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EFFECT OF SOME ANTIDEPRESSANTS ON THE LOW CORTICOSTERONE CONCENTRATION-INDUCED GENE TRANSCRIPTION IN LMCAT FIBROBLAST CELLS

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The aim of the present study was to investigate effects of some classical and new antidepressants on functional activity of the glucocorticoid receptor (GR) induced by low corticosterone concentration in mouse fibroblast cells stably transfected with mouse mammary tumor virus-chloramphenicol acetyltransferase plasmid (LMCAT cells). We found that the transcriptional activity of GR stimulated by 50 nM corticosterone was strongly attenuated by imipramine, desipramine, fluoxetine and tianeptine in a concentration-dependent way, whereas reboxetine had only a weak effect and venlafaxine was inactive. Further study revealed that the inhibitor of c-Jun N-terminal kinase - mitogen-activated protein kinase (JNK-MAPK), SP600125 (0.1 μ M), reversed the imipramine-induced suppression of GR function, whereas the inhibitor of extracellular signal-regulated kinase (ERK)-MAPK, PD 98059 (15 μ M), potentiated the antidepressant action. No effect of selective inhibitors of p38-MAPK, phosphatidylinositol 3-kinase (PI3-K)/Akt, and glycogen synthase kinase (GSK-3) on the imipramine-induced inhibition of GR function was detected. These data indicate that the functional activity of GR evoked by low corticosterone concentration in LMCAT cells is efficiently inhibited by tricyclic antidepressants. Moreover, it was found that JNK- and ERK-MAPK were oppositely involved in the regulation of the imipramine-induced inhibition of the GR functional activity. Thus, the present study supports the notion that the interaction of antidepressants with GR may play a role in attenuating pathological hyperactivity of HPA axis in depression.

Key words: *antidepressant drugs, glucocorticoid receptor-mediated gene transcription, mitogen-activated protein kinase, LMCAT cells*

INTRODUCTION

It is well documented that hyperactivity of hypothalamic-pituitary-adrenal (HPA) axis plays a significant role in the etiology of depression and in the mechanism of antidepressant drug action (1 - 7). Furthermore, an association between medication with corticosteroids and major depressive episode in the general population has been found (8). Also experimental data show that the corticosterone administration to animals evokes several behavioral, biochemical and morphological changes similar to those observed in depression (9 - 13). On the other hand, a dysfunction of the HPA axis is corrected during a clinically effective therapy with antidepressant drugs, while lack of HPA axis normalization is often associated with the risk of relapse or the lack of improvement (14, 15). Chronic treatment with antidepressant drugs inhibits most changes elicited by glucocorticoids in experimental animals, however, the mechanism of these drug action is poorly recognized, yet. Antidepressant drugs are known to enhance inhibitory mechanism of HPA axis regulation what leads to the decreased CRH and glucocorticoid secretion (16, 17). However, other data indicate that antidepressants inhibit glucocorticoid action *in vitro* and *in vivo* without interference with HPA axis activity (18, 19). In particular, it has been recently found that some antidepressants are able to directly affect the action of glucocorticoid receptor (GR)-hormone complex on gene transcription, but results of these study are not univocal. Previously, we found that classical antidepressants strongly inhibited corticosterone-induced gene transcription in fibroblast cells, while new generation drugs were less potent (20 - 22). In line with these data, other authors have shown that antidepressant drugs reduced dexamethasone-induced transcription in COS cells (23). On the other hand, Pariante *et al.* (24) based on experiments in the same cell cultures as used in our studies indicated that antidepressants when present in the culture medium for a shorter time and simultaneously with a glucocorticoid could potentiate dexamethasone-induced gene transcription. The above discrepancies may result from various concentrations of glucocorticoids and exposure times of cells to both glucocorticoids and antidepressants. These parameters are likely to affect processes regulating the GR function, such as synthesis and degradation of these receptors, their phosphorylation status, the amount and activity of GR-associated proteins and efficacy of GR translocation from cytosol to the nucleus. Previously, we studied the effect of antidepressants on gene transcription induced by high corticosterone concentration, *i.e.* concentration comparable to brain level of this hormone during stress. The aim of the present study was to investigate the effect of some antidepressants on gene transcription induced by low corticosterone concentration, *i.e.* comparable to brain concentration of this hormone during basal condition. Moreover, in order to exclude any possible effects of the cell pretreatment with antidepressants in the present study the drugs and corticosterone were added to cell culture simultaneously.

