with one of four doses of dexamethasone (1, 5, 25, and 50 $\mu\text{g}/\text{kg}$; $n = 4$ per group) and one group of rats was treated with vehicle ($n = 8$) in order to determine a dose of dexamethasone that could effectively suppress the corticosterone response to stress. Dexamethasone (Sigma, St. Louis, MO) or vehicle (1 ml/kg propylene glycol) were injected SC 90 min prior to acute stress. The stressor was one hour restraint in adjustable length, cylindrical, Plexiglas tubes: smaller tubes (5.1 cm diameter and 13.0 ± 2.5 cm length) were used for rats weighing approximately 250–300 g and larger tubes (6.3 cm diameter and $15.5 \pm$

2.5 cm length) were used for rats weighing approximately 300–350 g. Blood samples for measuring corticosterone were taken within 3 min after the onset of the restraint, 30 and 60 min into restraint, and 1 h after the termination of restraint.

2.3. Experiment 2: plasma dexamethasone concentration measurement

Dexamethasone plasma concentrations were determined for the 50 µg/kg dose of dexamethasone. Six rats were injected SC with the 50 µg/kg dose of dexamethasone and blood samples were taken just prior to and 30, 60, 120, and 180 min after injections.

2.4. Experiment 3: dexamethasone effect on available corticosteroid receptors

The acute effect of dexamethasone injection on available corticosteroid receptor levels was examined in peripheral tissues (pituitary and spleen) and brain tissues (hypothalamus, hippocampus, cortex) in order to estimate *in vivo* receptor occupancy by dexamethasone. For this experiment animals were adrenalectomized 24 h prior to death. Estimate of total corticosteroid receptors was provided by a group pretreated with vehicle only (1 ml/kg propylene glycol) ($n = 3$). The effect of a 50 µg/kg SC dose of dexamethasone on available corticosteroid receptors was examined in rats injected with dexamethasone 30 min ($n = 3$) and 90 min ($n = 4$) prior to death by rapid decapitation. The 90 min time point was chosen to coincide with the time after dexamethasone injection in which rats were challenged with restraint stress, CRH or ACTH in the other studies reported in this paper. Available MR and GR were measured by a cytosolic receptor binding assay (see below).

2.5. Experiment 4: CRH challenge

Eight treatment groups ($n = 4$ per group) were used in order to determine if a CRH challenge could short-circuit possible inhibitory effects at the level of the brain exerted by the 50 µg/kg SC dose of dexamethasone. Each CRH challenge dose was paired with dexamethasone (50 µg/kg SC) or vehicle (1 ml/kg propylene glycol SC) pretreatment. CRH (human/rat synthetic corticotropin releasing factor, Sigma, St. Louis, MO) was freshly dissolved in sterile 0.9% saline. CRH (0.3, 1.0, or 3.0 µg/kg) or vehicle (1 ml/kg saline) challenges were injected IP 90 min after dexamethasone or vehicle injections. Blood samples were taken 30, 60, and 120 min post CRH injections for measurement of plasma corticosterone.

2.6. Experiment 5: ACTH challenge

Four treatment groups ($n = 4$ per group) were used in order to determine if an ACTH challenge could short-circuit possible inhibitory effects at the level of the brain and pituitary exerted by the 50 µg/kg SC dose of dexamethasone. The challenge dose of ACTH (ACTH 1-39, corticotropin A from porcine pituitary, Sigma, St. Louis, MO; freshly dissolved in sterile 0.9% saline) was paired with

dexamethasone (50 µg/kg SC) or vehicle (1 ml/kg propylene glycol SC) pretreatment. The challenge dose of ACTH (2.5 I.U.) was determined from a pilot dose-response study which examined four doses of ACTH in the range of 2.5–20 I.U. We used the lowest dose examined since it produced a maximal stimulatory effect on corticosterone secretion (data not shown). ACTH (2.5 I.U./kg) or vehicle (1 ml/kg saline) were injected IP 90 min after dexamethasone/vehicle injections. Blood samples were taken 30 and 60 min post ACTH injections for measurement of plasma corticosterone.

2.7. Corticosterone assay

For blood samples the tail clip method was used to collect 0.1 ml blood in heparinized tubes. Samples were centrifuged and the plasma stored at -20°C for subsequent corticosterone measurement. On the day of the assay samples were diluted in buffer (0.01 M PBS) and then heated for 1 h in a water bath (75°C) in order to inactivate corticosteroid binding globulin. Radioimmunoassay was then performed on heat inactivated samples using rabbit antiserum raised against corticosterone-21-hemisuccinate BSA (B21-42; Endocrine Sciences, Tarzana, CA). Assay sensitivity was 0.5 µg/100 ml when assaying 20 µl plasma. The within assay coefficients of variation were 14 and 10%, for samples containing 5 and 20 µg/100 ml corticosterone, respectively. The between assay coefficients of variation were 12 and 8% for samples containing 5 and 20 µg/100 ml corticosterone, respectively.

2.8. Dexamethasone assay

Plasma samples were obtained and stored in the same fashion as those for the corticosterone assay. For radioimmunoassay, plasma samples were first diluted 1:50 in 0.01M PBS. Diluted sample (100 µl) was then mixed with a 100 µl solution containing 20 000 cpm of ^3H -dexamethasone (S.A. = 48 ci/mmol; Amersham, Arlington Heights, IL) and rabbit anti-dexamethasone serum (1:100 dilution, IgG Corporation, Nashville, TN), and incubated overnight at 4°C . Bound and free ^3H -dexamethasone were separated by centrifugation in the presence of dextran coated activated charcoal. The supernatant was mixed with scintillation cocktail and radioactivity was then measured with a scintillation counter. Dexamethasone (Sigma, St. Louis, MO) standards were assayed in parallel (0.01, 0.02, 0.05, 0.1, 0.2, 0.5 and 1 ng per assay tube).

