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# Increased Glucocorticoid Receptor Gene Promoter Activity after Antidepressant Treatment

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**SUMMARY**

We have tested the hypothesis that antidepressants affect the expression of the glucocorticoid receptor gene, by looking at glucocorticoid receptor gene promoter activity, glucocorticoid receptor mRNA levels, and glucocorticoid-binding activity after treatment of different cell lines with desipramine. Treatment of LTK$^-$ cells or Neuro 2A cells with desipramine produced a 50–200% increase in chloramphenicol acetyltransferase activity transcribed from a 2.7-kilobase glucocorticoid receptor gene promoter region. In cell lines derived from both neuronal and non-neuronal sources, glucocorticoid receptor mRNA concentration doubled after desipramine treatment, and this was associated with a 2-fold higher functional glucocorticoid binding capacity and increased glucocorticoid sensitivity, as measured with the reporter plasmid pMMTVCAT. Antidepressant-induced increases in glucocorticoid receptor gene promoter activity, glucocorticoid receptor mRNA levels, and functional glucocorticoid binding activity suggest a novel mechanism of action for these drugs on the hypothalamic-pituitary-adrenal axis.

Hypercortisolemia and a premature escape from the adrenocorticotropin- and cortisol-suppressant actions of exogenously administered dexamethasone have been reported in patients suffering from major depression (1–3). It is well known that glucocorticoid hormones restrain HPA axis activity by exerting negative feedback effects at various levels of the axis, including secretion of adrenocorticotropin from the pituitary gland and corticotropin-releasing factor from the hypothalamus (4–9). In depressive illness, there is an apparent lack of sensitivity to this glucocorticoid feedback inhibition (10, 11), and we hypothesize that this could stem from dysregulation of glucocorticoid receptor gene expression in limbic-hypothalamic areas. A decreased glucocorticoid receptor number in brain areas involved in the control of the HPA axis could explain the nonsuppression of cortisol secretion observed in depressed patients after injection of dexamethasone. Because, during the course of antidepressant pharmacotherapy of depression, the abnormal nonsuppressive response of serum cortisol to dexamethasone administration returns to normal suppression (12, 13) and because antidepressants can render the HPA axis more sensitive to inhibition by dexamethasone (14), we postulate that antidepressant drugs could modify the glucocorticoid receptor system of brain areas involved in control of the HPA axis. In support of this hypothesis, we have already demonstrated that antidepressants can modulate the type II glucocorticoid receptor mRNA content of both hypothalamus and hippocampus (15), as well as that of cells derived from certain brain areas (16). To investigate more conclusively the hypothesis that antidepressants modulate glucocorticoid receptors, we have looked at the effects of antidepressants on glucocorticoid receptor gene expression in mouse fibroblast LTK$^-$ cells and in mouse Neuro 2A neuroblastoma cells. Transfection of these cells with a plasmid DNA vector consisting of a glucocorticoid-responsive MMTV promoter-enhancer element fused to a reporter gene CAT enabled us to measure antidepressant-induced changes in glucocorticoid sensitivity, because, at constant glucocorticoid concentrations, we have previously demonstrated a linear relationship between MMTV-CAT activity and glucocorticoid receptor concentrations (17). With this chimeric construct, we observed a 2-fold increase in glucocorticoid-stimulated CAT activity when the cells were treated with desipramine. Additionally, we have used a chimeric gene construct, consisting of the type II glucocorticoid receptor gene promoter region fused to CAT gene (pHGR2.7CAT), to measure antidepressant action more directly. Up to a 3-fold increase in CAT activity was seen when cells transfected with pHGR2.7CAT were treated with desipramine. Finally, type II glucocorticoid receptor mRNA concentrations and glucocorticoid-binding activity of neuro-

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**ABBREVIATIONS:** HPA, hypothalamic-pituitary-adrenal; SDS, sodium dodecyl sulfate; SSC, standard saline citrate; MMTV, mouse mammary tumor virus; CAT, chloramphenicol acetyltransferase; LTR, long terminal repeat; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; kb, kilobases; bp, base pairs.

blastoma and fibroblast cells were measured after treatment with antidepressant. The results reported here support the conclusion that antidepressants can increase glucocorticoid receptor gene expression, thus opening the possibility that this action could be the basis for antidepressant-induced restoration of HPA axis sensitivity to circulating glucocorticoid hormones in depressive illness.

## Experimental Procedures

**Cell cultures.** LTK$^-$ mouse fibroblast cells and Neuro 2A mouse neuroblastoma cells were used as recipient cells in this study and were obtained from the American Type Culture Collection. LTK$^-$ cells were grown in minimum essential $\alpha$ medium supplemented with 10% fetal calf serum, and Neuro 2A cells were grown in minimum essential medium supplemented with 10% fetal calf serum (GIBCO Laboratories). All transfected cultures were maintained at 37° in a 5% $CO_2$/95% air atmosphere. Before transfection, cells were washed with an isotonic buffer (phosphate-buffered saline) and incubated in the same chemically defined medium containing $10^{-5}$ M ascorbic acid, with or without test substances. The antidepressant used (desipramine) was added at a final concentration of $10^{-6}$ M, for periods varying from 1 to 4 days.