We chose for this study imipramine, desipramine, fluoxetine, reboxetine, tianeptine and venlafaxine, *i.e.* both classical and new-generation antidepressant

drugs whose action on GR function under high corticosterone concentration was previously established (20, 22). Effect of antidepressants on GR function was investigated in mouse fibroblast cells (L929), stably transfected with mouse mammary tumor virus-chloramphenicol acetyltransferase (MMTV-CAT) plasmid (LMCAT cells). These cell line was employed, because it contains a high level of GR and it is most often used to study the GR function. Moreover, because some recent studies have demonstrated that mitogen-activated protein kinases (MAPK), glycogen synthase kinase (GSK-3) and protein kinase B (PKB, Akt) are involved in the regulation of GR transcriptional activity (25 - 30), the effects of inhibitors of these kinases on imipramine action were investigated.

MATERIALS AND METHODS

Cell culture conditions and treatments

The glucocorticoid receptor-mediated gene expression was determined in mouse fibroblast cells (L929), stably transfected with mouse mammary tumor virus-chloramphenicol acetyltransferase (MMTV-CAT) plasmid (LMCAT cells). The LMCAT cell line was generously provided by Dr E.R. Sanchez (Department of Pharmacology, Medical College of Ohio, Toledo, OH). The cells were grown in DMEM (Gibco-BRL) with a 10% heat-inactivated fetal bovine serum (Gibco-BRL) and a 0.02% geneticin (Gibco-BRL) at 37°C, in a 5% CO₂/95% air atmosphere.

In the preliminary experiment, the cells were treated with different concentration of corticosterone (25, 50, 100 and 500 nM) for 24-h. On the basis of this experiment, we chose corticosterone at 50 nM concentration for further study.

LMCAT cells, at final confluence of about 80%, were treated with imipramine hydrochloride (Polfa, Poland), desipramine hydrochloride (Sigma-Aldrich Co., USA), fluoxetine hydrochloride (Eli Lilly, England), tianeptine hydrochloride (Servier, France), reboxetine hydrochloride (Pharmacia-Upjohn, USA) and venlafaxine hydrochloride (Wyeth-Ayerst Research, USA) at concentrations of 3, 10 and 30 µM and with corticosterone (50 nM; Sigma Chemical Co, USA) for 24 h. The drugs were dissolved in medium, while corticosterone was dissolved in a small amount of ethanol, followed by dilution in medium (the final concentration of ethanol was below 0.5%) The control cultures were supplemented with the same amount of an appropriate vehicle.

In the next phase of the experiment, the influence of MAPK, GSK-3 and PKB inhibitors on imipramine-induced changes in GR-mediated gene transcription was determined. The following compounds were tested: PD 98059 (Tocris, UK), SP 600125 (Tocris, UK) and SB 203580 (Calbiochem, Germany), that are inhibitors of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38-MAP kinase, respectively; SB 216763 (Tocris, UK) - an inhibitor of GSK-3 and wortmannin (Sigma Chemical Co, USA) - an inhibitor of phosphatidylinositol 3-kinase (PI3-K). These compounds were dissolved in DMSO (the final concentration of DMSO was 0.5%) and added alone or 30 min before imipramine (10 µM). Next corticosterone (50 nM) was added to a culture medium for 24 h. The control cultures were supplemented with the same amount of DMSO.

