Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 495 (2004) 103–110

www.elsevier.com/locate/ejphar

Mood stabilizers inhibit glucocorticoid receptor function in LMCAT cells

Agnieszka Basta-Kaim, Bogusława Budziszewska, Lucylla Jaworska-Feil, Magdalena Tetich, Marta Kubera, Monika Leśkiewicz, Władysław Lasoń *

Department of Endocrinology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland

Received 19 March 2004; received in revised form 11 May 2004; accepted 20 May 2004
Available online 25 June 2004

Abstract

Mood stabilizers block some central effects induced by stress and glucocorticosteroids; however, little is known about interaction of these drugs with glucocorticoid receptor function. In the present study, we evaluated effects of lithium, valproate and carbamazepine on glucocorticoid receptor-mediated gene expression in mouse fibroblast cells (L929), stably transfected with mouse mammary tumor virus (MMTV)-chloramphenicol acetyltransferase reporter plasmid (LMCAT cells). Treatment of LMCAT cells with lithium (1–4 mM), valproate (0.1–3 mM) and carbamazepine (30 and 100 μM) inhibited corticosterone-induced activity of reporter gene in a time- and concentration-dependent manner. Furthermore, it was found that valproate, but not two other antimanic drugs, decreased the glucocorticoid receptor level in cytosolic and nuclear fraction, and its inhibitory effect on glucocorticoid receptor-mediated transcriptional activity was attenuated by c-Jun N-terminal kinase (JNK)-mitogen-activated protein kinase (MAPK) inhibitor. Protein kinase B (PKB), glycogen synthase kinase (GSK), p38-MAPK and depletion of inositol were not shown to be involved in the mechanism of mood-stabilizer action on glucocorticoid receptor function under present experimental condition. In contrast to mood stabilizers, amphetamine (1–100 μM) had no effect on glucocorticoid receptor-mediated transcriptional activity. These findings corroborate the hypothesis that direct effects of antidepressants and mood stabilizers on glucocorticoid receptor function is an important mechanism, by which these drugs may inhibit some deleterious effects of stress and glucocorticoids on the central nervous system.

Keywords: Mood stabilizer; Glucocorticoid receptor-mediated gene transcription; Glucocorticoid receptor; Protein kinase; Inositol; LMCAT cell; Amphetamine

1. Introduction

Depression is frequently associated with hyperactivity of hypothalamic–pituitary–adrenal axis, which is usually corrected during a clinically effective therapy with antidepressant drugs (Heuser et al., 1996; Holsboer et al., 1995; Linkowski et al., 1985). It has been suggested that antidepressants decrease hypothalamic–pituitary–adrenal axis activity by increasing the glucocorticoid receptor level in the brain, mainly in the hippocampus, which leads to the enhanced glucocorticoid receptor-mediated feedback inhibition, and consequently, to a decrease in the corticosterone level (Budziszewska, 2002; Budziszewska et al., 1994; Holsboer, 2000; Pariante and Miller, 2001). Additionally, these drugs are able to inhibit the corticotropin-releasing-hormone (CRH) gene activity directly, at least under in vitro condition (Budziszewska et al., 2004).

Apart from regulation of hypothalamic–pituitary–adrenal axis activity, antidepressants are known to exert opposite effects to those produced by glucocorticoids or to inhibit some glucocorticoid actions; however, molecular mechanism of the latter effect has not been fully elucidated (Jackson and Luo, 1998; Schaaf et al., 1998; Watanabe et al., 1992). Scarce data indicate that antidepressants can directly affect the glucocorticoid receptor-mediated gene transcription (Budziszewska et al., 2000; Okuyama-Tamura et al., 2003; Pariante et al., 1997). Glucocorticoid receptor is a hormone-activated transcription factor, which binds to a specific DNA sequence (glucocorticoid-responsive element, GRE), and acts as regulator of gene expression. Glucocorticoid receptor-mediated gene transcription can be modulated by various signal transduction pathways, e.g., cAMP/protein kinase A (PKA)-, phospholipase (PLC)/protein

* Corresponding author. Tel.: 48-12-637-40-22; fax: +48-12-637-45-00.
E-mail address: lason@rabbit.if-pan.krakow.pl (W. Lasoń).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.05.034

kinase C (PKC)-, calmodulin- and mitogen-activated protein kinase (MAPK)-mediated signal transduction pathway, whose activities are affected by antidepressant drugs (Mann et al., 1995; Nibuya et al., 1995; Schwaninger et al., 1995; Vetulani and Nalepa, 2000).

The mood-stabilizing agents lithium, valproate and carbamazepine are a group of drugs widely used in the treatment of manic-depressive illness, and their molecular mechanism of action is even better recognized than that of antidepressants (Chalecka-Franaszek and Chuang, 1999; Gottlicher et al., 2001; Klein and Melton, 1996; Mai et al., 2002; Manji and Lenox, 1999; Williams et al., 2002). However, their effects on glucocorticoid receptor function have not been elucidated so far.

The aim of the present study was to find out if lithium, valproate and carbamazepine regulate the glucocorticoid receptor-mediated gene expression in fibroblast cells (L929), stably transfected with a mouse mammary tumor virus (MMTV) promoter linked with chloramphenicol acetyltransferase (CAT) reporter gene. Moreover, an involvement of various intracellular signal transduction pathways, e.g., PLC/PKC, MAPK, protein kinase B (PKB, Akt), glycogen synthase kinase (GSK-3) and inositol level (Chen et al., 1994; Jensen and Mork, 1997; Manji and Chen, 2002), in mood-stabilizer effects on glucocorticoid receptor function was also examined. Additionally, the effect of these drugs on glucocorticoid receptor level in cytosolic and nuclear fraction was evaluated by Western blot method, because the MMTV-CAT activity depends on glucocorticoid receptor gene expression.