**Cell transfection.** Reporter plasmids were expressed either transiently or after stable integration in the host cell genome (18). For transient expression assays, the reporter plasmid pMMTVCAT (5 $\mu$g) (kindly provided by Dr. V. Giguère, University of Toronto) or the reporter plasmid pHGR2.7CAT (1 $\mu$g) (Fig. 1) was co-transfected with 2.5 $\mu$g of pRSV-LacZ (*Escherichia coli lac Z* gene, which encodes for $\beta$-galactosidase, fused to the Rous sarcoma virus LTR) by the calcium phosphate technique (19). The reporter plasmid pMMTVCAT consists of the CAT gene fused to the MMTV LTR and confers glucocorticoid inducibility in LTK$^-$ and Neuro 2A cells. The reporter plasmid pHGR2.7CAT consists of the same CAT gene under control of a 2.7-kb fragment of the glucocorticoid receptor gene promoter region (Fig. 1). Cells were treated with $10^{-6}$ M desipramine for 1–4 days and then transfected with plasmid. DNA was precipitated and introduced into cells by incubation for 5 hr with $2 \times 10^6$ cells, in 75-cm$^2$ flasks, followed by two washes with 5 ml of HEPES buffer (6.7 mM KCl, 0.14 M NaCl, 9.2 mM HEPES, pH 7.3). Cells transfected with pMMTVCAT were incubated with dexamethasone ($10^{-6}$ M) for 24 hr before harvesting for assay of $\beta$-galactosidase and CAT activities.

Stable transfectants were produced by co-precipitating the reporter plasmid (20 $\mu$g of pMMTVCAT or pHGR2.7CAT) and a neomycin resistance vector (pRSVNEO; 0.4 $\mu$g), with calcium phosphate (19), on $0.5 \times 10^6$ host cells (LTK$^-$ or Neuro 2A) cultured in 75-cm$^2$ flasks (20). After 24 hr, and every 3 days thereafter, the medium was replaced with fresh medium containing the neomycin analog G418 (155 $\mu$g/ml for LTK$^-$ cells and 180 $\mu$g/ml for Neuro 2A cells), in order to select for neomycin-resistant clones. Cells that had stably integrated either pMMTVCAT or pHGR2.7CAT into their genome were cloned and termed, respectively, LTK$^-$/pMMTVCAT/St, Neuro 2A/pMMTVCAT/St, LTK$^-$/pHGR2.7CAT/St, and Neuro 2A/pHGR2.7CAT/St. After 2 weeks, cell colonies were pooled and grown until treatment with $10^{-6}$ M desipramine for 1–4 days.

**CAT assay.** Cells were harvested in 40 mM Tris·HCl (pH 7.4), 1 mM EDTA, 0.15 M NaCl, pelleted, and resuspended in 0.25 M Tris·HCl (pH 7.8). They were subsequently lysed by three cycles of freezing-thawing and were centrifuged, and the supernatants were taken for protein, $\beta$-galactosidase, and CAT assays. $\beta$-Galactosidase activity was measured in an aliquot of the extract containing 50 $\mu$g of protein [determined by the method of Bradford (21)]. Hydrolysis of 0.8 mg of *o*-nitrophenyl-$\beta$-D-galactoside in 1 ml of 0.1 mM sodium phosphate (pH 7.0), 10 mM KCl, 1 mM $MgSO_4$, 50 mM $\beta$-mercaptoethanol, after reaction termination by the addition of 0.5 ml of 1 M $Na_2CO_3$, was determined by colorimetry at 420 nm. $\beta$-Galactosidase activity was used to normalize the quantity of supernatant subsequently taken for the CAT assay. In transiently transfected cells, CAT activity was measured in a final volume of 150 $\mu$l [0.25 M Tris·HCl, pH 7.8, 0.2 $\mu$Ci of [$^{14}$C]chloramphenicol (Amersham), 0.5 mM acetyl coenzyme A], and the incubation was at 37° for 45 min. Acetylated forms of [$^{14}$C]chloramphenicol were separated by thin layer chromatography on Whatman LK6D plates before quantification by liquid scintillation counting. CAT activity measurements in stable transfectants were performed with cell extracts containing equal amounts of protein (22). Results are expressed as percentage of chloramphenicol converted to acetylated forms per minute per unit of $\beta$-galactosidase activity or of protein content.