2.9. Corticosteroid receptor measures

Estimates of *in vivo* corticosteroid receptor occupancy by dexamethasone treatment were determined by examining the effect of dexamethasone treatment on available cytosolic corticosteroid receptors. Since only inactivated corticosteroid receptors can be measured in a cytosolic corticosteroid receptor binding assay (Chou and Lutttge, 1988; Litwack, 1988), a decrease in available receptors following acute dexamethasone treatment indicates receptor occupancy and activation by

dexamethasone (Spencer et al., 1990). Rats were adrenalectomized 24 h prior to sacrifice to prevent interference from endogenous corticosterone. Dexamethasone was administered subcutaneously at the times indicated prior to sacrifice. Immediately following sacrifice, tissues were rapidly removed and stored at -70°C . The available cytosolic MR and GR were measured using a radioligand receptor binding assay (Spencer et al., 1990). Tissues from individual animals were homogenized in a buffer solution comprised of 10 mM Tris, 1 mM EDTA, 10% glycerin, 20 mM molybdic acid, and 5 mM dithiothreitol at a pH of 7.4 at 4°C . The homogenate was centrifuged ($100\,000 \times g$, 30 min) and 150 μl of the supernatant fraction (cytosol) was incubated for 18–24 h in the presence of 200 μl of [^3H]-dexamethasone (10 nM, final concentration) \pm non-radioactive competitors. Available GR binding was determined from the amount of total [^3H]-dexamethasone binding that was displaced by the selective GR ligand RU28362 (0.5 μM). Available MR binding was determined by the amount of residual [^3H]-dexamethasone specific binding. Nonspecific binding was defined as the amount of [^3H]-dexamethasone binding that was not displaced by an excess of dexamethasone (10 μM). Non-specific binding was less than 10% of total binding. Bound [^3H]-dexamethasone was separated from unbound steroid by gel filtration (100 μl per incubate in triplicate) in 1 ml Sephadex (LH-20, Amersham Pharmacia, Piscataway, NJ) columns. The resulting eluate was mixed with scintillation cocktail and the radioactivity was measured using a scintillation counter (1600 Tri-Carb LSC, Packard, Meriden, CN). Specific binding was expressed as fmol/mg protein. Protein content was determined by the method of Bradford (1976), with use of bovine serum albumin as a reference point.

2.10. Statistical analysis

Overall treatment effects were determined by analysis of variance (ANOVA). For Experiments 1 and 3, determination of significant differences between individual groups for a given time-point or tissue were further examined by a Tukey's test. For Experiment 2, posthoc analysis tested the difference contrasts of adjacent time points with Bonferonni correction. For Experiments 4 and 5, determination of significant differences between vehicle and dexamethasone pretreatment for a given challenge dose of CRH or ACTH was determined by student's *t*-test. Data presented in figures and tables are group means \pm S.E.M.

3. Results

3.1. Experiment 1: dexamethasone dose response

The corticosterone response to an acute stressor after pretreatment with four different doses of dexamethasone was evaluated in order to determine a dose of dexamethasone that could effectively suppress the stress response (Fig. 1). Analysis of variance revealed significant effects for drug, $F(4,19) = 25.9$, $P < .001$; time,

$F(3,57) = 83$, $P < .001$; and time \times drug interaction, $F(12,57) = 4.4$, $P < .001$. The doses of 1 and 5 $\mu\text{g}/\text{kg}$ dexamethasone did not significantly decrease the stress response magnitude. Post-hoc tests indicate that the doses of 25 and 50 $\mu\text{g}/\text{kg}$ both significantly diminished the magnitude of the stress response. Moreover, only the latter dose was able to reduce the stress response to near basal levels. The 50 $\mu\text{g}/\text{kg}$ dose of dexamethasone reduced the corticosterone stress response by 80%. Due to the near maximal suppressive (functional) activity of this dose, it was used for the remainder of the studies.

3.2. Experiment 2: plasma dexamethasone concentration measurement

Plasma concentrations of dexamethasone were determined for the 50 $\mu\text{g}/\text{kg}$ dose of dexamethasone (Fig. 2). Peak dexamethasone concentration was observed 120 min after injection, although relatively high concentrations were observed at the earliest time point of 30 min after injection. Repeated measures analysis of variance revealed a significant effect for drug, $F(3,12) = 9.4$, $P < .001$. There were significantly greater dexamethasone levels at 120 min after injection than 60 min. None of the other post injection adjacent time points were significantly different from each other.

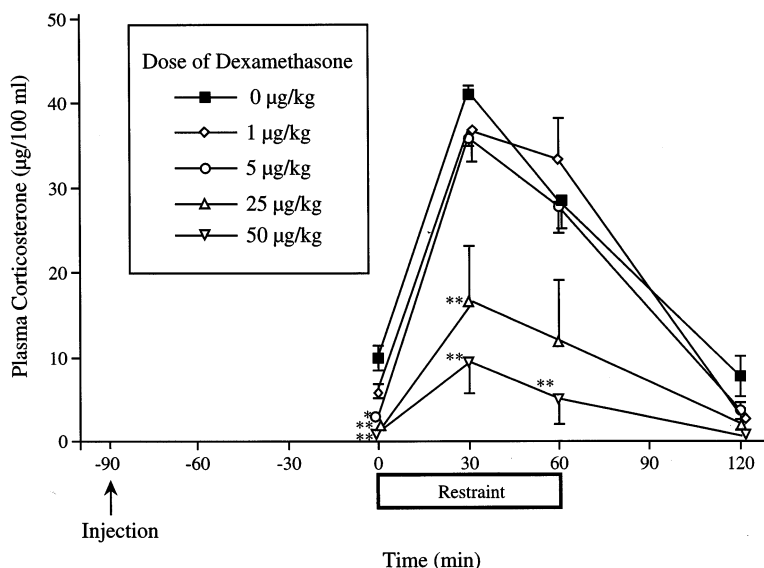


Fig. 1. Dexamethasone suppression of corticosterone release in response to restraint stress. Dexamethasone (1, 5, 25, or 50 $\mu\text{g}/\text{kg}$ SC; $n = 4$) or vehicle (propylene glycol, 1 ml/kg SC; $n = 8$) were injected 90 min prior to restraint stress. Blood samples were taken at the times indicated. * significantly different from vehicle group at the same time-point, $P < .01$, Tukey's test. ** significantly different from vehicle group at the same time-point, $P < .001$, Tukey's test.