Chloramphenicol acetyltransferase (CAT) activity assay

Cell lysates were prepared by a freezing/thawing procedure (22, 31). To determine CAT enzymatic activity, aliquots of lysate (after heating for 10 min at 60°C) were incubated in a 0.25 M Tris-HCl buffer (pH = 7.8) with 0.25 µCi D-threo-[dichloroacetyl-1-¹⁴C]-chloramphenicol and 0.2

mM n-butyryl coenzyme A for 1 h at 37°C. The butyrylated forms of chloramphenicol (in direct proportion to the CAT gene expression) were extracted twice with xylene, washed with 0.25 M Tris-HCl buffer, and radioactivity was measured in a β -counter (Beckmann LS 335 liquid scintillation counter). The results are calculated as dpm of a butyrylated fraction of chloramphenicol per 10 μ g of protein per hour of incubation, and are expressed as a percentage of the control value (compared to samples with corticosterone and appropriate vehicle, but without drug). The protein concentration in cell lysates was determined by the method of Lowry *et al.* (32).

Statistical analysis

The data are presented as the mean \pm S.E.M. of four independent experiments (in duplicate wells), and the significance of differences between the means was evaluated by the Dunnett's test following one-way or two-way analysis of variance, respectively.

RESULTS

Corticosterone present in the culture medium for 24 h increased CAT activity in a concentration-dependent manner (25 nM-500 nM) (*Fig. 1*). The 50 nM concentration of corticosterone, which evoked 24-fold induction of CAT activity was chosen for the next experiments. The effect of 50 nM corticosterone was blocked by a specific antagonist of the type II GR - RU-38486 (1 and 10 μ M) (data not shown). None of the antidepressant drug under study when given alone affected the low, non-stimulated CAT activity (data not shown).

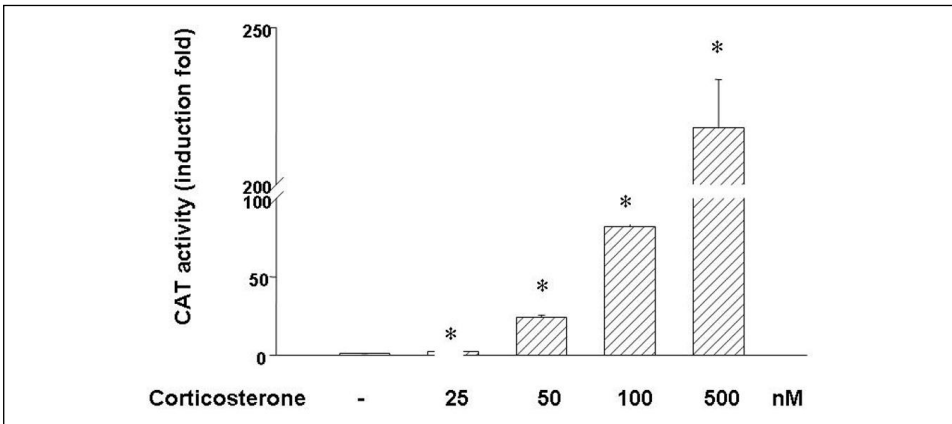


Fig. 1. The effect of corticosterone on the CAT gene transcription. Corticosterone at the indicated concentrations was added for 24 h and next the cells were harvested for an assay of CAT enzymatic activity. The data were calculated as dpm of the butyrylated fraction of chloramphenicol per 10 μ g of protein per hour of incubation averaged over two separate experiments and presented as an induction fold (\pm SEM) of control culture (with appropriate vehicle only). The significance of differences between the means was evaluated by the Dunnett's test following a one-way analysis of variance (* p <0.05 vs. control group; $n = 10$).

Treatment of cells with imipramine, fluoxetine or tianeptine (3-30 μM) for 24 h significantly and concentration-dependently inhibited the corticosterone-induced reporter gene transcription (*Fig. 2* and 3). Desipramine also inhibited the corticosterone-induced CAT activity, and the significant effects were observed after 10 and 30 μM but not at 3 μM concentrations of this drug. Reboxetine significantly decreased CAT activity at concentrations of 30 μM only, whereas venlafaxine (3 - 30 μM) failed to affect the corticosterone-induced gene transcription (*Fig. 4*).

