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Antidepressant Drug Action in a Transgenic Mouse Model of the Endocrine Changes Seen in Depression

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SUMMARY

We have created transgenic mouse lines with impaired glucocorticoid receptor function by expression of a type II glucocorticoid receptor antisense RNA in brain tissues. These animals have endocrinological characteristics similar to those seen in depression, including a hyperactive hypothalamic-pituitary-adrenal axis as indicated by elevated plasma corticosterone and adrenocorticotropin hormone levels. Treatment of transgenic animals with the tricyclic antidepressant desipramine increased hypothalamic glucocorticoid receptor mRNA concentration and dexamethasone-binding activity while decreasing plasma adrenocorticotropin hormone concentration and corticosterone levels. These results support the hypothesis that antidepressants exert action on the hypothalamic-pituitary-adrenal axis through modulation of glucocorticoid receptor gene expression.

Depressive disorders are estimated to disrupt the lives of up to 15% of the population at least once during their lifetimes and may be the most destructive group of mental illnesses in terms of prevalence, mortality, economic cost, and impact on families. Although antidepressive drug therapies produce improvement in 65–75% of patients, their mechanism of action remains unclear and only a smaller percentage of patients have essentially complete amelioration of symptomatology. Increased activity of the HPA axis, indicated by hypersecretion of ACTH and cortisol, and failure to suppress plasma cortisol concentrations after dexamethasone administration have been reported in patients with severe depression (1, 2). Glucocorticoid hormones normally restrain HPA axis function by exerting feedback effects in hypothalamic and extrahypothalamic brain areas, which ultimately cause a decrease in secretion of CRF, and also by inhibiting pituitary ACTH release (3–7). In order to exert this biological action, glucocorticoid hormones must first bind to a cellular receptor. This receptor has been purified (8, 9), its functional domains characterized (10), and the cDNAs cloned from rat, mouse, and human sources (11–13). The hormone-receptor complex, when translocated to the nucleus, recognizes specific DNA sequences (glucocorticoid response elements) to which it binds; by doing so, it modifies the transcription of downstream genes (14). Both the failure to suppress cortisol concentrations after a dexamethasone test dose or combined dexamethasone/CRF challenge (15) and the increased HPA axis activity, as indicated by hypersecretion of CRF and cortisol, have been reported in patients with severe depression (1, 2). This HPA axis dysfunction associated with depressive illness could be caused by ineffective glucocorticoid feedback inhibition leading to overactive hypothalamic CRF neurons and increased expression of the proopiomelanocortin gene. Because neuronal glucocorticoid receptors are necessary for the negative feedback action of glucocorticoid on the HPA axis, it is thus possible that the apparent lack of glucocorticoid sensitivity observed in depression could be due to an abnormality of glucocorticoid receptor regulation at the limbic-hypothalamic level. Support for this comes from the normalization of the hyperactive HPA axis that occurs during successful antidepressant pharmacotherapy of depressive illness (16, 17), and we have hypothesized that this could be brought about by an antidepressant-induced increase in glucocorticoid receptor rendering the HPA axis more susceptible to feedback inhibition by cortisol. This hypothesis is strengthened by the finding that antidepressants increase type II glucocorticoid receptor gene expression (18). Incorporation into the mouse genome of a transgene expressing antisense RNA complementary to a fragment of the glucocorticoid receptor cDNA has allowed us to develop an animal that has defective glucocorticoid feedback inhibition as a result of decreased glucocorticoid receptor production. These transgenic animals, which have reduced type II glucocorticoid receptor mRNA concentrations, a reduced type II glucocorticoid receptor binding capacity, and a hyperactive HPA axis, as demonstrated by elevated plasma corticosterone and ACTH levels (19), are an ideal model in which to test the

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ABBREVIATIONS: HPA, hypothalamic-pituitary-adrenal; PCR, polymerase chain reaction; ACTH, adrenocorticotropin hormone; CRF, corticotropin-releasing factor; SDS, sodium dodecyl sulfate; SSC, standard saline citrate.

hypothesis that antidepressants normalize the HPA axis by acting on glucocorticoid receptor gene expression. Treatment of these transgenic animals with an antidepressant drug resulted in an increase in hypothalamic type II glucocorticoid receptor mRNA concentration and in type II glucocorticoid receptor binding activity, a decrease in ACTH concentration, and a blood plasma corticosterone level not different from that of normal mice.