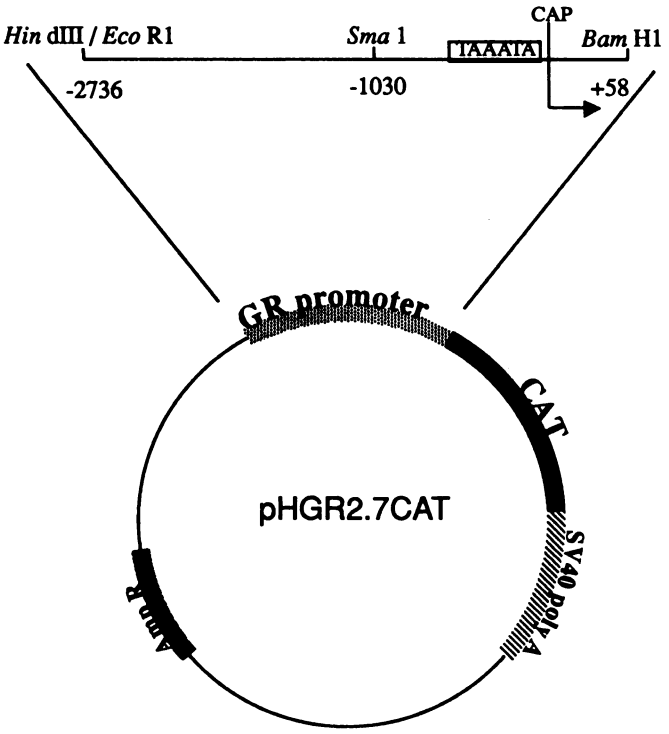



**Fig. 1.** Schematic representation of the reporter plasmid pHGR2.7CAT. This construct, which consists of the CAT gene under control of a 2.7-kb fragment of the glucocorticoid receptor gene promoter region, was used to study antidepressant-induced regulation of glucocorticoid receptor gene promoter element. A *Bam*HI site was introduced between +58 and +59 by oligonucleotide-directed mutagenesis, as described (39). HGR1.0CAT in M13mp18 was digested with *Hind*III and *Sma*I, and the *Hind*III-*Sma*I fragment (1700 bp) of the 5′ 2.7-kb human glucocorticoid receptor gene promoter was added at the *Hind*III/*Sma*I site (40). A resulting *Hind*III-*Bam*HI HGR gene fragment was isolated by sucrose gradient centrifugation and ligated to the promoterless pBL-CAT vector at the *Hind*III-*Bam*HI site.

**Northern (RNA) blot analysis.** RNA was prepared from cells ($15–20 \times 10^6$) by the guanidium isothiocyanate method (23). Total RNA was separated on 0.8% agarose-formaldehyde denaturing gels and blotted onto nylon filters (Hybond N; Amersham) before hybridization with glucocorticoid receptor and $\beta$-actin cRNA probes.

Glucocorticoid receptor cRNA probe was produced by T7 polymerase runoff transcription, with [$^{32}$P]UTP, of a 1815-bp glucocorticoid receptor cDNA fragment (24) subcloned into plasmid pGEM-1. A $\beta$-actin cRNA probe was generated from a 1500-bp $\beta$-actin cDNA *Pst*I fragment (25) inserted into pGEM-1.

Filters were prehybridized for 4 hr at 42° [in a mixture of 50% formamide, 5 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate), 6× Denhardt solution (50X Denhardt is 5% Ficoll, 5% polyviunylpyrrolidone, 5% BSA), 0.1% SDS, 50 mM sodium phosphate, 200 $\mu$g/ml yeast tRNA, and 200 $\mu$g/ml denaturated salmon sperm DNA] and hybridized at 65° for 20 hr. After hybridization under these stringency conditions, the filters were washed twice (30 min each) in 2× SSC containing 0.1% SDS at room temperature and twice (1 hr each) in

0.1× SSC, 0.1% SDS, at 70°. The filters were then exposed to Kodak X-Omat films, with intensifying screens for filters hybridized with glucocorticoid receptor cRNA probe.

***In vitro* binding assays of cytosolic receptor.** Glucocorticoid binding was measured with [$^3$H]dexamethasone. Cells ($1.5 \times 10^6$ cells/flask) were rinsed with an isotonic buffer (phosphate-buffered saline) and harvested 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 rpm for 15 min at 4°, an aliquot of the cytosol [protein content was determined by the method of Bradford (21)] was incubated with 10 nM [$^3$H]dexamethasone (specific activity, 44.7 Ci/mmol; New England Nuclear, Boston, MA) for 20–24 hr at 4°. The amount of nonspecific binding was determined in parallel incubations, which contained, in addition, a 200-fold excess of the unlabeled type II glucocorticoid receptor-specific agonist RU 28362. Sephadex LH20 (Pharmacia) columns (7 × 1 cm; equilibrated with TEDGM buffer), made from 1-ml disposable pipette tips, were used to separate bound from unbound steroid. After incubation, 100-μl aliquots were loaded onto the columns, washed with 100 μl of TEDGM, and eluted with 400 μl of TEDGM, into minivials. The vials were filled with 7.5 ml of aqueous counting cocktail Formula A-963 (New England Nuclear) and counted in a LKB scintillation counter at 40% efficiency.