None of the protein kinase inhibitors given alone at the concentrations shown in *Table 1* had any effect on the corticosterone-induced CAT activity. At higher concentrations wortmannin (100 nM) and SP 600125 (0.2 μM) increased, while PD 98059 (30 μM) decreased the CAT enzymatic activity (data not shown). It has been found that inhibition of p38-MAPK, GSK-3 and PKB had no effect, inhibition of ERK-MAPK enhanced while inhibition of JNK-MAPK attenuated the imipramine action on GR function (*Table 1*).

DISCUSSION

The present study showed that some antidepressant drugs inhibited the gene transcription induced by low corticosterone concentrations. These data are in line with our previous experiments where a high concentration of corticosterone was

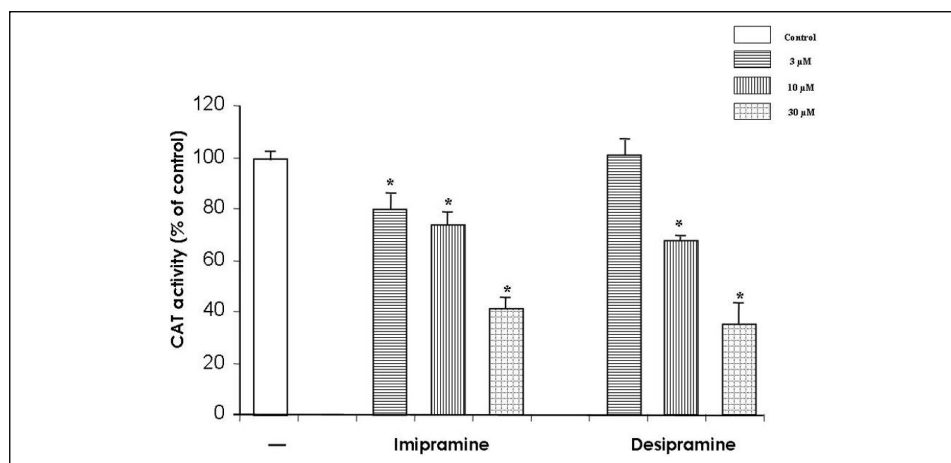


Fig. 2. The effect of imipramine and desipramine on the CAT gene transcription induced by corticosterone in LMCAT cells. Antidepressant drugs (at the indicated concentration) and corticosterone (50 nM) were applied for 24 h and the cells were harvested for an assay of CAT enzymatic activity. The data were calculated as dpm of the butyrylated fraction of chloramphenicol per 10 μg of protein per hour of incubation averaged over five separate experiments and presented as percentage (\pm SEM) of control culture (without antidepressant drug). The significance of differences between the means was evaluated by the Dunnett's test following a one-way analysis of variance (* $p < 0.05$ vs. control group; $n = 10$).

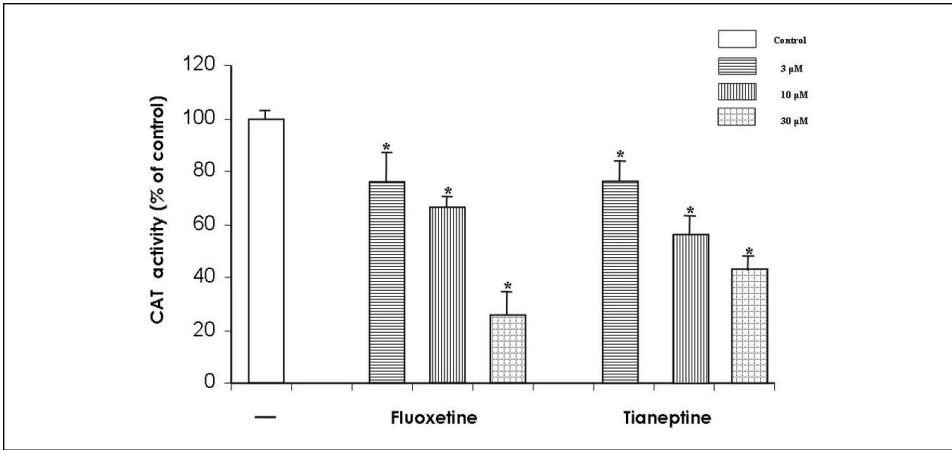


Fig. 3. The effect of fluoxetine and tianeptine on the CAT gene transcription induced by corticosterone in LMCAT cells. Antidepressant drugs (at the indicated concentration) and corticosterone (50 nM) were applied for 24 h and the cells were harvested for an assay of CAT enzymatic activity. The data were calculated as dpm of the butyrylated fraction of chloramphenicol per 10 µg of protein per hour of incubation averaged over five separate experiments and presented as percentage (± SEM) of control culture (without antidepressant drug). The significance of differences between the means was evaluated by the Dunnett's test following a one-way analysis of variance (*p<0.05 vs. control group; n = 10).