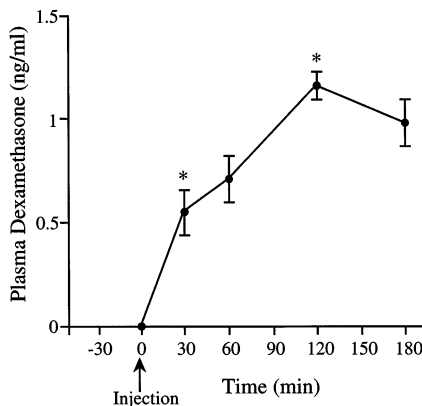


Fig. 2. Plasma concentration of dexamethasone after injection of 50 µg/kg dexamethasone SC ($n = 6$). Blood samples were taken at the times indicated. * significantly different from prior time-point, $P < .05$, posthoc analysis for difference contrasts of adjacent time-points with Bonferonni correction.

3.3. Experiment 3: dexamethasone effect on available corticosteroid receptors

In the first experiment, the 50 µg/kg SC dose of dexamethasone was able to effectively suppress the corticosterone response to an acute stressor. The third experiment utilized this functional dose in order to determine whether significant occupancy of MR and GR by dexamethasone could be detected in peripheral tissues (pituitary and spleen) and brain tissues (hypothalamus, hippocampus and cortex) of adrenalectomized rats. The 50 µg/kg SC dose of dexamethasone selectively decreased available GR binding in the two peripheral tissues examined (Fig. 3). In the pituitary there was a significant decrease in available GR binding 30 and 90 min after injection ($P < .01$, Tukey's test) and in the spleen there was a significant decrease 90 min after injection ($P < .05$, Fisher's test). There was no significant effect of dexamethasone treatment on GR binding in brain tissue (Fig. 3). In addition, dexamethasone had no significant effect on MR binding in any of the tissues examined.

3.4. Experiment 4: CRH challenge

A CRH challenge was administered with either the functional dose of dexamethasone (50 µg/kg SC) or vehicle pretreatment to determine if dexamethasone exerts an inhibitory effect at the level of the pituitary (Table 1). Analysis of variance revealed significant main effects for dexamethasone, $F(1,24) = 67.2$, $P < .001$; CRH, $F(3,24) = 4.7$, $P < .05$; and time, $F(2,48) = 16.8$, $P < .001$; and significant interactions for dexamethasone \times CRH, $F(3,24) = 4.2$, $P < .001$; time \times dexamethasone, $F(2,48) = 15.3$, $P < .001$; time \times CRH, $F(6,48) = 3.1$, $P < .05$; and time \times dexamethasone \times CRH, $F(6,48) = 3.2$, $P < .05$. The CRH challenge with vehicle pretreatment was able to stimulate corticosterone responses in a dose dependent manner.

The 1.0 $\mu\text{g}/\text{kg}$ and 3.0 $\mu\text{g}/\text{kg}$ doses of CRH stimulated corticosterone release comparable to typical stress response levels. Dexamethasone completely blocked the CRH induced corticosterone secretion for all three doses of CRH at all three timepoints. Fig. 4 shows the peak corticosterone responses to CRH at 30 min after CRH injections, and the complete blockade of those responses by dexamethasone.

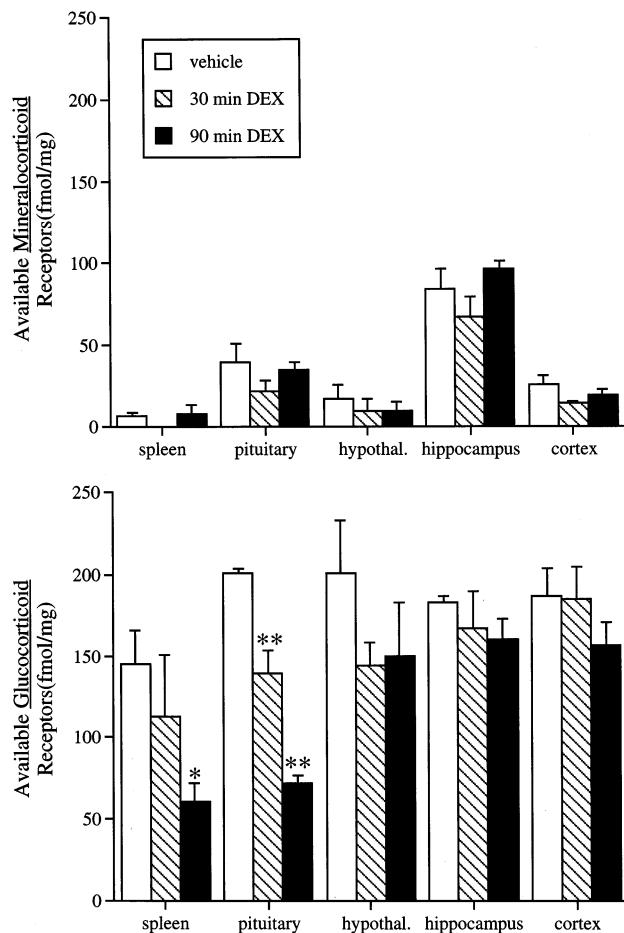


Fig. 3. Effect of dexamethasone on available corticosteroid receptors. All rats were adrenalectomized 24 h prior to sacrifice. Dexamethasone (50 $\mu\text{g}/\text{kg}$ SC) was administered 30 min ($n = 3$) or 90 min ($n = 4$) prior to death and was compared to a vehicle (propylene glycol 1 ml/kg SC; $n = 3$) injected group. Available MR and GR were measured by a cytosolic receptor binding assay (Section 2). Since the receptor assay can only measure unoccupied corticosteroid receptors, a dexamethasone induced decrease in available receptors indicates that those receptors were occupied and activated in vivo by dexamethasone treatment. * significantly different from vehicle group, $P < .05$, Fisher's test. ** significantly different from vehicle group, $P < .01$, Tukey's test.