2. Materials and methods

2.1. Cell culture conditions

Effects of drugs on the glucocorticoid receptor-mediated gene expression were determined in mouse fibroblast cells (L929), stably transfected with MMTV-CAT reporter plasmid (LMCAT cells). The LMCAT cell line was generously provided by Dr. E.R. Sanchez (Department of Pharmacology, Medical College of Ohio, Toledo, OH; Sanchez et al., 1994). The cells were grown in Dulbecco's modified Eagle's medium (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% CO_2/95% air atmosphere.

2.2. Drug treatments

The LMCAT cells (final confluency 80%) were treated with vehicle, lithium hydrochloride (0.01, 0.1, 0.5, 1.0, 2.0 and 4.0 mM, Merck), sodium valproate (0.01, 0.1, 0.5, 1.0 and 3.0 mM, Polfa), carbamazepine (3, 10, 30 and 100 μM, Polfa) or amphetamine sulfate (1, 10 and 100 μM, Sigma) for 1 and 5 days. We have studied the effects of mood stabilizers within their therapeutic blood concentration range, i.e., 0.5–1.2 mM for lithium, 0.5–1 mM for valproate and 20–50 μM for carbamazepine (Chen et al., 1996; Jensen and Mork, 1997; Yuan et al., 2001). The medium and drugs were changed once during the 5-day culture—on the third day. The CAT activity was stimulated by adding 1 μM corticosterone for 2 h.

In the next part of the experiment, the influence of some modulators on lithium-, valproate- and carbamazepine-evoked changes in the CAT activity induced by corticosterone (1 μM, 2 h) was determined. The following compounds were tested: phorbol 12-myristate 13-acetate (TPA, RBI) and tamoxifen citrate (Tocris)—an activator and inhibitor of PKC, respectively; PD 98059 (Tocris), SP 600125 (Tocris) and SB 203580 (Calbiochem)—inhibitors of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38-MAP kinases, respectively; SB 216763 (Tocris)—an inhibitor of GSK-3; wortmanin (Sigma)—an inhibitor of phosphatidylinositol 3-kinase (PI 3-K) and inositol (Sigma). These agents were added alone or 30 min before LiCl (4 mM), valproate (3 mM) or carbamazepine (30 μM) to a culture medium for 5 days. CAT activity was induced by corticosterone at 1 μM for 2 h.

2.3. Cell viability

Cell viability was determined by counting viable and nonviable (blue) cells in a hemocytometer. The cell suspensions were mixed (at the 1:1 ratio) with 0.4% trypan blue, and the number of nonviable cells per a total of 100 cells was determined.

2.4. CAT activity

Cell lysates were prepared by a freezing/thawing procedure (Budziszewska et al., 2000; Pariante et al., 1997). In order to determine CAT 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-^{14}C]-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) (Pariante et al., 1997) 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 were calculated as dpm of a butyrylated fraction of chloramphenicol per 10 μg of protein per an hour of incubation. The protein concentration in cell lysates was determined by a method of Lowry et al. (1951).

2.5. Western blot

The Western blot procedures for of PKC-α in cytosol and membrane fraction and glucocorticoid receptor in cytosolic

and nuclear fraction were previously described (Budziszewska et al., 1996; Basta-Kaim et al., 2002). LMCAT cells were washed twice with phosphate-buffered saline (PBS) and harvested by centrifuging at 800 × *g* for 5 min at a room temperature. For PKC-α determination, the pellet was homogenized in a Tris–HCl buffer (20 mM Tris–HCl, 2 mM EGTA, 5 mM EDTA, 2 mM DTT, 0.5 mM PMSF, 1.5 mM pepstatin, 2 mM leupeptin, pH 7.4), and the homogenate was centrifuged at 140 000 × *g* for 60 min at 4 °C. The supernatant was saved (the cytosolic fraction), and the pellet was homogenized with a Tris–HCl buffer containing a 0.2% IGEPAL, incubated for 60 min at 4 °C, centrifuged at 140 000 × *g* for 60 min at 4 °C, and the supernatant was saved (the membrane fraction). The equal volumes of samples (10 μg of protein per lane) and the buffer (100 mM Tris–HCl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.005% bromophenol blue, pH 6.8) were mixed and boiled for 5 min before loading on the gel. Proteins were separated by SDS-PAGE (4% stacking gel, 7.5% resolving gel) under constant voltage (60 V in stacking gel; 120 V in resolving gel) and were transferred electrophoretically to the PVDF membrane (Boehringer Mannheim) at a 60 V constant current for 2 h. The membranes were washed twice with the Tris-buffered saline (TBS), pH 7.5, blocked in a 1% blocking solution (Boehringer Mannheim) for 1 h and incubated overnight at 4 °C with the primary antibody (anti-PKC-α; 1:200), obtained from Santa Cruz Biotechnology. The blots were washed twice with TBS containing a 0.1% Tween-20 (TBST) and twice with a 0.5% blocking solution in TBS; they were than incubated with a horseradish peroxidase-linked secondary antibody (anti-rabbit IgG; 40 mU/ml; Boehringer Mannheim) for 1 h at a room temperature. Afterwards, the membranes were washed four times with large volumes of TBST, and immunoblots were visualized with a chemiluminescence detection kit (Boehringer Mannheim). The semiquantitative analysis of band intensity was performed using FujiLas 1000 and FujiGauge softwares.