## Results

**Increase by desipramine of the glucocorticoid sensitivity of cells.** The strategy used to measure the glucocorticoid sensitivity of the cells was to introduce into the cells a chimeric gene consisting of a glucocorticoid-responsive promoter-enhancer element linked to a reporter gene. We used the MMTV LTR fused to the CAT structural gene as reporter plasmid (pMMTVCAT), to measure the cellular response to dexamethasone that is mediated by glucocorticoid receptor. Glucocorticoid receptor, when bound to glucocorticoids, interacts with the glucocorticoid response elements localized in the MMTV LTR (26) and induces transcription of the CAT gene. We have previously demonstrated, by transfection of CV1 cells (cells that have no detectable glucocorticoid receptor and are not able to mediate the dexamethasone induction of CAT activity) with different amounts of a glucocorticoid receptor expression vector (Rous sarcoma virus-glucocorticoid receptor), that MMTV-CAT promoter activity is proportional to the amount of functional glucocorticoid receptor present in the cell (17). This indicated that an increase in MMTV-CAT activity could be observed if, in cells that normally contain glucocorticoid receptor, the glucocorticoid receptor level is up-regulated by antidepressants.

The reporter plasmid (pMMTVCAT) was expressed either transiently or after stable integration in the host cell genome (22). In transient transfections, LTK$^-$ or Neuro 2A cells were treated with $10^{-6}$ M desipramine for 1–4 days before introduction of the reporter plasmid pMMTVCAT and were then treated with $10^{-6}$ M dexamethasone for 24 hr before harvesting. Stable transfectants were treated for 1–4 days with $10^{-6}$ M desipramine and then treated with dexamethasone for 24 hr before harvesting. In both transient (Fig. 2A) and stable (Fig. 2B) transfectants of LTK$^-$ cells, we observed increases in CAT activity produced by desipramine treatment, compared with control cells. This increase in CAT activity was observed in cells treated for up to 4 days with desipramine.

The stable transfectants Neuro 2A/pMMTVCAT/St and Neuro 2A cells transiently transfected (not shown) with pMMTVCAT increased by 1.8–2-fold the level of CAT transcription from the MMTV LTR promoter, when incubated

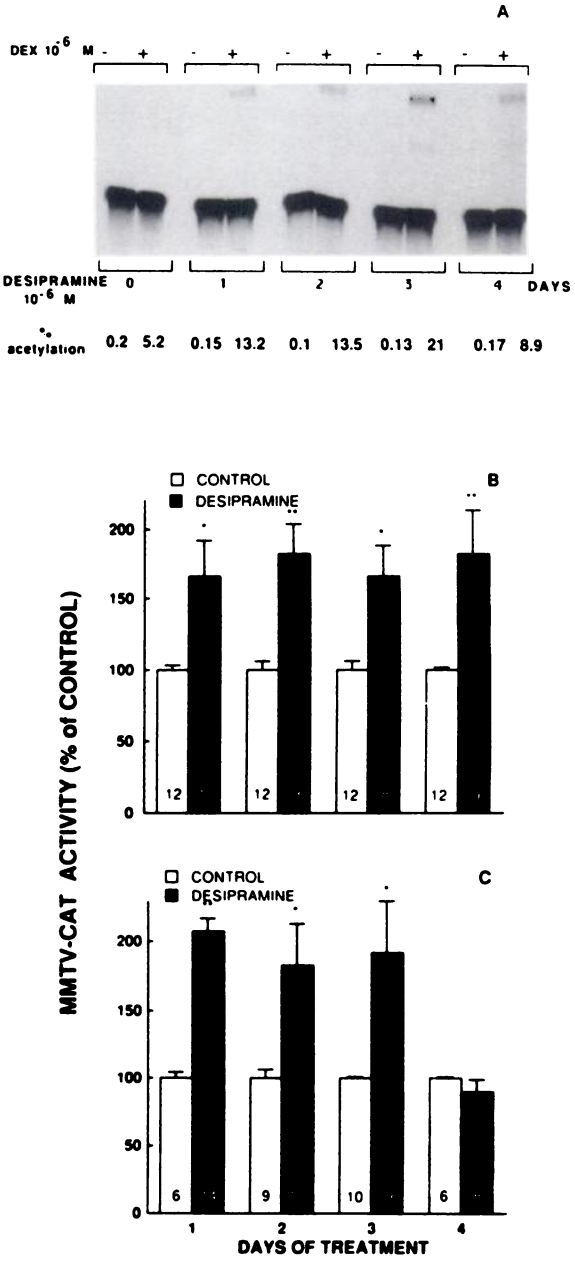



**Fig. 2.** Promoter activity of the reporter plasmid pMMTVCAT. A, LTK$^-$ cells were incubated with $10^{-6}$ M desipramine for up to 4 days before transient transfection with the pMMTVCAT plasmid. The reporter plasmid pMMTVCAT (5 μg) was precipitated with pRSV-LacZ (2.5 μg) for 5 hr, on $2 \times 10^6$ cells. Dexamethasone (*DEX*) ($10^{-6}$ M) was added 24 hr after transfection, and the cells were harvested 24 hr later for assay of CAT activity (at constant β-galactosidase activity for each CAT assay). The CAT activity of cell extracts (containing equivalent amounts of protein) of stable transfectants LTK$^-$/pMMTVCAT/St (B) or Neuro 2A/pMMTVCAT/St (C) was measured after treatment for 1–4 days with $10^{-6}$ M desipramine, followed by incubation with $10^{-6}$ M dexamethasone for 24 hr before harvesting. Results are shown as the mean ± standard error (numbers of experiments are indicated in the columns), and the significance of differences between means was evaluated by the Duncan-Kramer test after analysis of variance (41). **, $p < 0.01$; *, $p < 0.05$.

with $10^{-6}$ M desipramine. This increase in CAT activity returned to basal levels after 4 days of treatment (Fig. 2C).