Table 1

Corticosterone plasma concentrations (mean \pm S.E.M.) in vehicle or CRH challenged rats receiving either vehicle or dexamethasone pretreatment ($n = 4$).

CRH ($\mu\text{g}/\text{kg}$)	Vehicle pretreatment (min)			Dexamethasone pretreatment (min)		
	30	60	120	30	60	120
0	1.9 \pm 0.4	11.6 \pm 3.7	4.2 \pm 2.4	0.2 \pm 0.2	0.7 \pm 0.2	0.5 \pm 0.5
0.3	13.0 \pm 5.9	15.3 \pm 6.6	8.7 \pm 6.4	0.1 \pm 0.1	0.3 \pm 0.1	0.1 \pm 0.1
1.0	23.7 \pm 3.1*	18.2 \pm 4.2	8.4 \pm 2.8	0.9 \pm 0.9	0.8 \pm 0.3	0.1 \pm 0.1
3.0	27.5 \pm 2.9*	29.8 \pm 1.6	12.0 \pm 4.0	1.2 \pm 1.0	0.5 \pm 0.4	0.7 \pm 0.4

* Significantly different from zero CRH (vehicle treatment) at same time-point with same pretreatment, $P < .01$, Tukey's test.

3.5. Experiment 5: ACTH challenge

An ACTH challenge was administered after pretreatment with either vehicle or dexamethasone (50 $\mu\text{g}/\text{kg}$ SC) to determine if dexamethasone exerted an inhibitory effect on corticosterone secretion at the level of the adrenal glands (Fig. 5). Analysis of variance indicates that ACTH was able to stimulate a corticosterone response, $F(1,16) = 21.3$, $P < .05$. There was also a significant interaction for time \times pretreatment \times challenge, $F(1,16) = 5.0$, $P < .05$; and pretreatment \times challenge, $F(1,16) = 5.5$, $P < 0.05$. No significant effects were present for pretreatment. The interaction

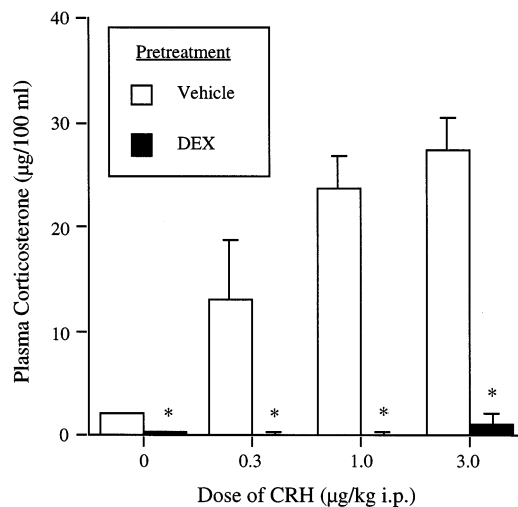


Fig. 4. Effect of dexamethasone pretreatment on the corticosterone response 30 min after CRH challenge. Rats were pretreated with dexamethasone (50 $\mu\text{g}/\text{kg}$ SC) or vehicle (propylene glycol, 1 ml/kg SC), and 90 min later were challenged with vehicle (saline, 1 ml/kg IP) or three different doses of CRH (0.3, 1.0, or 3.0 $\mu\text{g}/\text{kg}$ IP). Blood samples were taken 30 min after vehicle or CRH challenge ($n = 4$). * significantly different from vehicle pretreated group at the same time-point, $P < .01$, Student's t -test.

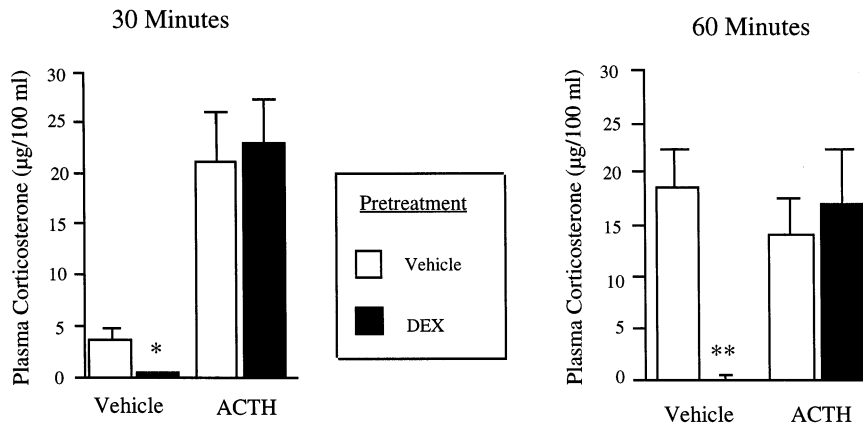


Fig. 5. Effect of dexamethasone pretreatment on corticosterone response 30 and 60 min after ACTH challenge. Rats were pretreated with dexamethasone (50 µg/kg SC) or vehicle (propylene glycol, 1 ml/kg SC), and 90 min later were challenged with vehicle (saline, 1 ml/kg IP) or ACTH (2.5 I.U./kg IP). Blood samples were taken 30 and 60 min after vehicle or ACTH challenge ($n = 4$). * significantly different from vehicle group, $P < .05$, Student's t -test. ** significantly different from vehicle group, $P < .001$, Student's t -test.