Experimental Procedures

Animals. Normal and transgenic mice (19) were maintained according to institution guidelines and were injected once per day either with vehicle (0.9% sodium chloride) or with 20 mg/kg of body weight desipramine dissolved in vehicle, for 10 days before sacrifice by decapitation.

Northern blot analysis of type II glucocorticoid receptor mRNA. Hypothalamus, frontal cortex, and other organs were rapidly frozen in liquid nitrogen and stored at −80° until used. RNA was prepared from these tissues by the guanidium/isothiocyanate method (20), separated on 0.8% agarose-formaldehyde denaturing gels, and blotted onto nylon filters (Hybond N; Amersham) before hybridization with type II glucocorticoid receptor cRNA and β-actin cRNA probes. Type II glucocorticoid receptor cRNA antisense probe was produced by T7 polymerase run-off transcription with [^{32}P]UTP of a 1.8-kilobase type II glucocorticoid receptor cDNA fragment (11) subcloned into the plasmid pGEM-1. A β-actin cRNA probe was generated from a 1.5-kilobase β-actin cDNA *Pst*I fragment (21) inserted in pGEM-1. Filters were prehybridized for 4 hr at 42° (in 50% formamide, 5× SSC, 6× Denhardt, (0.12% bovine serum albumin, 0.12% Ficoll 400, 0.12% polyvinyl pyrrolidone), 0.1% SDS, 50 mM phosphate, 200 μg/ml yeast tRNA, 200 μg/ml denatured salmon sperm DNA) and hybridized at 65° for 20 hr. After hybridization, under stringent conditions, filters were washed twice (30 min each time) in 2× SSC/0.1% SDS at room temperature and twice (1 hr each time) in 0.1× SSC/0.1% SDS at 70°. Filters were wrapped in Saran Wrap and exposed to Kodak X-OMAT films with (for filters hybridized with type II glucocorticoid receptor cRNA probe) or without (for β-actin cRNA probe) intensifying screens.

Measurement of type II glucocorticoid receptor binding capacity. Type II glucocorticoid binding activities in brain and other organs were measured using [^{3}H]dexamethasone. Tissue was homogenized in 30 mM Tris, 1 mM EDTA, 10 mM molybdate, 10% (v/v) glycerol, 1 mM dithiothreitol (TEDGM, pH 7.4). After centrifugation at 55,000 × *g* for 15 min at 4°, an aliquot of the cytosol was incubated with 10 nM [^{3}H]dexamethasone (specific activity, 44.7 Ci/mmol; Amersham) for 20–24 hr at 4°. The amount of nonspecific binding was determined in parallel incubations with the labeled steroid in the presence of a 300-fold excess of unlabeled type II glucocorticoid receptor-specific agonist RU 28362. Sephadex LH20 (Pharmacia) columns equilibrated with TEDGM buffer were used to separate bound from unbound steroid. After incubation, 100-μl aliquots of the incubates were loaded onto the columns, washed with 100 μl of TEDGM, and eluted with 400 μl of TEDGM into minivials. The vials were then filled with 5 ml of aqueous counting cocktail Formula A-963 (New England Nuclear, Boston, MA) and counted in a LKB scintillation counter at 40% efficiency. Protein content was determined by the method of Bradford (22).