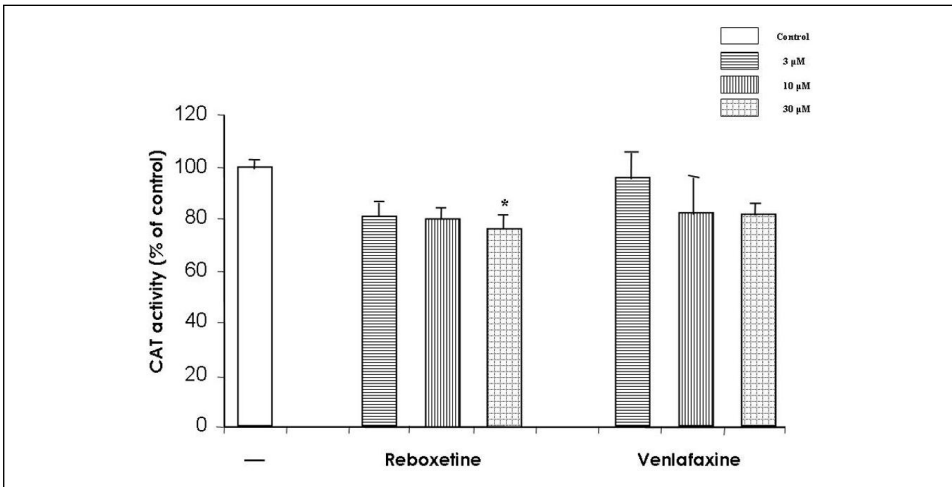


Fig. 4. The effect of reboxetine and venlafaxine on the CAT gene transcription induced by corticosterone in LMCAT cells. Antidepressant drugs (at the indicated concentration) and corticosterone (50 nM) were applied for 24 hours and the cells were harvested for an assay of CAT enzymatic activity. The data were calculated as dpm of the butyrylated fraction of chloramphenicol per 10 µg of protein per hour of incubation averaged over five separate experiments and presented as percentage (± SEM) of control culture (without antidepressant drug). The significance of differences between the means was evaluated by the Dunnett's test following a one-way analysis of variance (*p<0.05 vs. control group; n = 10).

Table 1. The effect of mitogen-activated protein kinase, protein kinase B and glycogen synthase kinase inhibitors on imipramine-induced inhibition of CAT gene transcription

| Treatment | CAT activity | |
|--------------------|--------------|--------------|
| | Vehicle | Imipramine |
| Vehicle | 100.0 ± 4.8 | 73.2 ± 6.3 |
| SP 600125 (0.1 µM) | 107.9 ± 6.3 | 99.3 ± 6.5 * |
| SB 203580 (1 µM) | 93.7 ± 4.2 | 64.9 ± 9.8 |
| PD 98059 (15 µM) | 84.7 ± 3.5 | 47.8 ± 8.1 * |
| Wortmannin (10 nM) | 119.6 ± 11.2 | 87.4 ± 11.9 |
| SB 216763 (1 µM) | 93.3 ± 4.2 | 62.6 ± 9.3 |

SP 600125 (JNK-MAPK inhibitor), SB 203580 (p38-MAPK inhibitor), PD 98059 (ERK-MAPK inhibitor), wortmannin (PI3-K inhibitor) and SB 216763 (GSK-3 inhibitor) were added at the indicated concentrations alone or 30 minutes before imipramine (10 µM) and next corticosterone (50 nM) was applied for 24 h. The data were calculated as dpm of the butyrylated fraction of chloramphenicol per 10 µg of protein per hour of incubation averaged over four separate experiments and presented as percentage (± SEM) of control culture (with appropriate vehicle only). The significance of differences between the means was evaluated by the Dunnett's test following a two-way analysis of variance. *p<0.05 vs. vehicle/imipramine-treated cells; n = 8.

