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Effects of mood stabilizers on adult dentate gyrus-derived neural precursor cells

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Abstract

Neurogenesis in the adult dentate gyrus (DG) is considered to be partly involved in the action of mood stabilizers. However, it remains unclear how mood stabilizers affect neural precursor cells in adult DG. We have established a culture system of adult rat DG-derived neural precursor cells (ADP) and have shown that lithium, a mood stabilizer, and dexamethasone, an agonist of glucocorticoid receptor, reciprocally regulate ADP proliferation. Neurogenesis constitutes not only proliferation of neural precursor cells but also apoptosis and differentiation. To develop further understanding of mood stabilizer effects on neural precursor cells in adult DG, we investigated and compared the effects of four common mood stabilizers—lithium, valproate, carbamazepine, and lamotrigine—on ADP proliferation, apoptosis, and differentiation. ADP proliferation, decreased by dexamethasone, was examined using Alamar Blue assay. Using TUNEL assay, ADP apoptosis induced by staurosporine was examined. The differentiated ADP induced by retinoic acid was characterized by immunostaining with anti-GFAP or anti-Tuj1 antibody. Lithium and valproate, but not carbamazepine and lamotrigine, recovered ADP proliferation decreased by dexamethasone. All four mood stabilizers decreased ADP apoptosis. Retinoic acid differentiated ADP into both neurons and astrocytes. Lithium and carbamazepine increased the ratio of neurons and decreased that of astrocytes. However, valproate and lamotrigine increased the ratio of astrocytes and decreased that of neurons. Therefore, these four stabilizers exhibited both common and differential effects on ADP proliferation, apoptosis, and differentiation.

Key Words: neurogenesis, proliferation, differentiation, apoptosis, mood disorder, hippocampus, dexamethasone, staurosporine, retinoic acid

Abbreviations: Li, lithium; VPA, valproate; CBZ, carbamazepine; LTG, lamotrigine; DG, dentate gyrus; ADP, adult rat DG-derived neural precursor cell; DEX, dexamethasone; STS, staurosporine; RA, retinoic acid; PHT, phenytoin; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; DAPI, 4',6-diamino-2-phenylindole

1. Introduction

Drugs of a group including lithium (Li), valproate (VPA), carbamazepine (CBZ), and lamotrigine (LTG), known as mood stabilizers, are commonly used to treat bipolar disorder (Goodwin, 2003). Although the biochemical effects of mood stabilizers have been investigated extensively (Schloesser et al., 2007; Schloesser et al., 2008), the essence of their mood-stabilizing effects remains unclear.

Recently, neurogenesis has been confirmed to occur in the adult hippocampus (Kempermann, 2006; Gage et al., 2008). Stem cells (Type-1 cells) are located in the subgranular zone between the granular cell layer and hilus in the dentate gyrus (DG), and differentiate to amplify progenitor cells (Type-2a and Type-2b cells). Thereafter, numerous newborn cells die, presumably by the apoptosis (Kempermann et al., 2003). New surviving neurons mature morphologically and electrophysiologically. They ultimately integrate into active neural circuits. Although the function of these newborn cells remains unclear, they are reportedly involved in the therapeutic action of antidepressants (Santarelli et al., 2003; Surget et al., 2008; David et al., 2009). Moreover, recent reports have described that Li and VPA affect neurogenesis through increasing cell proliferation and/or promotion of neuronal differentiation of neural precursor cells (Chen et al., 2000; Son et al., 2003; Hao et al., 2004; Hsieh et al., 2004; Kim JS et al., 2004; Laeng et al., 2004; Wexler et al., 2008) and that Li blocks the effects of stress on depression-like behaviors through increasing hippocampal neurogenesis in adult rodent models (Silva et al., 2008). Results of these studies suggest that adult hippocampal neurogenesis plays an important role in the therapeutic action of mood stabilizers as well.

We have already established the culture system of adult DG-derived neural precursor cell (ADP), which approximately corresponds to Type-2a amplifying progenitor cells (Boku et al., 2009). Li reported a lack of an effect on ADP proliferation but recovered ADP proliferation decreased by dexamethasone (DEX), a specific agonist of glucocorticoid receptor (Boku et al., 2009). To expand knowledge about the effect of mood stabilizers on adult neurogenesis, we examined and compared the effects of four mood stabilizers—Li, VPA, CBZ, and LTG—on ADP proliferation, apoptosis and differentiation (cell-fate determination). Although these four stabilizers had varied effects on ADP proliferation and differentiation, all commonly decreased ADP apoptosis.