Analysis of transgene expression. Reverse transcriptase/PCR amplification assay methods were used to measure transgene (type II glucocorticoid receptor antisense RNA) and β-actin mRNA levels. RNA prepared by the guanidium/isothiocyanate method (20) was treated with an amount of DNase (10 units of DNase/2.5 μg of RNA for 10 min at 37°) shown to prevent amplification from DNA (19). A 15-base poly(dT) oligomer was used as primer for reverse transcriptase. For the glucocorticoid receptor antisense RNA amplification, a 21-base oligomer corresponding to the sequence (bases 1824–1844) immediately upstream from the polyadenylation signal in the VP1 gene of bacteriophage SV40, used in conjunction with a 20-base oligomer primer corresponding to positions 381–400 of the type II glucocorticoid receptor cDNA antisense fragment, served for PCR amplification of reverse transcriptase reaction product using a Perkin-Elmer Cetus PCR kit (GeneAmp). A pair of forward and reverse 20-base oligomer primers, corresponding to sequences 292–311 and 807–826 of the mouse β-actin cDNA, were used for PCR amplification. Conditions for reverse transcriptase and PCR amplification were as described by the manufacturer. After electrophoresis and transfer to Hybond N$^+$, amplified products were identified by hybridization to a ^{32}P-labeled 20-base oligomer corresponding to positions 1733–1752 of the glucocorticoid receptor antisense cDNA and to positions 564–583 of the β-actin cDNA.

Results

Effect of desipramine on type II glucocorticoid receptor gene activity in the brain of normal and transgenic mice. Glucocorticoid receptor mRNA concentration was measured in the brain of untreated or desipramine-treated (20 mg/kg of body weight, for 10 days) normal or transgenic mice. Although in both normal mice and transgenic mice type II glucocorticoid receptor mRNA concentration was increased after desipramine treatment, the percentage of stimulation in antidepressant-treated transgenic mice was greater than that seen in antidepressant-treated normal mice (Table 1).

When two different brain regions were analyzed, the antidepressant-induced changes in transgenic mouse type II glucocorticoid receptor mRNA concentration were found to be more pronounced in hypothalamus than in cerebral cortex (Fig. 1). The type II glucocorticoid receptor mRNA concentration in the liver of transgenic or antidepressant-treated transgenic mice (Fig. 1) was not significantly different from that of normal mice.

Antidepressant effects on glucocorticoid binding activity in transgenic mice. Type II glucocorticoid receptor binding activity of transgenic mice treated with desipramine was measured to determine whether the antidepressant-induced increase in type II glucocorticoid receptor mRNA concentration was associated with an increase in functional type II glucocorticoid receptors. Antidepressant treatment increased the total type II glucocorticoid receptor binding capacity in normal and transgenic mouse brain by, respectively, 27% and 33% (Table 1). In different brain regions of transgenic mice, a

TABLE 1

Effect of desipramine on type II glucocorticoid receptor mRNA concentrations and type II glucocorticoid receptor binding activity in brain

Normal or transgenic mice (line 1.3) (19) were given injections of either vehicle or desipramine (20 mg/kg) for 10 days before sacrifice. Type II glucocorticoid receptor mRNA concentration and [^{3}H]dexamethasone binding were measured as described in Experimental Procedures. The results shown are the changes in type II glucocorticoid receptor mRNA concentration and in [^{3}H]dexamethasone binding after desipramine treatment and are expressed as a percentage of values seen in untreated normal mouse or untreated transgenic mouse control groups. Differences between means were tested by the Duncan-Kramer test (30) after analysis of variance (*n* is shown in parenthesis).

Animals	Glucocorticoid receptor mRNA/β-actin mRNA	[^{3}H]Dexamethasone bound
	% of untreated control	*% of untreated control*
Desipramine-treated normal mice	190 ± 36 (8)[a]	127 ± 7 (10)[a]
Desipramine-treated transgenic mice	273 ± 27 (4)[b]	133 ± 12 (20)[a]

[a] $p < 0.05$.
[b] $p < 0.01$.