between pretreatment and challenge does not represent a significant difference between the vehicle and dexamethasone pretreatments for the ACTH challenge, as is clear by the substantial overlap of their standard error bars. An independent t -test revealed a significant difference between the dexamethasone and vehicle pretreatments for the saline challenged groups at both time points, thereby accounting for the significant interaction between pretreatment and ACTH. Thus, dexamethasone did not show an ability to block the corticosterone response to an ACTH challenge. On the other hand, dexamethasone (50 µg/kg) pretreatment effectively suppressed the corticosterone response to vehicle challenge. This is likely due to dexamethasone's suppression of the corticosterone response to the stress of injections and blood sampling.

4. Discussion

The data from these experiments indicate that a dose of dexamethasone (50 µg/kg SC) that effectively suppresses the corticosterone response to an acute stressor produces its inhibitory effect primarily by binding to GR in the anterior pituitary. Thus, available corticosteroid receptor measurements revealed that this dose of dexamethasone selectively decreased available GR in the pituitary while having no effect on available MR in the pituitary nor available MR and GR in the brain. Dexamethasone suppression of a CRH challenge provided functional confirmation that dexamethasone acted at the level of the anterior pituitary. Finally, the corticosterone response to an ACTH challenge was unaffected by dexamethasone

pretreatment indicating that inhibitory effects of dexamethasone did not take place directly at the level of the adrenal gland.

The inability of systemic doses of dexamethasone in our study to activate corticosteroid receptors in the brain is consistent with the results of several other studies. De Kloet and colleagues found that intravenous injection of ^3H -dexamethasone produced a very limited uptake of ^3H -dexamethasone in the nuclear fraction of brain tissue in contrast to a substantial accumulation of ^3H -dexamethasone in the pituitary (de Kloet et al., 1974, 1975). Miller et al. (1992) found that low doses of dexamethasone administered through the drinking water produced a significant activation of GR in the pituitary while having no effect on available corticosteroid receptors in the brain. The differential effect of dexamethasone on corticosteroid receptors in the pituitary versus the brain may reflect differential access of systemic dexamethasone to cells within these tissues. On the other hand, dexamethasone may interact differently with corticosteroid receptors in these two tissues. Several lines of evidence point to the former case, thus, there appears to be a limited ability of dexamethasone to cross the blood-brain barrier. In support of this conclusion, intracerebroventricular injection of rats with ^3H -dexamethasone or *in vitro* incubation of brain tissue slices with ^3H -dexamethasone resulted in a high degree of cellular uptake of ^3H -dexamethasone in brain tissue (de Kloet et al., 1974, 1975). Furthermore, high concentrations of peripherally administered dexamethasone were able to activate GR in hippocampus and hypothalamus (Reul et al., 1987; Miller et al., 1992). Recent studies have provided evidence for a mechanism that may largely account for the poor access of dexamethasone into brain tissue. Meijer et al. (1998) believe that a multiple drug resistance transporter protein (mdr 1a gene product) located in the endothelial cells of cerebral blood vessels actively exports dexamethasone from brain tissue. They have found that a subcutaneous injection of ^3H -dexamethasone produced a fivefold higher accumulation of radioactivity in brain tissue of mdr 1a knock-out mice compared to wild-type mice.

The receptor binding assay used in this study does not provide the cellular resolution to conclude that none of the 50 $\mu\text{g}/\text{kg}$ dose of dexamethasone gained access to cells within subregions of the brain. For example, although we did not see a significant decrease in available corticosteroid receptor binding in a hypothalamic block of tissue, it is possible that some dexamethasone selectively bound receptors within a subregion of the hypothalamus, such as the paraventricular nucleus. In a study examining the uptake of ^3H -dexamethasone in brain and pituitary of rats after subcutaneous injection a relatively high level of ^3H -dexamethasone was detected in the medial basal hypothalamus (ventral caudal arcuate nucleus and adjacent infundibular region). No significant levels of ^3H -dexamethasone were found in other hypothalamic nuclei (Warembourg, 1975). This hypothalamic distribution pattern of dexamethasone is consistent with entry into the portion of the hypothalamus that lacks a blood brain barrier. A regulatory effect of neuron cell bodies located in this medial basal hypothalamic region on CRH or ACTH secretion has yet to be demonstrated.

Regardless of whether some dexamethasone penetrates into various brain regions, the CRH challenge experiment in this study indicates that the actions of

dexamethasone at the pituitary were sufficient to completely block the corticosterone response to relatively high doses of CRH. Thus, the present study strongly points toward lower systemic doses of dexamethasone exerting their chief inhibitory effect on corticosteroid secretion at the level of the pituitary. This conclusion has some notable implications for the interpretation of both basic research and preclinical studies utilizing the systemic administration of dexamethasone. First, lower doses of dexamethasone can not probe the effects of glucocorticoids centrally because they are unable to penetrate the brain. In addition lower doses of dexamethasone may at the level of the brain effectively produce a 'chemical adrenalectomy'. Functional support for this is provided by studies demonstrating that dexamethasone treatment will substitute for adrenalectomy in producing apoptosis of dentate gyrus granule cells (Hassan et al., 1996; Hornsby et al., 1996). Second, the use of higher doses of dexamethasone in order to produce GR occupancy in the brain undoubtedly results in extraordinary levels of GR activation in the periphery. Moreover, studies that utilize chronic dexamethasone treatment raise concerns of extremely high levels of dexamethasone accumulating in the periphery. In addition, during conditions by which dexamethasone is able to enter the brain, the concurrent decreased levels of corticosterone (due to dexamethasone suppression of HPA activity) may lead to a very non-physiological situation of GR occupancy and less than normal MR occupancy (Spencer et al., 1990). Consequently, all of these factors contribute to an ambiguous experimental situation in which it is difficult to sort out the direct site of action (central or peripheral) and the corticosteroid receptor type (increased GR activation or decreased MR activation) responsible for dexamethasone's effects.