2. Materials and Methods

Drugs Dexamethasone (DEX) was purchased from Sigma Chemical Co. (St. Louis, MO). Retinoic acid (RA) was purchased from Invitrogen Corp. (Carlsbad, CA). Staurosporine (STS) was kindly donated by Asahi-Kasei Medical Co. Ltd. (Shizuoka, Japan). Lithium chloride (Li) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Sodium valproate (VPA) was kindly donated by Kyowa Hakko Kogyo Co. Ltd. (Tokyo, Japan). Carbamazepine (CBZ) was kindly donated by Nihon Ciba-Geigy K.K. (Tokyo, Japan). Lamotrigine was kindly donated by Glaxo SmithKline plc. (London, UK). Phenytoin (PHT) was kindly donated by Dainippon Sumitomo Pharma Co. Ltd. (Osaka, Japan).

Isolation and culture of ADP ADP was isolated from the dentate gyrus of adult male Sprague–Dawley rats (8 weeks old), as described in a previous report (Boku et al., 2009). The ADPs were maintained with Neurobasal (Invitrogen Corp., Carlsbad, CA)/ B27 supplement minus vitamin A (Invitrogen Corp.)/ 1 mM L-glutamine (Invitrogen Corp.)/ 20 ng/ml bFGF (Invitrogen Corp.) (proliferation medium) at 37°C on laminin (Invitrogen Corp.)-ornithine

(Sigma)-coated dishes and fed with new medium every two or three days by replacing 50% of the medium. When cell confluency reached 80–90%, cells were passaged by trypsinization, and the cell density for plating was approximately 1×10^4 cells/cm².

Cell counting Alamar Blue assay is a rapid and simple non-radioactive assay used to estimate the number of living cells (Ahmed et al., 1994). Alamar Blue dye is a fluorogenic redox indicator is converted from the oxidized form to the reduced form in cells. The reduced form of Alamar Blue dye is highly fluorescent; fluorescence in Alamar Blue assay reflects the number of cells. Although BrdU-based assays are often used for cell counting, BrdU-positiveness reflects the duplication of DNA, but not the number of cells. Additionally, we confirmed that fluorescence in Alamar Blue assay is proportional to the simply counted number of ADPs (data not shown). Therefore, we used Alamar Blue assay to estimate the effects of drugs on the number of cells. First, 1×10^4 cells/well were put in laminin–ornithine coated 96-well plates in 100 μ l/well of proliferation medium. After overnight incubation, cells were treated with each drug at each concentration. After 3 days, 10 μ l/well of Alamar Blue solution (Invitrogen Corp.) was added to medium, and cells were incubated at 37°C for 3 hr. Subsequently, 50 μ l of medium were dispensed into plates and the fluorescence of samples were measured and calculated as described in the manufacturer’s manual. Statistical analysis was performed using one-way ANOVA and Dunnet’s post hoc test. Significance was inferred for $p < 0.05$. Data are expressed as means $\pm$ SEM.

TUNEL assay First, 2×10^4 cells/well were put in laminin–ornithine coated 8-chamber slides (Lab-Tek II; Nalge Nunc International, Naperville, IL) with proliferation medium. After overnight incubation, cells were treated in proliferation medium with STS, PHT, Li, VPA, CBZ, and/or LTG. After 2 days, cells were fixed in 4% paraformaldehyde for 15 min. Permeabilization was performed with PBS containing 0.2% Triton X-100 for 30 min. Subsequently, terminal

deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed with a DeadEnd Fluorometric TUNEL System (Promega Corp., Madison, WI), as described in the manufacturer's manual. Fluorescent signals were detected using a fluorescence microscope system (IX-71; Olympus Corp.). The quantities of both TUNEL and 4',6-diamino-2-phenylindole (DAPI; Vector Laboratories Inc., Burlingame, CA) signals were counted in four randomly selected fields/well. Then the ratio of TUNEL signals/ DAPI signals was calculated. Statistical analysis was performed using Student's *t*-test. Significance was defined as $p < 0.05$. Data are expressed as means $\pm$ SEM.