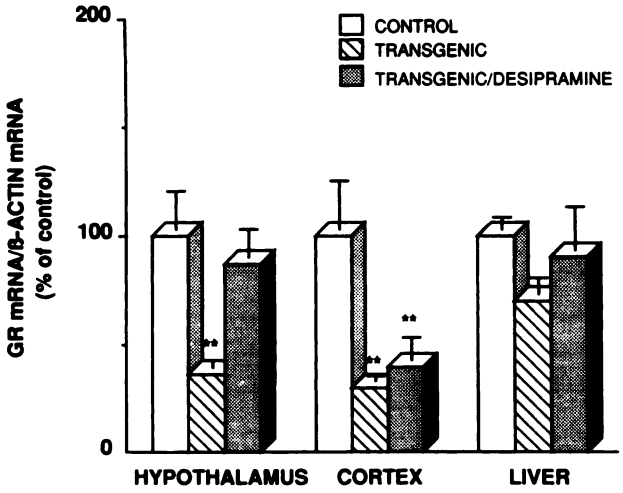

Fig. 1. Effect of desipramine on type II glucocorticoid receptor mRNA concentration in transgenic mouse tissues. Northern blot analysis of glucocorticoid receptor mRNA was performed as described in Experimental Procedures. The results shown are the ratio of type II glucocorticoid receptor (*GR*) mRNA to β-actin mRNA in normal mice (*CONTROL*), transgenic mice (line 5.9) (19), and antidepressant-treated transgenic mice. The number of animals in each group was between 6 and 30 and the significance of differences between means was evaluated by the Duncan-Kramer test (30) after analysis of variance. **, $p < 0.01$.

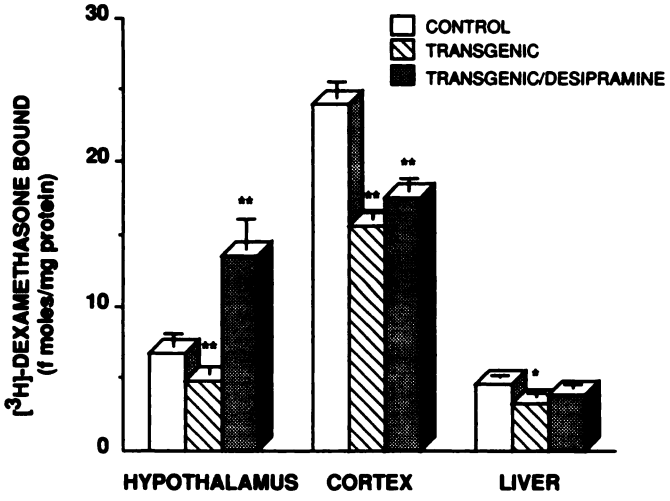

Fig. 2. Effect of desipramine on type II glucocorticoid receptor binding activity in transgenic mouse tissues. [^{3}H]Dexamethasone binding assays were performed as described in Experimental Procedures. The results shown are for normal mice (*CONTROL*), transgenic mice (line 5.9) (19), and antidepressant-treated transgenic mice. Values are the mean ± standard error of a minimum of 24 different determinations. The significance of differences between means was evaluated by the Duncan-Kramer test (30) after analysis of variance. **, $p < 0.01$; *, $p < 0.05$.

maximum of 15.5 (cortex) and 4.8 (hypothalamus) fmol of [^{3}H] dexamethasone/mg of protein was bound. Antidepressant treatment increased these binding capacities by 15% (cortex) and 180% (hypothalamus) to, respectively, 17.5 and 13.5 fmol of [^{3}H]dexamethasone/mg of protein (Fig. 2). No significant change in glucocorticoid binding capacity was observed in the liver of transgenic mice after desipramine treatment (Fig. 2).

Type II glucocorticoid receptor antisense RNA and β-actin mRNA in antidepressant-treated transgenic mice. Antidepressant effects could be exerted primarily on expression of the type II glucocorticoid receptor antisense transgene, followed by secondary changes in glucocorticoid receptor mRNA concentrations. To exclude this possibility we measured type II glucocorticoid receptor antisense RNA, produced by transgene transcription, in different tissues of transgenic mice. The effect of desipramine on β-actin mRNA, which is used as an internal standard for Northern blot analysis, was assessed in these same tissues. No modification of either β-actin mRNA or type II glucocorticoid receptor antisense RNA levels was seen in desipramine-treated transgenic mice, compared with the non-treated group of transgenic mice (Fig. 3). Antisense RNA expression, directed by a neurofilament promoter element, thus remains constant during antidepressant treatment and does not block the functional use of additional type II glucocorticoid receptor mRNA induced by antidepressant treatment.