These conclusions may also have some important implications for the interpretation of DST outcomes. In light of the characteristics of dexamethasone identified by this study and others, the DST is most likely an indicator of impaired ability of dexamethasone to suppress HPA activity at the level of the pituitary. Although it may be risky to draw direct parallels between the distribution and action of dexamethasone in rats and humans, it is worth noting that our relatively high dose of dexamethasone produced peak blood levels in the rat (1.16 ± 0.07 ng/ml 2 h after injection SC) that are comparable to levels (0.5–4 ng/ml) observed in humans around 1600 h the afternoon following dexamethasone treatment (Klein and Berger, 1987; Asnis et al., 1989; O'Sullivan et al., 1989), a time when non-suppression or escape from suppression is most evident (Carroll, 1982). On the other hand, initial peak dexamethasone blood levels are substantially higher in humans after a 1 mg oral dexamethasone challenge (4.3–14.1 ng/ml; O'Sullivan et al., 1989). These higher circulating dexamethasone levels, however, still do not appear to be in the range necessary to occupy a significant proportion of corticosteroid receptors in rat brain (Miller et al., 1992).

There is considerable interest among biological psychiatrists as to the basis of the dexamethasone resistance in individuals with a major depressive episode. As has been well delineated by Kathol et al. (1989), the prevailing hypotheses can be subdivided into two general possibilities. First, there may be a decreased responsiveness to dexamethasone as a result of altered pharmacokinetic (e.g. increased metabolism of dexamethasone) or pharmacodynamic (e.g. glucocorticoid receptor

down-regulation) related factors. Second, there may be an increased response of HPA components to stimulatory input that at least partially overwhelms the suppressive effect of dexamethasone. There is accumulating indirect evidence supporting this second prospect (Heit et al., 1997; Kathol et al., 1989; Von Bardeleben and Holsboer 1989).

Assuming that dexamethasone suppression of cortisol is a result of a direct pituitary site of action, an impaired DST result may point to an increased amount of ACTH secretagogues that overcome the dexamethasone suppression. This condition may functionally be expressed as a shift to the right in the dexamethasone dose–response curve. In support of this shift, higher levels of dexamethasone are able to suppress cortisol in some individuals that are non-suppressors to a lower dose of dexamethasone (O’Sullivan et al., 1989). In addition, some suppressors become non-suppressors when given CRH challenge in combination with a DST (Von Bardeleben and Holsboer 1989). Presumably in these individuals the combination of exogenous CRH with hypersecretion of endogenous ACTH secretagogues overcomes the dexamethasone suppression. Our results suggest that the endogenous secretagogues that are able to overcome dexamethasone suppression may include factors other than CRH. This conclusion is based on the fact that dexamethasone was able to completely suppress the corticosterone response to a relatively high dose of CRH (3.0 µg/kg). Hatzinger et al. (1996) also found that dexamethasone effectively suppressed the corticosterone response to CRH challenge in rats, but interestingly, in aged rats it did not fully suppress the ACTH response to CRH challenge. The notion that an ACTH secretagogue besides CRH may be responsible for dexamethasone non-suppression in depressives has been advanced by Von Bardeleben and Holsboer (1989) and Von Bardeleben et al. (1985). They provide evidence in support of vasopressin as a factor that can combine with CRH to overcome dexamethasone suppression of cortisol.

Finally, we want to point out that if the impairment in DST non-suppressors is due to an increased central drive resulting in ACTH secretagogue hypersecretion, the increased drive could still be a result of impaired central glucocorticoid negative feedback (Sapolsky and Plotsky, 1990). But, it appears that the DST is unable to directly probe for such an effect. Instead, the dexamethasone resistance expressed at the level of the pituitary may be an indirect consequence of impaired centrally mediated negative feedback effects of endogenous corticosteroids. Such a situation may explain the impaired dexamethasone suppression of corticosterone evident in rats with hippocampal lesions (Feldman and Conforti, 1980b) or in transgenic mice with reduced GR levels (Stec et al., 1994; Barden et al., 1997). Perhaps the use of a cortisol or corticosterone challenge would be a good strategy for probing central sensitivity to glucocorticoids. Gispen-de Wied et al. (1987, 1993, 1998) using this strategy found that acute cortisol treatment for the most part parallels dexamethasone treatment effects on HPA axis activity in control, depressed and child psychiatric populations. Importantly, in some cases cortisol treatment revealed differences between control and depressed patients that were not evident with dexamethasone treatment. Young et al. (1991) found that cortisol infusions in depressed patients also produced an impairment in the fast feedback effects of

cortisol on the corticotrope products, beta-endorphin/beta-lipotropin levels. The use of naturally occurring glucocorticoids has an advantage over dexamethasone in that cortisol and corticosterone activate both mineralocorticoid and glucocorticoid receptors. In addition, due to the high levels of corticosteroid binding globulins that are associated with pituitary tissue, cortisol and corticosterone appear to have preferential access to brain tissue over the pituitary (de Kloet et al., 1977). Corticosterone may be an especially attractive probe in humans since it crosses the blood-brain barrier better than cortisol (Pardridge, 1981), and still allows for the measurement of endogenous cortisol as an endpoint.

Acknowledgements

This work was supported by NIH grants MH54742 and DK49143, and by the University of Colorado Hughes Initiative for undergraduate research training.