**Higher glucocorticoid receptor gene transcription in cells treated with desipramine than in nontreated cells.** To observe the effect of antidepressant on the regulation of

glucocorticoid receptor gene transcription, we used a reporter plasmid consisting of a 2.7-kb fragment of the glucocorticoid receptor gene promoter region linked to the CAT gene (Fig. 1). This construct permitted the detection of changes in the regulation of the glucocorticoid receptor gene produced by antidepressant treatment. LTK$^-$ cells that had stably integrated pHGR2.7CAT into their genome (labeled LTK$^-$/pHGR2.7CAT/St) increased CAT transcription by 3-fold after incubation with $10^{-6}$ M desipramine (Fig. 3A). This increase in transcription lasted for 3 days, after which a return to basal levels of CAT activity was observed. In the stable transfectants Neuro 2A/pHGR2.7CAT/St, the effect of antidepressant on CAT activity was less pronounced (Fig. 3B) than it was in LTK$^-$ cells, with a 50% increase in CAT activity being evident on the first day of treatment with desipramine and a return to normal occurring the following day. A dose-dependent effect of desipramine on CAT activity directed by the 2.7-kb glucocorticoid receptor gene promoter element is indicated in Fig. 4.

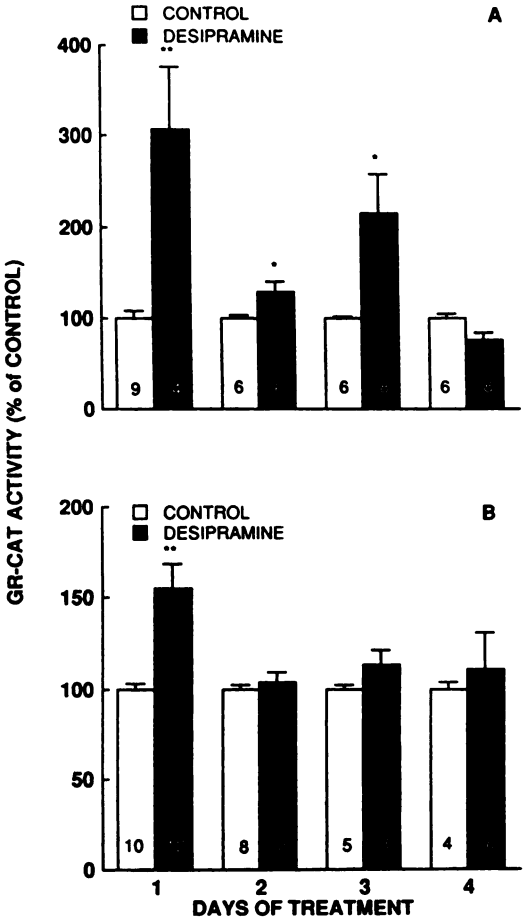



Fig. 3. Promoter activity of the reporter plasmid pHGR2.7CAT when stably transfected in LTK$^-$ (A) or Neuro 2A (B) cells incubated with $10^{-6}$ M desipramine for 1–4 days. The stable transfectants of LTK$^-$ cells (LTK$^-$/pHGR2.7CAT/St) and the stable transfectants of Neuro 2A cells (Neuro 2A/pHGR2.7CAT/St) were produced by calcium phosphate coprecipitation of phGR2.7CAT (20 μg) with a neomycin resistance vector, pRSV-Neo (0.4 μg). Clones that had incorporated the recombinant plasmids into the host cell genome were selected by growth in medium containing the neomycin analog G418. Results are shown as the mean ± standard error (numbers of experiments are indicated in the columns), and the significance of differences between means was evaluated by the Duncan-Kramer test (41) after analysis of variance. **, $p < 0.01$; *, $p < 0.05$.