HPA axis modification in transgenic animals. The type II glucocorticoid receptor antisense transgene, designed to disrupt normal HPA axis function, produced elevated ACTH and corticosterone levels in transgenic mice (Table 2). Treatment of these animals with desipramine resulted in a partial reversal of these elevated ACTH concentrations and a return of the serum corticosterone concentration to within the range of normal mice.

Discussion

Among the most prominent neuroendocrine findings in depression and other affective disorders is the failure of dexamethasone to adequately suppress ACTH and cortisol and to prevent increased release of these hormones after stimulation with CRF. Disturbed glucocorticoid feedback is one event that could explain the pathophysiology of the HPA axis dysfunction in patients with major depression, who present these kind of endocrine symptoms in about 60% of cases (23). One hypothesis proposed to explain these phenomena in patients with depression is a decreased sensitivity to dexamethasone of cells involved in HPA axis control. Incorporation into the mouse genome of a gene fragment directing expression of an antisense RNA complementary to the type II glucocorticoid receptor mRNA has allowed us to develop an animal model that has decreased glucocorticoid receptor gene expression. This transgene causes neuroendocrinological changes reminiscent of those seen in depression, including an apparent reduced neuronal sensitivity to glucocorticoids that leads to high ACTH and corticosterone levels. With these transgenic mice we have studied, at the molecular level, changes in glucocorticoid receptor gene activity after treatment with the tricyclic antidepressant desipramine.

Among several antidepressants shown to increase glucocorticoid receptor mRNA concentrations (24, 25) we selected for these studies desipramine, a monoamine reuptake inhibitor that preferentially acts on the blockade of norepinephrine reuptake with very slight action on serotonin reuptake (26). We tested the hypothesis that antidepressants affect glucocorticoid receptor function by monitoring type II glucocorticoid receptor mRNA levels, type II glucocorticoid receptor binding activity, and HPA axis modification after treatment with desipramine. Type II glucocorticoid receptor mRNA concentration, measured in the brain of normal or transgenic mice, was increased after desipramine treatment. This increase in type II glucocorticoid receptor mRNA was translated into functional glucocorticoid receptors, as shown by elevated type II glucocorticoid receptor binding activity in the brain of antidepressant-treated normal or transgenic mice. Finally, presumably as a

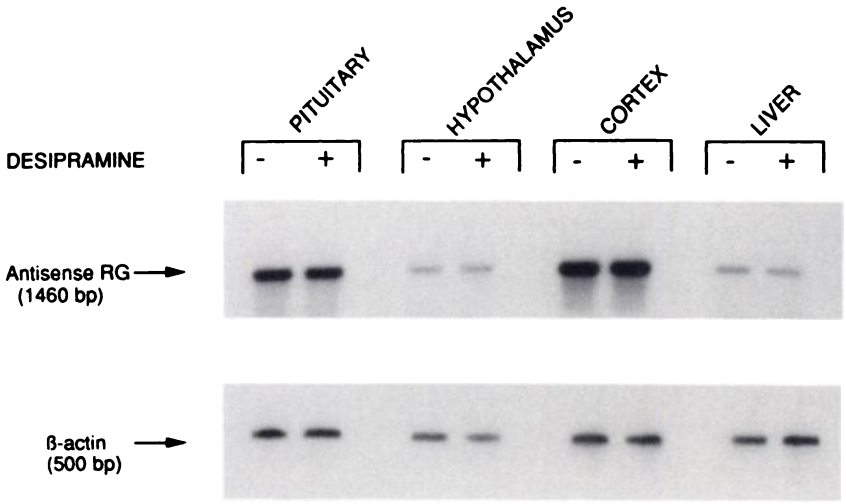

Fig. 3. Effect of desipramine on type II glucocorticoid receptor antisense RNA and on β-actin mRNA in transgenic mice. Animals were given injections of saline or desipramine for 10 days before sacrifice. Reverse transcriptase/PCR amplification of transgene (type II glucocorticoid receptor antisense RNA) and β-actin mRNA was performed as described in Experimental Procedures. No modification of the type II glucocorticoid receptor antisense transgene expression after desipramine treatment of transgenic mice was evident.