For glucocorticoid receptor determination, the cells were harvested, homogenized in ice-cold Tris–HCl buffer (5 mM Tris–HCl, 1 mM EDTA, 1 mM DTT, 10 mM sodium molybdate, 10% glycerol, pH 7.4) using a Teflon glass Thomas homogenizer (1200 rpm) and centrifuged at 800 × *g* for 10 min. Next, the supernatant was centrifuged at 140 000 × *g* for 60 min at 2 °C to obtain a supernatant which represented the cytosolic fraction. The nuclear precipitate (from 800 × *g*) was washed three times in Tris–HCl buffer, extracted with 10 mM Tris–HCl buffer containing 0.4 M KCl, 1.5 mM EDTA, 0.5 mM DDT, and centrifuged at 140 000 × *g* for 60 min at 2 °C to obtain a nuclear extract. The equal volumes of samples (15 μg of protein per lane) were separated on 10% resolving gel. Glucocorticoid receptor was analyzed using the anti-glucocorticoid receptor polyclonal antibody (anti-GR 1:200, Santa Cruz Biotechnology), a horseradish peroxidase-linked secondary antibody (anti-rabbit IgG; 40 mU/ml; Boehringer Mannheim) and immunoblots were visualized with a chemiluminescence detection kit (Boehringer Mannheim).

2.6. Statistical analysis

The data are presented as a mean ± S.E.M. of three to five 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.

3. Results

3.1. Effect of mood stabilizers and amphetamine on corticosterone-induced gene transcription

We evaluated CAT activity, which correlated to glucocorticoid receptor-mediated CAT gene expression. Addition of corticosterone at a concentration of 1 μM for 2 h caused 34-fold increase in CAT activity (basal CAT activity was 495 ± 85 dpm/10 μg of protein/h; while corticosterone-induced CAT activity was 17 312 ± 1148 dpm/10 μg of protein/h). As we described previously (Budziszewska et al., 2000), the effect of corticosterone was completely blocked by addition of 10 μM RU 38486 [11β-(4-dimethylamino)-phenyl-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one], a specific antagonist of type II glucocorticoid receptors, which confirms involvement of the glucocorticoid receptor in this response. Mood stabilizers at concentrations up to 100-fold higher than that used in the cultures did not affect CAT activity in vitro (data not shown).

Treatment of cells with mood stabilizers for 5 days inhibited the stimulatory effect of corticosterone on the glucocorticoid receptor-mediated gene transcription. Corticosterone-induced CAT activity was significantly inhibited by lithium (max. by ca. 35%) at 1, 2 and 4 mM, but not at 0.01, 0.1 and 0.5 mM concentrations (Fig. 1). Valproate at concentrations of 0.1, 0.5, 1.0 and 3.0 mM (by ca. 20–70%) attenuated corticosterone-induced CAT activity (Fig. 2), whereas carbamazepine (30 and 100 μM) inhibited this parameter by ca. 30–40% as compared to control culture (Fig. 3). Lower concentrations of the mood stabilizers were without effect. Amphetamine (1–100 μM) had no effect on corticosterone-induced CAT gene transcription (Fig. 4).

In contrast to 5-day treatment, the 1-day incubation of LMCAT cells with mood stabilizers or amphetamine had no effect on corticosterone-induced CAT activity (data not shown).

3.2. Effect of inositol and protein kinase modulators on mood-stabilizer-induced inhibition of CAT gene transcription

Inositol, tamoxifen (inhibitor of PKC), TPA (PKC activator), SP 600125 (JNK-MAPK inhibitor), SB 203580

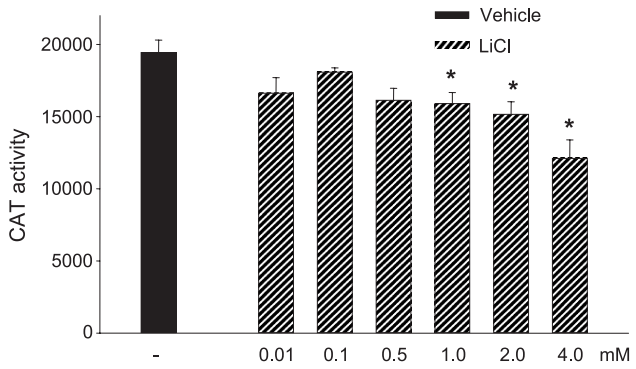

Fig. 1. The effect of lithium hydrochloride (LiCl) on the CAT gene transcription induced by corticosterone [1 μM, 2 h] in LMCAT cells. LiCl was applied at the indicated concentrations for 5 days. Corticosterone [1 μM] was added 2 h before harvesting the cells for an assay of CAT activity. The data are presented as the mean ± S.E.M. (dpm of the butyrylated fraction of chloramphenicol per 10 μg of protein per hour of incubation). 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).

(p38-MAPK inhibitor), PD 98059 (ERK-MAPK inhibitor), wortmanin (PI 3-K inhibitor) and SB 216763 (GSK-3 inhibitor) given alone at the concentrations shown in Table 1 had no effect on the corticosterone-induced CAT activity. SP 600125, an inhibitor of JNK-MAP kinase, attenuated valproate effect on glucocoirticoid receptor-mediated gene transcription in LMCAT, but had no effect on lithium and carbamazepine action. Among other studied agents only PD 98059, an inhibitor of ERK-MAP kinase, enhanced significantly the inhibitory effect of lithium, valproate and carbamazepine on corticosterone-induced CAT activity.