References

- APA Task Force on Laboratory Tests in Psychiatry, 1987. The dexamethasone suppression test. An overview of its current status in psychiatry. *Am. J. Psychiatry* 144, 1253–1268.
- Asnis, G.M., Friedman, J.M.H., Miller, A.H., 1989. Plasma dexamethasone and cortisol levels in depressed outpatients. *J. Affect. Disord.* 16, 5–10.
- Barden, N., Stec, I.S.M., Montkowski, A., Holsboer, F., Reul, J.M.H.M., 1997. Endocrine profile and neuroendocrine challenge tests in transgenic mice expressing antisense RNA against the glucocorticoid receptor. *Neuroendocrinology* 66, 212–220.
- Bradbury, M.J., Akana, S.F., Dallman, M.F., 1994. Roles of type I and type II corticosteroid receptors in regulation of basal activity in the hypothalamo–pituitary–adrenal axis during the diurnal trough and the peak: evidence for a nonadditive effect of combined receptor occupation. *Endocrinology* 134, 1286–1296.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Calogero, A.E., Kamilaris, T.C., Johnson, E.O., Tartaglia, M.E., Chrousos, G., 1990. Recovery of the rat hypothalamus–pituitary–adrenal axis after discontinuation of prolonged treatment with the synthetic glucocorticoid agonist dexamethasone. *Endocrinology* 127, 1574–1579.
- Carroll, B.J., 1982. The dexamethasone suppression test for melancholia. *Br. J. Psychiat.* 140, 292–304.
- Chou, Y.C., Luttge, W.G., 1988. Activated Type II receptors in brain cannot rebind glucocorticoids: relationship to progesterone's antiglucocorticoid actions. *Brain. Res.* 361, 212–216.
- de Kloet, R., van der Vies, J., de Wied, D., 1974. The site of suppressive action of dexamethasone on pituitary-adrenal activity. *Endocrinology* 94, 61–73.
- de Kloet, R., Wallach, G., McEwen, B.S., 1975. Differences in corticosterone and dexamethasone binding to rat brain and pituitary. *Endocrinology* 96, 589–609.
- de Kloet, R., Burbach, P., Mulder, G.H., 1977. Localization and role of transcortin-like molecules in the anterior pituitary. *Mol. Cell. Endocrinol.* 7, 261–273.
- de Kloet, R., Oitzl, M.S., Joels, M., 1993. Functional implications of brain corticosteroid receptor diversity. *Cell. Mol. Neurobiol.* 13, 433–455.
- de Wied, D., 1964. The site of the blocking action of dexamethasone on stress-induced pituitary ACTH release. *J. Endocrin.* 29, 29–37.
- Feldman, S., Conforti, N., Chowers, I., 1973. Effect of dexamethasone on adrenocortical responses in intact and hypothalamic deafferented rats. *Acta Endocrinol.* 73, 660–664.

- Feldman, S., Conforti, N., 1980a. Adrenocortical responses in dexamethasone-treated rats with septal, preoptic and combined hypothalamic lesions. *Horm. Res.* 12, 289–295.
- Feldman, S., Conforti, N., 1980b. Participation of the dorsal hippocampus in the glucocorticoid feedback effect on adrenocortical activity. *Neuroendocrinology* 30, 52–55.
- Feldman, S., Siegel, R.A., Weidenfeld, J., Conforti, N., Melamed, E., 1983. Role of medial forebrain bundle catecholaminergic fibers in the modulation of glucocorticoid negative feedback. *Brain Res.* 260, 297–300.
- Feldman, S., Weidenfeld, J., 1991. Depletion of hypothalamic norepinephrine and serotonin enhances the dexamethasone negative feedback effect on adrenocortical secretion. *Psychoneuroendocrinology* 16, 397–405.
- Feldman, S., Weidenfeld, J., 1993. The dorsal hippocampus modifies the negative feedback effect of glucocorticoids on the adrenocortical and median eminence CRF-41 responses to photic stimulation. *Brain. Res.* 614, 227–232.
- Gispens-de Wied, C.C., D'Haenen, H., Verhoeven, W.M.A., et al., 1993. Inhibition of the pituitary-adrenal axis with dexamethasone and cortisol in depressed patients and healthy subjects: a dose-related study. *Psychoneuroendocrinology* 18, 191–204.
- Gispens-de Wied, C.C., Jansen, L.M.C., Wynne, H.J., et al., 1998. Differential effects of hydrocortisone and dexamethasone on cortisol suppression in a child psychiatric population. *Psychoneuroendocrinology* 23, 295–305.
- Gispens-de Wied, C.C., Westenberg, H.G.M., Thijssen, J.H.H., van Ree, J.M., 1987. The dexamethasone and cortisol suppression test in depression: beta-endorphin as a useful marker. *Psychoneuroendocrinology* 12, 355–366.
- Guillaume-Gentil, C., Rohner-Jeanrenaud, F., Abramo, F., Bestetti, G.E., Giovanni, L.R., Jeanrenaud, B., 1990. Abnormal regulation of the hypothalamo-pituitary-adrenal axis in the genetically obese fa/fa rat. *Endocrinology* 126, 1873–1879.
- Haracz, J.L., Minor, T.R., Wilkins, J.N., Zimmerman, E.G., 1988. Learned helplessness: an experimental model of the DST in rats. *Biol. Psychiat.* 23, 388–396.
- Hassan, A.H., von Rosenstiel, P., Patchev, V.K., Holsboer, F., Almeida, O.F., 1996. Exacerbation of apoptosis in the dentate gyrus of the aged rat by dexamethasone and the protective role of corticosterone. *Exp. Neurol.* 140, 43–52.
- Hatzinger, M., Reul, J.M.H.M., Landgraf, R., Holsboer, F., Neumann, I., 1996. Combined dexamethasone/CRH test in rats: hypothalamo-pituitary-adrenocortical system alterations in aging. *Neuroendocrinology* 64, 349–356.
- Heit, S., Owens, M.J., Plotsky, P., Nemeroff, C.B., 1997. Corticotropin-releasing factor, stress, and depression. *Neurosci.* 3, 186–194.
- Holsboer, F., 1983. The dexamethasone suppression test in depressed patients: clinical and biochemical aspects. *J. Steroid Biochem.* 19, 251–257.
- Hornsby, C.D., Grootendorst, J., de Kloet, E.R., 1996. Dexamethasone does not prevent seven-day ADX-induced apoptosis in the dentate gyrus of the rat hippocampus. *Stress* 1, 51–64.
- Kathol, R.G., Jaeckle, R.S., Lopez, J.F., Meller, W.H., 1989. Consistent reduction of ACTH responses to stimulation with CRH, vasopressin and hypoglycemia in patients with depression. *Br. J. Psychiatry* 155, 468–478.
- Klein, H.E., Berger, M., 1987. The pitfalls of the dexamethasone suppression test: A biological marker for endogenous depression? *Hum. Psychopharmacol.* 2, 85–103.
- Litwack, G., 1988. The glucocorticoid receptor at the protein level. *Cancer Res.* 48, 2636–2640.
- Loose, D.S., Do, Y.S., Chen, T.L., Feldman, D., 1980. Demonstration of glucocorticoid receptors in the adrenal cortex: evidence for a direct dexamethasone suppressive effect on the rat adrenal gland. *Endocrinology* 107, 137–146.
- Maes, M., Meltzer, H.Y., D'Hondt, P., Cosyns, P., Blockx, P., 1995. Effects of serotonin precursors on the negative feedback effects of glucocorticoids on hypothalamic-pituitary-adrenal axis function in depression. *Psychoneuroendocrinology* 20, 149–167.
- Meijer, O.C., de Lange, E.C.M., Breimer, D.D., de Boer, A.G., Workel, J.O., de Kloet, E.R., 1998. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in *mdr1A* P-glycoprotein knockout mice. *Endocrinology* 139, 1789–1793.