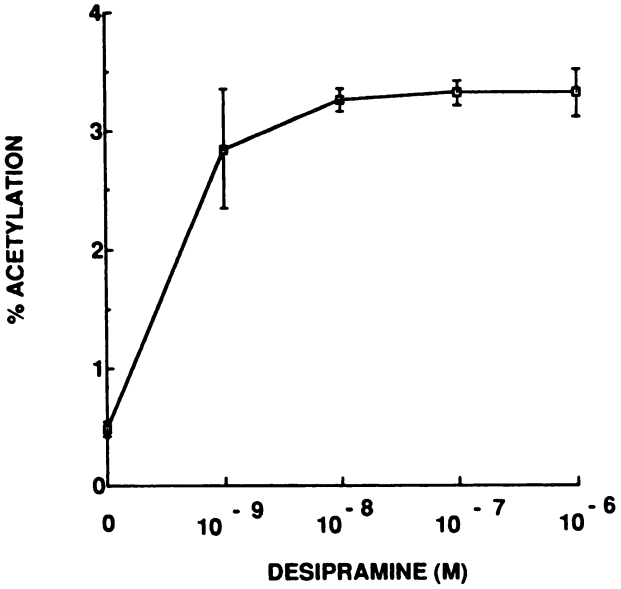
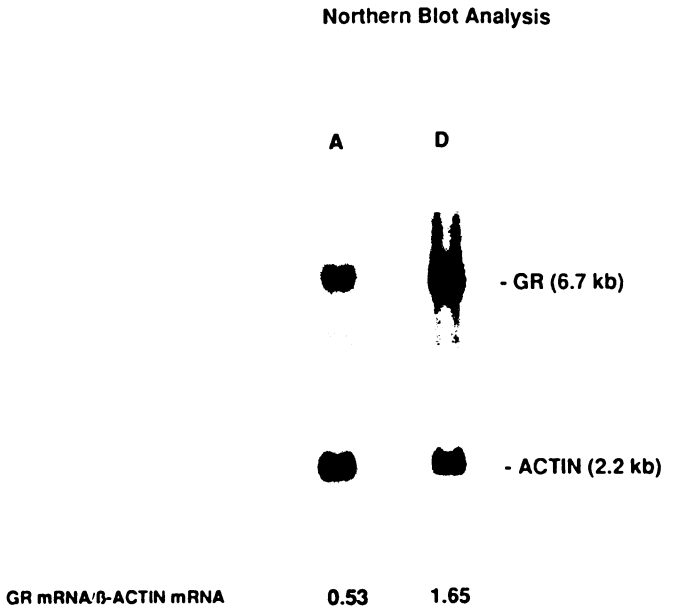



Fig. 4. Desipramine dose-response curve for glucocorticoid receptor promoter activity measured with the reporter plasmid pHGR2.7CAT. LTK$^-$ cells were incubated with increasing concentrations of desipramine for 24 hr before transient transfection with the pHGR2.7CAT plasmid. The reporter plasmid pHGR2.7CAT (5 μg) was precipitated with pRSV-LacZ (2.5 μg) for 5 hr, on $2 \times 10^6$ cells, which were harvested 24 hr later for assay of CAT activity (at constant β-galactosidase activity for each CAT assay). Results are shown as the mean ± standard error (four experiments).

Maximal stimulation of CAT activity was achieved at a desipramine concentration of $10^{-8}$ M.

**Northern blot analysis of glucocorticoid receptor mRNA in cells treated with desipramine.** An approximately 6.7-kb glucocorticoid receptor mRNA, corresponding to the one observed in rat liver, pituitary gland, and brain (24, 27, 28), was detected by Northern blot analysis of total RNA extracted from Neuro 2A cell cultures (Fig. 5, *upper*). Treatment of cells with $10^{-6}$ M desipramine for 24 hr produced a maximum 75% increase in glucocorticoid receptor mRNA concentrations. Although after 48 hr of treatment a transient return to basal levels of glucocorticoid receptor mRNA concentrations was seen, this was followed by a more persistent stimulatory phase for up to 4 days of treatment (Fig. 5, *lower*). Similar cyclic changes in glucocorticoid receptor mRNA concentrations induced by antidepressants have been noted in primary neuronal cultures derived from fetal rat brain areas (16).

**Antidepressant effects on glucocorticoid binding in Neuro 2A cells.** Type II glucocorticoid binding activity of Neuro 2A cells treated with $10^{-6}$ M desipramine was measured, to determine whether the antidepressant-induced increase in glucocorticoid receptor mRNA concentrations was associated with an increase in functional glucocorticoid receptors. Whereas in control cells (Neuro 2A cells treated with $10^{-5}$ M ascorbic acid) a maximum of 47 fmol of [$^3$H]dexamethasone/mg of protein was bound, addition of desipramine increased the binding activity by 2-fold, to 91 fmol of [$^3$H]dexamethasone/mg of protein (Table 1). This 100% increase in the glucocorticoid-binding capacity of antidepressant-treated cells is comparable to the observed increase in CAT activity in response to dexamethasone (Fig. 2).