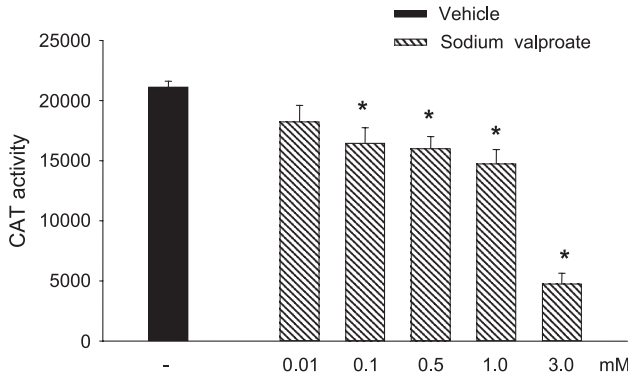

Fig. 2. The effect of sodium valproate on the CAT gene transcription induced by corticosterone [1 μM, 2 h] in LMCAT cells. Valproate was applied at the indicated concentrations for 5 days. Corticosterone [1 μM] was added 2 h before harvesting the cells for an assay of CAT activity. The data are presented as the mean ± S.E.M. (dpm of the butyrylated fraction of chloramphenicol per 10 μg of protein per hour of incubation). 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).

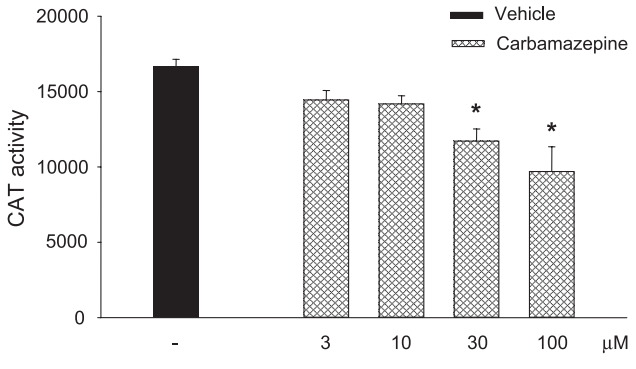

Fig. 3. The effect of carbamazepine on the CAT gene transcription induced by corticosterone [1 μM, 2 h] in LMCAT cells. Carbamazepine was applied at the indicated concentrations for 5 days. Corticosterone [1 μM] was added 2 h before harvesting the cells for an assay of CAT activity. The data are presented as the mean ± S.E.M. (dpm of the butyrylated fraction of chloramphenicol per 10 μg of protein per hour of incubation). 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).

3.3. Effect of mood stabilizers on glucocorticoid receptor and PKC-α concentration

As shown by Western blot study, lithium (4 mM) and carbamazepine (30 μM) had no effect on glucocorticoid receptor concentration in any fraction, while valproate (3 mM) decreased this parameter in both cytosolic and nuclear fraction (Fig. 5). Furthermore, lithium (4 mM) and carbamazepine (30 μM) had no effect on PKC-α level, while valproate (3 mM) decreased this isoenzyme concentration in membrane, but not cytosolic fraction (Fig. 6).

3.4. Effect of mood stabilizers and amphetamine on cell viability

The exposure of LMCAT cells to lithium chloride, sodium valproate, carbamazepine and amphetamine sulfate

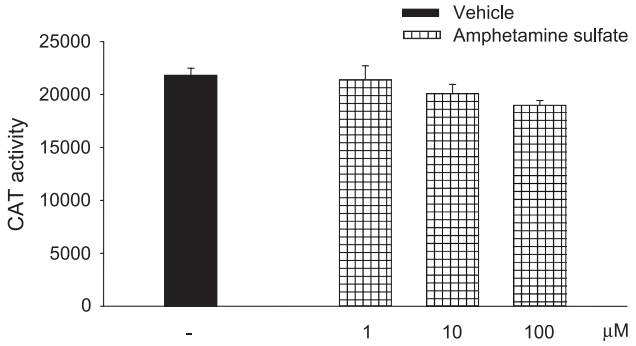

Fig. 4. The effect of amphetamine sulfate on the CAT gene transcription induced by corticosterone [1 μM, 2 h] in LMCAT cells. Amphetamine was applied at the indicated concentrations for 5 days. Corticosterone [1 μM] was added 2 h before harvesting the cells for an assay of CAT activity. The data are presented as the mean ± S.E.M. (dpm of the butyrylated fraction of chloramphenicol per 10 μg of protein per hour of incubation). The significance of differences between the means was evaluated by the Dunnett's test following a one-way analysis of variance.

Table 1
The effect of inositol and protein kinase modulators on mood stabilizers-induced inhibition of CAT gene transcription

Pretreatment	Treatment	CAT activity		
	Vehicle	LiCL [4 mM]	Valproate [3 mM]	Carbamazepine [30 μM]
Vehicle	17312 ± 1148	10780 ± 1119	4257 ± 332	8428 ± 428
Inositol [1 mM]	18724 ± 595	6252 ± 1056	3061 ± 455	6480 ± 1187
Tamoxifen [1 μM]	22787 ± 1150	7928 ± 1179	3683 ± 386	6150 ± 650
TPA [0.1 μM]	21306 ± 1305	7691 ± 1050	4377 ± 358	8730 ± 1420
SP 600125 [1 μM]	20416 ± 1359	9411 ± 380	6002 ± 343*	7300 ± 489
SB 203580 [10 μM]	20772 ± 1609	8436 ± 653	3482 ± 320	8802 ± 1220
PD 98059 [15 μM]	14550 ± 1196	4093 ± 880*	1491 ± 72*	4695 ± 780*
Wortmanin [0.1 μM]	20569 ± 2522	8382 ± 132	4022 ± 657	7303 ± 546
SB 216763 [1 μM]	21414 ± 2610	9869 ± 892	3967 ± 776	6158 ± 1503

Inositol, tamoxifen (PKC inhibitor), TPA (PKC activator), SP 600125 (JNK-MAPK inhibitor), SB 203580 (p38-MAPK inhibitor), PD 98059 (ERK-MAPK inhibitor), wortmanin (PI 3-K inhibitor) and SB 216763 (GSK-3 inhibitor) were added at indicated concentrations alone or 30 min before LiCl [4 mM], valproate [3 mM] or carbamazepine [30 μM] for 5 days. Corticosterone [1 μM] was added 2 h before harvesting the cells for an assay of CAT enzyme activity. The data are presented as the mean ± S.E.M. (dpm of the butyrylated fraction of chloramphenicol per 10 μg of protein per hour of incubation) from two separate experiments. 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. respective control group.

for 5 days did not change the number of nonviable cells (data not shown).