TABLE 2

Plasma ACTH and corticosterone concentrations in normal and transgenic mice after desipramine treatment

Hormone content of blood plasma from individual animals (*n* is shown in parenthesis) was measured by specific radioimmunoassay as previously described (31, 32). Differences between means were tested by the Duncan-Kramer (30) test after analysis of variance.

Animals	ACTH	Corticosterone
	pg/ml of plasma	*nmol/liter of plasma*
Normal mice	131 ± 30 (16)	100 ± 20 (15)
Normal mice/desipramine	70 ± 17 (20)	169 ± 25 (16)
Transgenic mice	623 ± 100 (12)[a]	230 ± 40 (15)[a]
Transgenic mice/desipramine	388 ± 75 (16)[a,b]	129 ± 30 (8)

[a] $p < 0.01$ versus untreated normal mice.
[b] $p < 0.01$ versus untreated transgenic mice.

result of increased glucocorticoid receptor binding activity and more efficient glucocorticoid negative feedback action, a reduction in the HPA axis activity of transgenic mice was produced.

The correlation between glucocorticoid receptor mRNA levels and functional glucocorticoid binding activity in brain regions strongly suggests that antidepressants increase type II glucocorticoid receptor gene expression *in vivo* as they have been shown to do *in vitro* (18). Moreover, a role for these receptors in regulation of the HPA axis is supported by the normalization of plasma corticosterone concentration and by the 2-fold decrease in ACTH observed in antidepressant-treated transgenic mice. Although the stimulatory effect of antidepressant drugs on glucocorticoid receptor mRNA and glucocorticoid binding capacity is observed in both brain areas analyzed, it is more pronounced in the hypothalamus. This region is more directly involved in HPA axis control than is the cerebral cortex and may represent a preferential site of action for antidepressant drugs. Desipramine treatment of transgenic mice increased both the type II glucocorticoid receptor mRNA concentration and the type II glucocorticoid receptor binding capacity to levels higher than those seen in normal mice. This increase in glucocorticoid receptors failed, however, to produce a complete reversal to normal levels of the elevated ACTH concentrations. The reason for this is not known but may suggest that factors other than glucocorticoid receptors are also involved in the disturbed HPA axis of these transgenic animals. The decreased glucocorticoid receptor levels of transgenic mice are likely to cause modifications in the expression of the numerous genes that are regulated by glucocorticoid hormones. Included in this category are genes coding for enzymes that are rate limiting in the biosynthesis of neurotransmitters known to regulate the HPA axis. Continued transgene expression, even after antidepressant treatment, may prevent a complete return to normal homeostasis.

It is interesting to note that, although desipramine increases type II glucocorticoid receptor mRNA concentration and dexamethasone binding in normal mouse brain (but to a lesser degree than in transgenic mouse brain), this does not result in any significant modification of plasma ACTH or corticosterone concentrations. It is possible that in normal mice glucocorticoid receptor levels are sufficient to enable a maximal retroinhibitory effect of circulating glucocorticoid hormones on the HPA axis.

The precise molecular mechanism of action of antidepressant drugs on glucocorticoid receptor gene expression remains to be elucidated. The present demonstration of phenotypic changes in the expression of the type II glucocorticoid receptor gene, which is a key regulator of the HPA axis, caused by desipramine suggests that the mechanism of action of this antidepressant may be found at a more basic level than that of synaptic transmissions. It remains to be determined whether this action of antidepressant drugs is exerted directly or through other *trans*-acting factors involved in regulation of the steroid receptor gene expression.