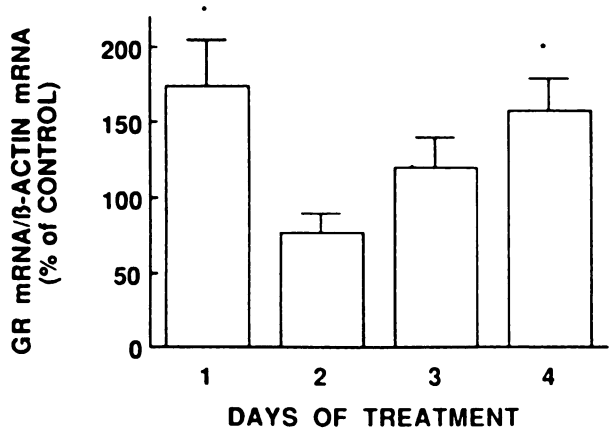




**Fig. 5.** Effect of desipramine on glucocorticoid receptor mRNA concentrations in Neuro 2A cells. *Upper*, cells were incubated with vehicle ($10^{-5}$ M ascorbic acid) (*lane A*) or with $10^{-6}$ M desipramine dissolved in vehicle (*lane D*). Total RNA, extracted from 15–20 × $10^6$ cells, was analyzed by Northern blot hybridization with a glucocorticoid receptor cRNA and a β-actin cRNA probe. A 6.7-kb glucocorticoid receptor mRNA and a 2.2-kb β-actin mRNA were detected and quantified. *Lower*, ratios of glucocorticoid receptor mRNA/β-actin mRNA in cells treated with $10^{-6}$ M desipramine, expressed as a percentage of the ratio found in control incubations without desipramine. Results are the mean ± standard error of eight determinations derived from separate experiments, and the significance of differences between means was evaluated by the Duncan-Kramer test (41) after analysis of variance. **, $p < 0.01$; *, $p < 0.05$.

## Discussion

We have studied, at the molecular level, changes in glucocorticoid receptor gene activity of different cell lines after treatment with an antidepressant. The antidepressant used here, desipramine, is a monoamine reuptake inhibitor that acts predominantly to block norepinephrine reuptake and has only a very slight action on serotonin reuptake (29). We have previously reported that this and other antidepressant drugs increase glucocorticoid receptor mRNA concentrations in primary cultures of neurons derived from brain areas involved in control of the HPA axis (16), as well as in hypothalamic and hippocampal areas of rat brain (15). Normalization of hyperactive HPA axis parameters occurs during successful antidepressant pharmacotherapy of depressive illness (12, 13, 30), and we have hypothesized that this could be brought about by antidepressant-induced increases in glucocorticoid receptor, rendering the HPA axis more susceptible to feedback inhibition by cortisol. We have tested the hypothesis that antidepressants affect the expression of glucocorticoid receptor by looking at glucocorticoid receptor gene promoter activity, glucocorticoid receptor mRNA levels, and glucocorticoid binding activity, after treatment of different cells lines with desipramine. Although it is difficult to correlate these actions directly between the different systems used (nontransfected and transiently and stably transfected cell lines), in all cases only stimulatory effects of desipramine were seen. When LTK$^-$ cells or Neuro 2A cells were treated with desipramine, we observed a 50–200% increase in CAT activity transcribed from the 2.7-kb glucocorticoid receptor gene promoter region. The glucocorticoid receptor mRNA concentration in neuroblastoma cells doubled after desipramine treatment, and up to a 2-fold higher glucocorticoid binding capacity was seen in these cells. This consistency in stimulatory effects of desipramine on glucocorticoid receptor gene promoter activity, glucocorticoid receptor mRNA levels, and functional glucocorticoid binding activity strongly suggests that this antidepressant increases glucocorticoid receptor gene expression. Although effects of desipramine on CAT activity directed by the glucocorticoid receptor gene promoter element are not sustained, particularly in Neuro 2A cells, longer lasting increases in glucocorticoid receptor mRNA concentrations or MMTV-CAT activity are seen. These discrepancies may result from the different types of cells used or from differences in the half-lives of various classes of substances (mRNA, receptor protein). Thus, whereas effects of antidepressants on glucocorticoid receptor mRNA concentrations were determined in wild-type Neuro 2A cells, effects on glucocorticoid receptor gene promoter were measured in cells transiently or stably transfected with a chimeric construct. The cyclic variations in glucocorticoid receptor mRNA concentrations seen were similar

TABLE 1

**Effects of desipramine on [$^3$H]dexamethasone binding to Neuro 2A and LTK$^-$ cells**

Binding assays were performed as described in Experimental Procedures. Results shown are the mean ± standard error of eight different determinations. The significance of differences between means was determined by the Duncan-Kramer test after analysis of variance.

| Cell line | Treatment | [$^3$H]Dexamethasone bound | |
|---|---|---|---|
| | | Control | Desipramine |
| | *days* | *fmol/mg of protein* | |
| Neuro 2A | 1 | 38.4 ± 2.0 | 46.4 ± 1.0[a] |
| Neuro 2A | 2 | 24.4 ± 2.0 | 84.0 ± 0.8[a] |
| Neuro 2A | 3 | 46.3 ± 0.3 | 84.2 ± 5.0[a] |
| Neuro 2A | 4 | 46.8 ± 0.8 | 90.8 ± 0.8[a] |
| LTK$^-$ | 1 | 35.0 ± 4.0 | 45.0 ± 2.0[b] |
| LTK$^-$ | 2 | 49.0 ± 4.0 | 51.0 ± 2.0[b] |
| LTK$^-$ | 3 | 25.0 ± 7.0 | 51.0 ± 1.0[a] |
| LTK$^-$ | 4 | 30.0 ± 3.0 | 78.0 ± 5.0[a] |

[a] $p < 0.01$.
[b] Not significant.

to those reported previously in primary neuronal cultures treated with the antidepressant used here (16), and the return of glucocorticoid receptor mRNA to basal levels after 48 hr of treatment may be due to autoregulation of endogenous glucocorticoid receptor gene expression, which may not be operative on the transfected gene.