4. Discussion

The present data showed that all three mood stabilizers at clinically relevant concentrations attenuated the glucocorticoid receptor-mediated functions in LMCAT cells in a time- and concentration-dependent manner. Assuming similar regulation of glucocorticoid receptor-mediated gene transcription in LMCAT and neurons, the inhibitory effect of mood stabilizers on glucocorticoid receptor function may be a mechanism by which these drugs block some effects induced by stress or glucocorticoids (Couturier et al., 2001; Terao et al., 1997; Wada et al., 2000). In line with our results, it has been reported that lithium inhibits the activation of glucocorticoid receptors (Junker et al., 1984), although that study did not evaluate its effect on glucocorticoid receptor function. The significant inhibition of glucocorticoid receptor function was observed after 5 days but not after 1 day of incubation, which resembles time of manifestation of antidepressant drug effects. The delayed onset of action of mood stabilizers is in line with observations that clinical efficacy of both antidepressant

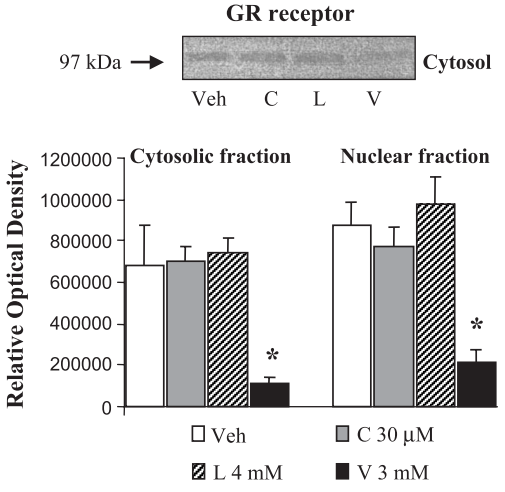

Fig. 5. Western blot of the glucocorticoid receptor in cytosolic and nuclear fraction of LMCAT cells treated for 5 days with vehicle (veh), carbamazepine (C; 30 μM), lithium (L; 4 mM) or valproate (V; 3 mM). The polyclonal antibody (anti-glucocorticoid receptor) recognized a band at ~ 97 kDa. Top: A representative Western blots in cytosolic fraction. Bottom: The semiquantitative analysis of glucocorticoid receptor bands. Results (mean ± S.E.M.) are expressed as a relative optical density units. 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).

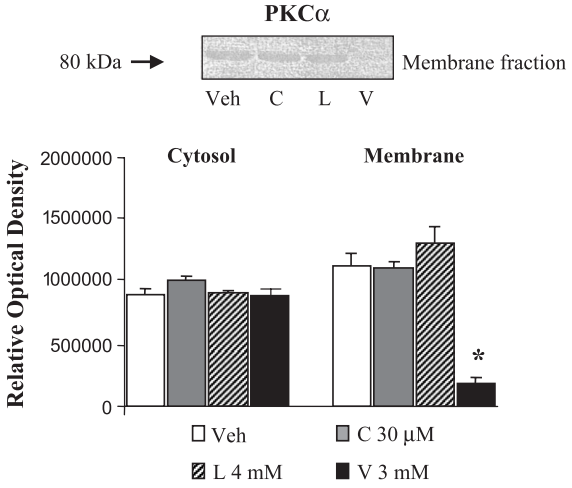

Fig. 6. Western blot of the PKC-α isozyme in cytosolic and membrane fraction of LMCAT cells treated for 5 days with vehicle (veh), carbamazepine (C; 30 μM), lithium (L; 4 mM) or valproate (V; 3 mM). The polyclonal antibody (anti-PKC-α) recognized a band at ~ 80 kDa. Top: A representative Western blot in membrane fraction. Bottom: The semiquantitative analysis of PKC-α bands. Results (mean ± S.E.M.) are expressed as a relative optical density units. 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).

and antimanic drugs can be observed after their long-term administration.

On the other hand, amphetamine, included in the present study for comparison, which is sometimes administered to animals to model manic disorders, and whose pharmacological effects are reversed by mood stabilizers (Chen et al., 1996; Gould et al., 2001; Lee et al., 2000), had no effect on glucocorticoid receptor function, which excludes its direct effect on glucocorticoid receptor-mediated gene transcription in LMCAT cells.

The delayed onset of action of mood stabilizers suggests that these drugs act not directly on the MMTV-CAT reporter gene, but affect a downstream processes involved in glucocorticoid receptor action, such as the binding of hormones with receptors, dissociation of the steroid–receptor complex from other cytosolic proteins, translocation to the nucleus, phosphorylation of glucocorticoid receptor or binding to DNA. The observed inhibitory effect of mood stabilizers on CAT activity may result from a decrease in glucocorticoid receptor expression in the cytoplasm or from its translocation to the nucleus, as was previously observed with some antidepressants (Heiske et al., 2003; Pariante et al., 1997). However, valproate decreases the number of glucocorticoid receptor both in the cytosolic and nuclear fraction, which excludes its effect on translocation and suggests that the main mechanism of this drug action in the present study involves inhibition of glucocorticoid receptor synthesis or enhancement of the receptor degradation. In contrast to valproate, lithium and carbamazepine did not change glucocorticoid receptor expression, which indicates that mood stabilizers inhibit glucocorticoid receptor function by different mechanisms.