Considering that HPA axis hyperactivity returns to normal after successful antidepressant therapy of depression (17, 27, 28) and because neuronal glucocorticoid receptors are necessary for the negative feedback action of glucocorticoid hormones on the HPA axis to occur, we propose that the antidepressant-induced increase in glucocorticoid receptor gene expression could be a part of the mechanism whereby these agents restore HPA axis sensitivity to circulating glucocorticoid hormones in depressive illness. Recent work suggests that hypercortisolemia may contribute to behavioral aspects of depressive illness (23, 29), and antidepressant-induced reversal of this state may thus facilitate mood improvement. Our results, which indicate that antidepressants can reverse the hormonal imbalance of the transgenic mice, validate this animal model of the neuroendocrinological changes that accompany depression and suggest that it could be valuable for development of new antidepressants directed towards effects on HPA axis parameters.

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References

1. Carroll, B. J., G. C. Curtis, and J. Mendels. Neuroendocrine regulation in depression. I. Limbic system-adrenocortical dysfunction. *Arch. Gen. Psychiatry* **33**:1039–1050 (1976).
2. Linkowski, P., J. Mendlewicz, R. Leclercq, M. Brasseur, P. Hubain, J. Golstein, G. Copinschi, and E. V. Cauter. The 24-hour profile of adrenocorticotropin and cortisol in major depressive illness. *J. Clin. Endocrinol. Metab.* **61**:429–438 (1985).
3. Calogero, A. E., W. T. Galluchi, P. W. Gold, and G. P. Chrousos. Multiple feedback regulation loops upon rat hypothalamic corticotropin-releasing hormone secretion: potential clinical implications. *J. Clin. Invest.* **82**:767–774 (1988).
4. Carnes, M., C. M. Barksdale, N. H. Kalin, M. S. Brownfield, and S. J. Lent. Effects of dexamethasone on central and peripheral ACTH systems in the rat. *Neuroendocrinology* **45**:160–164 (1987).
5. de Kloet, E. R., J. Van der Vies, and D. De Wied. The site of the suppression of dexamethasone on pituitary-adrenal activity. *Endocrinology* **94**:61–73 (1974).
6. Jingami, H., S. Matsukura, S. Numa, and H. Imura. Effects of adrenalectomy and dexamethasone administration on the level of preprocorticotropin-releasing factor messenger ribonucleic acid (mRNA) in the hypothalamus and adrenocorticotropin-β-lipotropin precursor mRNA in the pituitary in rats. *Endocrinology* **117**:1314–1320 (1985).
7. Keller-Wood, M. E., and M. F. Dallman. Corticosteroid inhibition of ACTH secretion. *Endocr. Rev.* **5**:1–24 (1984).
8. Wrange, O., C. Carlstedt-Duke, and J.-Å. Gustafsson. Purification of the glucocorticoid receptor from rat liver cytosol. *J. Biol. Chem.* **254**:9284–9290 (1979).
9. Wrange, O., S. Okret, M. Radojéiè, C. Carlstedt-Duke, and J.-Å. Gustafsson. Characterization of the purified activated glucocorticoid receptor from rat liver cytosol. *J. Biol. Chem.* **259**:4534–4541 (1984).
10. Gustafsson, J.-Å., J. Carlstedt-Duke, L. Poellinger, A. Okret, A.-C. Wikström, M. Brönnegård, N. M. Gillner, Y. Dong, K. Fuxe, A. Cintra, A. Harfstrand, and L. F. Agnati. Biochemistry, molecular biology, and physiology of the glucocorticoid receptor. *Endocr. Rev.* **8**:185–234 (1987).
11. Miesfeld, R., S. Okret, A.-C. Wikström, O. Wrange, J.-Å. Gustafsson, and K. R. Yamamoto. Characterization of a steroid hormone receptor gene and mRNA in wild-type and mutant cells. *Nature* (Lond.) **312**:779–781 (1984).
12. Miesfeld, R., S. Rusconi, P. J. Godowski, B. A. Maler, S. Okret, A.-C. Wikström, J.-Å. Gustafsson, and K. R. Yamamoto. Genetic complementation of a glucocorticoid receptor deficiency by expression of cloned receptor cDNA. *Cell* **46**:389–399 (1986).