The action of antidepressants on glucocorticoid receptor gene expression is seen in at least two different types of cells, namely mouse fibroblast and mouse neuroblastoma cells, and is consistent with an increase in glucocorticoid receptor activity. This latter aspect was confirmed by results from transfection of these cells with a reporter plasmid (pMMTVCAT) that is able to measure the glucocorticoid sensitivity of the cells. Using this latter approach, we evaluated the effect of any increase in functional glucocorticoid receptors caused by incubation of the cells with antidepressants. When $LTK^-$ cells were transfected either transiently or stably with pMMTVCAT, a 60–400% increase in CAT activity was seen after treatment with desipramine. The slightly greater response to desipramine in the transiently transfected cells may result from the presence of a larger number of pMMTV-CAT plasmids in these cells than the copy number that becomes stably integrated into the genome. Because we observed an increase of the glucocorticoid receptor gene promoter element activity in $LTK^-$ cells treated with antidepressant and we know that these fibroblast cells do not contain any catecholamines, it appears likely that the effects of desipramine on glucocorticoid receptor gene activity reported here are not a result of antidepressant effects on monoamine reuptake. We suggest that one action of antidepressants may be exerted at the genomic level, to stimulate the transcription of the glucocorticoid receptor gene, and that this action may not be limited to neuronal cells. The mechanism of this genomic action remains to be elucidated. The involvement of transcription factors in the mechanism of action of antidepressants on glucocorticoid receptor gene expression cannot be excluded. It is possible that antidepressants modify these *trans*-acting factors, which, in turn, regulate the glucocorticoid receptor gene. In this respect, it is interesting to compare antidepressant action with that of lithium, an effective agent used in the treatment of manic depressive illness that has also been reported to augment the clinical effects of medication in depressed patients who do not respond to antidepressant therapy alone (31, 32). Despite its extensive clinical use and after much investigation into the molecular basis of its action, the major target of the therapeutic effects of lithium remains unclear, but the effects could be related to actions on GTP-binding proteins and the phospholipase C/phosphatidylinositol second messenger system (33–35). Recently, Peiffer *et al.* (15) have shown that lithium, as well as different antidepressants (imipramine and desipramine), can up-regulate the glucocorticoid receptor mRNA content in rat brain. In addition, Kalasapudi *et al.* (36) have demonstrated that lithium induces *fos* protooncogene activity in PC12 pheochromocytoma cells. It is, thus, possible that the ability of lithium to increase expression of the transcription factor *fos* could be a factor involved in the mechanisms responsible for its clinical effectiveness. Because lithium can modulate glucocorticoid receptor mRNA in the same manner as does desipramine (15), it is possible that antidepressants that modulate glucocorticoid receptor gene expression act via other transcriptional factors, such as *fos* protooncogene. Further experiments, using pHGR2.7CAT and an expression vector for c-*fos*, could demonstrate whether, effectively, this transcription factor is implicated in the up-regulation of the glucocorticoid receptor gene expression induced by antidepressants.

Maximum effects of desipramine were seen at concentrations of $10^{-8}$ M and above, which correlates well with the minimum plasma concentration necessary for clinical efficacy (37). Discrepancies between rapid actions of antidepressants on monamine reuptake and delayed actions on humor are well known. A similar discrepancy is evident in our results, because desipramine action on glucocorticoid receptor gene expression occurs within 24 hr. It is possible that effects in cell cultures are more rapid than are those seen *in vivo* and that a delay is necessary before any substantial increase in glucocorticoid receptor activity can be detected. Additional time may be required to permit this increase in glucocorticoid receptors to sensitize brain areas to glucocorticoid feedback and thus reduce the HPA axis hyperdrive. Kinetic studies *in vivo*, outside the scope of this study, will be required to resolve these questions.

Our hypothesis that the apparent lack of sensitivity to glucocorticoids seen in depression could be due to an abnormality of glucocorticoid receptor gene regulation at the level of the limbic-hypothalamic system is increasingly supported by experimental evidence. A strong argument in favor of this hypothesis is a transgenic mouse line created in our laboratory, which has decreased glucocorticoid receptor gene expression associated with neuroendocrinological changes reminiscent of those seen in depression (38). Because, in humans, HPA axis hyperactivity returns to normal after successful antidepressant therapy of depression (12, 13) and because neuronal glucocorticoid receptors are necessary for the negative feedback action of glucocorticoid hormones on the HPA axis to be exerted, we propose that the antidepressant-induced increase in glucocorticoid receptor gene expression could be a part of the mechanism whereby antidepressants restore HPA axis sensitivity to circulating glucocorticoid hormones in depressive illness.

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