Apart from glucocorticoid receptor number, glucocorticoid-mediated gene transcription depends on activity of various kinase pathways. Lithium and valproate enhance MAP kinase activity in various cell cultures and rat brain regions; on the other hand, MAP kinases, via phosphorylation, inhibit glucocorticoid receptor-mediated gene transcription (Einat et al., 2003; Irusen et al., 2002; Manji and Lenox, 1999; Rogatsky et al., 1998a; Yuan et al., 1999, 2001). Therefore, one might expect that MAP kinase inhibitors should reverse effect of mood stabilizers on glucocorticoid receptor function. In fact, only valproate action was attenuated by inhibition of JNK MAPK, which indicates that besides suppression of glucocorticoid receptor level, this drug may affect glucocorticoid receptor function also via an increase in JNK MAPK activity. Unexpectedly, an inhibitor of ERK MAPK enhanced inhibitory effect of all three mood stabilizers on MMTV-CAT activity. This phenomenon is difficult to explain; however, it should be kept in mind that in contrast to JNK and p38, participation of ERK MAP kinase in the regulation of glucocorticoid receptor function has not been fully elucidated (Irusen et al., 2002; Krstic et al., 1997; Rogatsky et al., 1998a). Some data also indicate that there are functional differences between stress-activated protein kinases (JNK, p38) and ERK, depending on cell type and particular process under study; for example, they show opposite effects in some apoptotic changes (Kamiguti et al., 2003; Xia et al., 1995).

Inhibitors of PKB and GSK-3 kinases, which have been reported to regulate the glucocorticoid receptor-mediated gene transcription activity (Rogatsky et al., 1998b; Schmidt et al., 2001) proved inactive under our experimental conditions. Since the concentrations of inhibitors were chosen to assure selective inhibition of particular enzyme, this strongly suggests that PKB, GSK-3 and p38 are not involved in mood-stabilizer effect on glucocorticoid receptor function in LMCAT cells. Furthermore, PLC/PKC pathway does not seem to be engaged in the mechanism of mood-stabilizer effect on glucocorticoid receptor function, since neither PKC activator (TPA) nor PKC inhibitor (tamoxifen) modified their action. This suggestion is supported by the results of Western blot analysis, which showed that lithium and carbamazepine did not change the PKC-α isozyme level in membrane and cytosol of LMCAT cells. PKC-α and PKC-ε are regarded by some authors as target enzymes for antipsychotic drugs, but only the former isoenzyme is present at high concentration in L929 fibroblasts (Manji and Lenox, 1999). Only valproate decreased PKC-α concentration; however, this effect seems to be functionally meaningless, since activation of PKC did not attenuate valproate action on glucocorticoid receptor function. Furthermore, PKA pathway does not seem to play a role in mood-stabilizer effects on glucocorticoid receptor function, because our previous findings showed that neither activation nor inhibition of this kinase had any effect on corticosterone-mediated gene transcription in LMCAT cells (Budziszewska et al., 2000).

It has been suggested that inositol depletion may underlie lithium activity in the bipolar affective disorder. Inositol has been reported to abolish mood-stabilizer-induced morphological changes in cultures of sensory neurons (Williams et al., 2002). However, this intracellular pathway may not be involved in regulation of glucocorticoid receptor transcriptional activity, since inositol at millimolar concentration failed to affect glucocorticoid receptor function and did not modulate inhibitory effect of lithium, valproate and carbamazepine on this parameter.

Mood stabilizers used in the present study, even at the highest concentration, produced no toxic effect, as estimated by nonviable cell count. Interestingly, these drugs exerted neuroprotective effects in various experimental models, and recent research suggested that neuroprotection could be important for their therapeutic action (Chuang et al., 2002; Manji and Chen, 2002; Yuan et al., 2001).

Regardless of molecular mechanisms, which are yet to be understood, the present study demonstrated unidirectional inhibitory effect of three mood stabilizers on glucocorticoid receptor function. This strengthens the hypothesis that direct effects of antidepressants and mood stabilizers on glucocorticoid receptor function is an important mechanism, by which these drugs may inhibit some of deleterious effects

of stress and glucocorticoids in the central nervous system (Jacobs et al., 2000; Manji and Chen, 2002; McEwen and Magarinos, 2001). In the light of new hypothesis accentuating significance of hypercortisolemia associated with disturbance of neuronal plasticity in pathogenesis of depression (McEwen and Magarinos, 2001), the mood-stabilizer-induced inhibition of glucocorticoid receptor function may significantly contribute to their therapeutic effects.

Acknowledgements

The authors are grateful to Dr. E.R. Sanchez for generously providing an LMCAT cell line. They are also indebted to Ms. B. Korzeniak for her skillful technical assistance. This study was supported by the Statutory Funds of the Institute of Pharmacology of the Polish Academy of Sciences, Kraków, Poland.

References

Basta-Kaim, A., Budziszewska, B., Jaworska-Feil, L., Tetich, M., Leśkiewicz, M., Kubera, M., Lasoń, W., 2002. Chlorpromazine inhibits the glucocorticoid receptor-mediated gene transcription in a calcium-dependent manner. Neuropharmacology 43, 1035–1043.

Budziszewska, B., 2002. Effect of antidepressant drugs on the hypothalamic–pituitary–adrenal axis activity and glucocorticoid receptor function. Pol. J. Pharmacol. 54, 343–349.