used (20, 22), and indicate that independently of the degree of GR stimulation, antidepressant drugs can attenuate the GR function at least in *in vitro* conditions. A controversy exists whether simultaneous exposure of cells to antidepressants and glucocorticoids enhances or inhibits the GR function. Some authors observed that under such conditions antidepressants increased the sensitivity of GR to glucocorticoids, whereas the pretreatment of cells with antidepressants had opposite effects possibly via enhancing the translocation of GR from cytosol to the nucleus (31). Our data from previous and the present study showed that in various experimental conditions, *i.e.* 5 day pretreatment of the cells with antidepressants or simultaneous administration of the drugs and corticosterone as well as application of corticosterone at different concentrations, antidepressants inhibited the GR function. This suggests that many target genes which possess the functionally active GRE sequence could be inhibited by antidepressant drugs not only during stress but also when the concentrations of corticosterone are lower. The latter fact may be clinically relevant since in depressed patients a prolonged secretion of cortisol after stress and its disturbed circadian rhythm lead to maintenance of elevated, though lower than that occurring during stress, level of this hormone for a long time (3, 15).

Like in the previous study (20, 22), we found that the effects exerted by low corticosterone concentration were strongly attenuated by imipramine, desipramine, fluoxetine and tianeptine, whereas new antidepressant drugs had only weak effect. The lack of any effect of venlafaxine and the weak action of reboxetine suggest that these antidepressants may show a lower, than tricyclic

drugs and fluoxetine, clinical efficacy in the treatment of depression associated with hyperactivity of HPA axis.

With respect to intracellular mechanism of antidepressant drug action on GR function, only imipramine, as a classical and the most widely used drug, was studied. It has been observed that the selective JNK-MAPK kinase inhibitor, SP600125, reversed the imipramine-induced suppression on GR function. It should be emphasized that we used this inhibitor at the concentrations which alone did not change the GR-mediated function. As shown by us and other investigators, higher concentrations of this inhibitor elevate the GR functional activity (30). In contrast to SP600125, the ERK-MAPK inhibitor (PD98059) at higher concentrations (30 μM) attenuated, when given alone, the corticosterone-induced gene transcription, whereas at a lower dose (15 μM) it potentiated the inhibitory action of imipramine. Thus, these data strongly suggest that the activation of JNK-MAPK inhibits and ERK-MAPK enhances GR function in LMCAT cells. JNK-MAPK and ERK-MAPK, which are activated in response to cellular stress and pro-inflammatory cytokines, have been found to inhibit GR-mediated transcription in HeLa cells (26, 27). Those authors also reported that JNK phosphorylated the GR primarily at Ser-246, which resulted in the inhibition of GR function, while suppression of GR transcription activity by ERK was rather connected with a modification of a GR cofactor. As shown by the present study, also in LMCAT cells, the JNK inhibition leads to an increase in GR function, whereas attenuation of ERK activity decreases the functional activity of this receptor. This indicates that JNK plays a similar role in regulatory mechanism of GR function in various cell models, whereas ERK activation may produce even opposite effects depending on the cell type.

In contrast to JNK- and ERK-MAPK, the lack of any effect of selective inhibitors of p38-MAPK, PI3-K/Akt, and GSK-3 on imipramine-induced inhibition of GR function speak against an involvement of these kinases in intracellular mechanism of antidepressants-GR interaction in LMCAT cells.

Summing up, this study indicates that independently of corticosterone concentration some antidepressant drugs inhibit functional activity of GR in fibroblast LMCAT cells. Moreover, it was found that JNK- and ERK-MAPK were oppositely involved in the regulation of the imipramine-induced inhibition of the GR functional activity. Thus, these data strongly support the notion that the interaction of antidepressants with GR may play a role in attenuating pathological hyperactivity of HPA axis in depression.

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