- Miller, A.H., Spencer, R.L., Pulera, M., Kang, S., McEwen, B.S., Stein, M., 1992. Adrenal steroid receptor activation in rat brain and pituitary following dexamethasone: implications for the dexamethasone suppression test. *Biol. Psychiatry* 32, 850–869.
- Murphy, B.E., Dhar, V., Ghadirian, A.M., Chouinard, G., Keller, R., 1991. Response to steroid suppression in major depression resistant to antidepressant therapy. *J. Clin. Psychopharmacol.* 11, 121–126.
- O'Sullivan, B.T., Hunt, G.E., Johnson, G.F., Caterson, I.D., 1989. The plasma dexamethasone window: evidence supporting its usefulness to validate dexamethasone suppression test results. *Biol. Psychiatry* 25, 739–754.
- Pardridge, W.M., 1981. Transport of protein-bound hormones into tissues in vivo. *Endocr. Rev.* 2, 103–123.
- Pepin, M.C., Pothier, F., Barden, N., 1992a. Antidepressant drug action in a transgenic mouse model of the endocrine changes seen in depression. *Mol. Pharmacol.* 42, 991–995.
- Pepin, M.C., Pothier, F., Barden, N., 1992b. Impaired type II glucocorticoid-receptor function in mice bearing antisense RNA transgene. *Nature* 355, 725–728.
- Reul, J.M.H.M., van den Bosch, F.R., de Kloet, E.R., 1987. Relative occupation of type-I and type-II corticosteroid receptors in rat brain following stress and dexamethasone treatment: functional implications. *J. Endocr.* 115, 459–467.
- Ribeiro, S.C.M., Tandon, R., Grunhaus, L., Greden, J.F., 1993. The DST as a predictor of outcome in dexamethasone: a meta-analysis. *Am. J. Psychiatry* 150, 1618–1629.
- Rupprecht, R., Kornhuber, J., Wodarz, N., et al., 1991. Disturbed glucocorticoid receptor autoregulation and corticotropin response to dexamethasone in depressives pretreated with metyrapone. *Biol. Psychiatry* 29, 1099–1109.
- Sapolsky, R.M., Plotsky, P.M., 1990. Hypercortisolism and its possible neural bases. *Biol. Psychiatry* 27, 937–952.
- Spencer, R.L., Young, E.A., Choo, P.H., McEwen, B.S., 1990. Adrenal steroid type I and II receptor binding: estimates of in vivo receptor number, occupancy, and activation with varying level of steroid. *Brain Res.* 514, 37–48.
- Stec, I., Barden, N., Reul, J.M., Holsboer, F., 1994. Dexamethasone nonsuppression in transgenic mice expressing antisense RNA to the glucocorticoid receptor. *J. Psychiat. Res.* 28, 1–5.
- Von Bardeleben, U., Holsboer, F., 1989. Cortisol response to a combined dexamethasone-human corticotrophin-releasing hormone challenge in patients with depression. *J. Neuroendocrinol.* 1, 485–488.
- Von Bardeleben, U., Holsboer, F., Stalla, G.K., Muller, O.A., 1985. Combined administration of human corticotrophin-releasing factor and lysine vasopressin induces cortisol escape from dexamethasone suppression in healthy subjects. *Life Sci.* 37, 1613–1618.
- Warembourg, M., 1975. Radioautographic study of the rat brain and pituitary after injection of 3H dexamethasone. *Cell. Tiss. Res.* 161, 183–191.
- Weidenfeld, J., Feldman, S., 1993. Glucocorticoid feedback regulation of adrenocortical responses to neural stimuli: role of CRF-41 and corticosteroid type I and type II receptors. *Neuroendocrinology* 58, 49–56.
- Young, E.A., Haskett, R.F., Murphy-Weinberg, V., Watson, S.J., Akil, H., 1991. Loss of glucocorticoid fast feedback in depression. *Arch. Gen. Psychiatry* 48, 693–699.