Budziszewska, B., Siwanowicz, J., Przegaliński, E., 1994. The effect of chronic treatment with antidepressant drugs on the corticosteroid receptor levels in the rat hippocampus. Pol. J. Pharmacol. 46, 147–152.

Budziszewska, B., Leśkiewicz, M., Jaworska-Feil, L., Lasoń, W., 1996. Repeated cocaine administration down-regulates glucocorticoid receptors in the rat brain cortex and hippocampus. Pol. J. Pharmacol. 48, 575–581.

Budziszewska, B., Jaworska-Feil, L., Kajta, M., Lasoń, W., 2000. Antidepressant drugs inhibit glucocorticoid receptor-mediated gene transcription—a possible mechanism. Br. J. Pharmacol. 130, 1385–1393.

Budziszewska, B., Jaworska-Feil, L., Tetich, M., Basta-Kaim, A., Kubera, M., Leśkiewicz, M., Lasoń, W., 2004. Regulation of the human corticotropin-releasing-hormone gene promoter activity by antidepressant drugs in Neuro-2A and AtT-20 cells. Neuropsychopharmacology 29, 785–794.

Chalecka-Franaszek, E., Chuang, D.M., 1999. Lithium activates the serine/threonine kinase Akt-1 and suppresses glutamate-induced inhibition of Akt-1 activity in neurons. Proc. Natl. Acad. Sci. U. S. A. 96, 8745–8750.

Chen, G., Manji, H.K., Hawver, D.B., Wright, C.B., Potter, W.Z., 1994. Chronic sodium valproate selectively decreases protein kinase C alpha and epsilon in vitro. J. Neurochem. 63, 2361–2364.

Chen, G., Pan, B., Hawver, D.B., Wright, C.B., Potter, W.Z., Manji, H.K., 1996. Attenuation of cyclic AMP production by carbamazepine. J. Neurochem. 67, 2079–2086.

Chuang, D.M., Chen, R.W., Chalecka-Franaszek, E., Ren, M., Hashimoto, R., Senatorov, V., Kanai, H., Hough, C., Hiroi, T., Leeds, P., 2002. Neuroprotective effects of lithium in cultured cells and animal models of diseases. Bipolar Disord. 4, 129–136.

Couturier, J., Steele, M., Hussey, L., Pawliuk, G., 2001. Steroid-induced mania in an adolescent: risk factors and management. Can. J. Clin. Pharmacol. 8, 109–112.

Einat, H., Yuan, P., Gould, T.D., Li, J., Du, J., Zhang, L., Manji, H.K., Chen, G., 2003. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. J. Neurosci. 23, 7311–7316.

Gottlicher, M., Minucci, S., Zhu, P., Kramer, O.H., Schimpf, A., Giavara, S., Sleeman, J.P., Lo Coco, F., Nervi, C., Pelicci, P.G., Heinzel, T., 2001. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J. 20, 6969–6978.

Gould, T.J., Keith, R.A., Bhat, R.V., 2001. Differential sensitivity to lithium's reversal of amphetamine-induced open-field activity in two inbred strains of mice. Behav. Brain Res. 118, 95–105.

Heiske, A., Jesberg, J., Krieg, J.C., Vedder, H., 2003. Differential effects of antidepressants on glucocorticoid receptors in human primary blood cells and human monocytic U-937 cells. Neuropsychopharmacology 28, 807–817.

Heuser, I.J.E., Schweiger, U., Gotthardt, U., Schmider, J., Lammers, C.H., Dettling, M., Yassouridis, A., Holsboer, F., 1996. Pituitary-adrenal-system regulation and psychopathology during amitriptyline treatment in elderly depressed patients and normal comparison subjects. Am. J. Psychiatr. 153, 93–99.

Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23, 477–501.

Holsboer, F., Lauer, C.J., Schreiber, W., Krieg, J.C., 1995. Altered hypothalamic–pituitary–adrenocortical regulation in healthy subjects at high familial risk for affective disorders. Neuroendocrinology 62, 340–347.

Irusen, E., Matthews, J.G., Takahashi, A., Barnes, P.J., Chung, K.F., Adcock, I.M., 2002. p38 Mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. J. Allergy Clin. Immunol. 109, 649–657.

Jackson, I.M., Luo, L.G., 1998. Antidepressants inhibit the glucocorticoid stimulation of thyrotropin releasing hormone expression in cultured hypothalamic neurons. J. Invest. Med. 46, 470–474.

Jacobs, B.L., van Praag, H., Gage, F.H., 2000. Adult brain neurogenesis and psychiatry: a novel theory of depression. Mol. Psychiatry 5, 262–269.

Jensen, J.B., Mork, A., 1997. Altered protein phosphorylation in the rat brain following chronic lithium and carbamazepine treatments. Eur. Neuropsychopharmacol. 7, 173–179.

Junker, K., Svenson, M., Junker, S., 1984. Lithium inhibits the cytolytic glucocorticoid effect on S49 mouse lymphoma cells. J. Steroid Biochem. 20, 725–731.

Kamiguti, A.S., Harris, R.J., Slupsky, J.R., Baker, P.K., Cawley, J.C., Zuzel, M., 2003. Regulation of hairy-cell survival through constitutive activation of mitogen-activated protein kinase pathways. Oncogene 22, 2272–2284.

Klein, P.S., Melton, D.A., 1996. A molecular mechanism for the effect of lithium on development. Proc. Natl. Acad. Sci. U. S. A. 93, 8455–8459.

Krstic, M.D., Rogatsky, I., Yamamoto, K.R., Garabedian, M.J., 1997. Mitogen-activated and cyclin-dependent protein kinases selectively and differentially modulate transcriptional enhancement by the glucocorticoid receptor. Mol. Cell. Biol. 17, 3947–3954.