13. Hollenberg, S. M., C. Weinberger, E. S. Ong, G. Cerelli, A. Oro, R. Lebo, E. B. Thompsen, M. G. Rosenfeld, and R. M. Evans. Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* (*Lond.*) **318**:635–641 (1985).
14. Yamamoto, K. R. Steroid receptor regulated transcription of specific genes and gene networks. *Annu. Rev. Genet.* **19**:209–252 (1985).
15. von Bardeleben, U., and F. Holsboer. Cortisol response to a combined dexamethasone-human corticotrophin-releasing hormone challenge in patients with depression. *J. Neuroendocrinol.* **1**:485–488 (1989).
16. Christensen, L., A. Lolk, L. F. Gram, P. Kragh-Sorensen, O. L. Pedersen, and S. Nielsen. Cortisol and treatment of depression: predictive values of spontaneous and suppressed cortisol levels and course of spontaneous plasma cortisol. *Psychopharmacology* **97**:471–475 (1989).
17. Holsboer, F., R. Liebl, and E. Hofschuster. Repeated dexamethasone suppression test during depressive illness: normalization of test result compared with clinical improvement. *J. Affective Disord.* **4**:93–101 (1982).
18. Pepin, M.-C., M. V. Govindan, and N. Barden. Increased glucocorticoid receptor gene promoter activity following antidepressant treatment. *Mol. Pharmacol.* **41**:1016–1022 (1992).
19. Pepin, M.-C., F. Pothier, and N. Barden. Impaired glucocorticoid receptor function in transgenic mice expressing antisense RNA. *Nature* (*Lond.*) **355**:725–728 (1992).
20. Chirgwin, J., A. Pryzbala, R. MacDonald, and W. Rutter. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **8**:5294–5299 (1979).
21. Farmer, S. R., K. M. Wan, A. Ben-Ze'ev, and S. Penman. Regulation of actin mRNA levels and translation responds to changes in cell configuration. *Mol. Cell. Biol.* **3**:182–189 (1983).
22. Bradford, M. M. A rapid method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
23. Holsboer, F. Psychiatric implications of altered limbic-hypthalamic-pituitary-adrenorcortical activity. *Arch. Psychiatry Neurol. Sci.* **238**:302–322 (1989).
24. Pepin, M.-C., S. Beaulieu, and N. Barden. Antidepressants regulate glucocorticoid receptor messenger RNA concentrations in primary neural cultures. *Mol. Brain Res.* **6**:77–83 (1989).
25. Peiffer, A., S. Veilleux, and N. Barden. Antidepressant and other centrally-acting drugs regulate glucocorticoid receptor messenger RNA levels in rat brain. *Psychoneuroendocrinology* **16**:505–515 (1991).
26. Potter, W. Z., H. M. Calil, I. Extein, G. Muscettola, and F. K. Goodwin. Cross-over study of zimelidine and desipramine in depression: evidence for amine specificy. *Psychopharmacol. Bull.* **17**:26–28 (1981).
27. Greden, J. F., R. Gardner, D. King, L. Grunhaus, B. J. Carroll, and Z. Kronfol. Dexamethasone suppression test in antidepressant treatment of melancholia: the process of normalization and test-retest reproducibility. *Arch. Gen. Psychiatry* **40**:493–500 (1983).
28. Holsboer-Trachsler, E., R. Stohler, and M. Hatzinger. Repeated administration of the combined dexamethasone-human corticotropin releasing hormone stimulation test during treatment of depression. *Psychiatry Res.* **38**:163–171 (1991).
29. Murphy, B. E. P. Treatment of major depression with steroid suppressive drugs. *J. Steroid Biochem.* **39**:239–244 (1991).
30. Kramer, C. Y. Extension of multiple range tests to group means with unequal number of replications. *Biometrics* **12**:307–310 (1956).
31. Giguère, V., J. Côté, and F. Labrie. Specific inhibition by glucocorticoids of the α1-adrenergic stimulation of adrenocorticotropin release in rat anterior pituitary cells. *Endocrinology* **110**:1225–1230 (1982).
32. Provencher, P., A. Lorrain, A. Bélanger, and F. Fiet. Steroid biosynthesis by zona glomerulasa-fasciculata cells in primary culture of guinea-pig adrenals. *J. Steroid Biochem.* **36**:589–596 (1990).

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