Lee, Y., Hamamura, T., Ohashi, K., Miki, M., Fujiwara, Y., Kuroda, S., 2000. Carbamazepine suppresses methamphetamine-induced Fos expression in a regionally specific manner in the rat brain. Possible neural substrates responsible for antimanic effects of mood stabilizers. Neuropsychopharmacology 22, 530–537.

Linkowski, P., Mendlewicz, J., LeClerc, R., Brasseur, M., Hubain, P., Goldstein, J., Copinschi, G., Van Cauter, E., 1985. The 24-hour profile of ACTH and cortisol in major depressive illness. J. Clin. Endocrinol. Metab. 61, 429–438.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Mai, L., Jope, R.S., Li, X., 2002. BDNF-mediated signal transduction is modulated by GSK3β and mood stabilizing agents. J. Neurochem. 82, 75–83.

Manji, H.K., Chen, G., 2002. PKC, MAP kinases and the bcl-2 family of proteins as long-term targets for mood stabilizers. Mol. Psychiatry 7 (Suppl 1), S46–S56.

Manji, H.K., Lenox, R.H., 1999. Protein kinase C signaling in the brain: molecular transduction of mood stabilization in the treatment of manic–depressive illness. Biol. Psychiatry 46, 1328–1351.

Mann, C.D., Vu, T.B., Hrdina, P.D., 1995. Protein kinase C in rat brain cortex and hippocampus: effect of repeated administration of fluoxetine and desipramine. Br. J. Pharmacol. 115, 595–600.

McEwen, B.S., Magarinos, A.M., 2001. Stress and hippocampal plasticity: implications for the pathophysiology of affective disorders. Hum. Psychopharmacol. 16, S7–S19.

Nibuya, M., Morinobu, S., Duman, R.S., 1995. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J. Neurosci. 15, 7539–7547.

Okuyama-Tamura, M., Mikuni, M., Kojima, I., 2003. Modulation of the human glucocorticoid receptor function by antidepressive compounds. Neurosci. Lett. 342, 206–210.

Pariante, C.M., Miller, A.H., 2001. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol. Psychiatry 49, 391–404.

Pariante, C.M., Pearce, B.D., Pisell, T.L., Owens, M.J., Miller, A.H., 1997. Steroid-independent translocation of the glucocorticoid receptor by the antidepressant desipramine. Mol. Pharmacol. 52, 571–581.

Rogatsky, I., Logan, S.K., Garabedian, M.J., 1998a. Antagonism of glucocorticoid receptor transcriptional activation by the c-Jun N-terminal kinase. Proc. Natl. Acad. Sci. U. S. A. 95, 2050–2055.

Rogatsky, I., Waase, C.L., Garabedian, M.J., 1998b. Phosphorylation and inhibition of rat glucocorticoid receptor transcriptional activation by glycogen synthase kinase-3 (GSK-3). Species-specific differences between human and rat glucocorticoid receptor signaling as revealed through GSK-3 phosphorylation. J. Biol. Chem. 273, 14315–14321.

Sanchez, E.R., Hu, J.L., Zhong, S., Shen, P., Greene, M.J., Housley, P.R., 1994. Potentiation of glucocorticoid receptor-mediated gene expression by heat and chemical shock. Mol. Endocrinol. 8, 408–421.

Schaaf, M.J.M., de Jong, J., de Kloet, E.R., Vreugdenhil, E., 1998. Down-regulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. Brain Res. 813, 112–120.

Schmidt, P., Holsboer, F., Spengler, D., 2001. β_2-Adrenergic receptors potentiate glucocorticoid receptor transactivation via G protein beta gamma-subunits and the phosphoinositide 3-kinase pathway. Mol. Endocrinol. 15, 553–564.

Schwaninger, M., Schofl, C., Blume, R., Rossig, L., Knepel, W., 1995. Inhibition by antidepressant drugs of cyclic AMP response element-binding protein/cyclic AMP response element-directed gene transcription. Mol. Pharmacol. 47, 1112–1118.

Terao, T., Yoshimura, R., Shiratuchi, T., Abe, K., 1997. Effects of lithium on steroid-induced depression. Biol. Psychiatry 41, 1225–1226.

Vetulani, J., Nalepa, I., 2000. Antidepressants: past, present and future. Eur. J. Pharmacol. 405, 351–363.

Wada, K., Yamada, N., Suzuki, H., Lee, Y., Kuroda, S., 2000. Recurrent cases of corticosteroid-induced mood disorder: clinical characteristics and treatment. J. Clin. Psychiatry 61, 261–267.

Watanabe, Y., Gould, E., Daniels, D.C., Cameron, H., McEwen, B.S., 1992. Tianeptine attenuates stress-induced morphological changes in the hippocampus. Eur. J. Pharmacol. 222, 157–162.

Williams, R.S., Cheng, L., Mudge, A.W., Harwood, A.J., 2002. A common mechanism of action for three mood-stabilizing drugs. Nature 417, 292–295.

Xia, Z., Dickens, M., Raingeaud, J., Davis, R.J., Greenberg, M.E., 1995. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270, 1326–1331.

Yuan, P., Chen, G., Manji, H.K., 1999. Lithium activates the c-Jun NH2-terminal kinases in vitro and in the CNS in vivo. J. Neurochem. 73, 2299–2309.

Yuan, P.X., Huang, L.D., Jiang, Y.M., Gutkind, J.S., Manji, H.K., Chen, G., 2001. The mood stabilizer valproic acid activates mitogen-activated protein kinases and promotes neurite growth. J. Biol. Chem. 276, 31674–31683.