Immunocytochemistry First, 2×10^4 cells/well were put in laminin–ornithine coated Lab-Tek II eight-chamber slides with proliferation medium without bFGF (differentiation medium). After overnight incubation, cells were treated in differentiation medium with 1 μ M RA/ 0.5% fetal bovine serum (Invitrogen) and Li, VPA, CBZ, or LTG. After 7 days, cells were fixed 4% paraformaldehyde for 15 min. Permeabilization was performed with PBS containing 0.2% Triton X-100 for 30 min. Subsequently, samples were blocked in PBS containing 3% goat serum for 20 min at room temperature (RT), incubated in PBS containing 3% goat serum containing primary antibodies at 4°C overnight, and incubated in PBS containing secondary antibodies for 1 hr at RT. Samples were coverslipped with Vectashield containing DAPI. Fluorescent signals were detected using the IX-71 fluorescent microscope system described above. Primary antibodies were used at the following concentrations: mouse anti-nestin (1:2000; BD Biosciences, Franklin Lakes, NJ), rabbit anti-glial fibrillary acidic protein (GFAP) (1:2000; Dako, Glostrup, Denmark), mouse anti-Tuj1 (1:5000; Covance Inc., Princeton, NJ). Secondary antibodies were used at the following concentrations: FITC-conjugated goat anti-mouse IgG antibody (1:100; Jackson Immuno Research Laboratories, Inc., West Grove, PA), Cy3-conjugated goat anti-rabbit IgG antibody (1:100; Jackson Immuno Research Laboratories, Inc.). The quantities of signals of each marker

gene and DAPI were counted in four randomly selected fields/ well. Then the ratio of each marker gene-derived signals/DAPI signals was calculated. Statistical analysis was performed using Student's *t*-test. Significance was defined as $p < 0.05$. Data are expressed as means $\pm$ SEM.

3. Results

Effects of mood stabilizers on ADP proliferation

Adult neurogenesis in DG is decreased in rodent models for stress-related disorders (Malberg and Duman, 2003; Jayatissa et al., 2006; Silva et al., 2008). Although it remains unclear how adult neurogenesis in DG is decreased in these models, reports of some studies have suggested that glucocorticoids are involved in them (Cameron and McKay, 1999; Kim JB et al., 2004). We have already shown that Li has no effect on ADP proliferation but recovers ADP proliferation decreased by dexamethasone (DEX), a specific agonist of glucocorticoid receptor (Boku et al., 2009). Following our previous study, we used Alamar Blue assay to examine the effects of Li, VPA, CBZ and LTG on ADP proliferation in the absence or presence of 5 μ M DEX. Results showed that ADP proliferation decreased significantly with 5 μ M DEX. Furthermore, 0.3–3 mM Li and 30–1000 μ M VPA showed no effect on ADP proliferation in the absence of DEX but 1–3 mM Li and 100–300 μ M VPA recovered ADP proliferation decreased by 5 μ M DEX in a dose-dependent manner (Figs. 1A and 1B). However, 3–30 μ M CBZ and 30–300 nM LTG had no effect on ADP proliferation, either in the absence or presence of 5 μ M DEX (Figs. 1C and 1D). The ADP proliferation was decreased remarkably more than 30 μ M CBZ and 300 nM LTG (data not shown).

Effects of mood stabilizers on ADP apoptosis

Staurosporine (STS) is a common inhibitor of Protein Kinase C that is often used to induce apoptosis on culture cells (Sanchez et al., 1992). Apoptosis has two pathways: The internal

pathway via mitochondria and external pathways via death receptors (Adams, 2003); STS is well known as an inducer of internal pathway (Ferrari et al., 1998). All four of these stabilizers increase the expression of Bcl-2, a key regulator of the internal pathway (Chen et al., 1999; Chang et al., 2008). In addition, internal pathway, but not external pathway, is involved in the apoptosis of neural progenitor cells (Ekdahl et al., 2003; Ceccatelli et al., 2004). Our preliminary data showed that Tumor Necrosis Factor- α , a ligand of death receptors and an inducer of external pathway, did not induce apoptosis on ADP (data not shown). Therefore, we specifically examined the internal pathway in the present study. First, the effect of STS on ADP apoptosis was examined using TUNEL assay at 0, 100, 300 nM, and 1 μ M. Only a few TUNEL signals were found at 0 and 100 nM STS. Most of the cells were removed from the bottom of the eight-well chamber at 1 μ M STS (data not shown). However, around 70% of cells were TUNEL-positive with 300 nM STS (Fig. 2). Therefore, we investigated the effects of Li, VPA, CBZ, and LTG on ADP apoptosis induced by 300 nM STS. Next, the effects of Li (1, 3, 10 mM), VPA (100, 300 μ M, 1 mM), CBZ (1, 3, 10, 30 μ M), and LTG (10, 30, 100, 300 nM) on ADP apoptosis induced by 300 nM STS were examined using TUNEL assay. All of 1 mM Li, 1 mM VPA, 3 μ M CBZ, and 30 nM LTG decreased the ratio of TUNEL-positive cells to around 30% (Figs. 2A–2D). Nevertheless, none of these four mood stabilizers had any effect on the ratio of TUNEL-positive cells or peeled off many cells from the bottom of the eight-well chamber at other doses (data not shown). We also examined the effect of 5 μ M phenytoin (PHT), an antiepileptic drug that is not used as a mood stabilizer, on ADP apoptosis induced by 300 nM STS. It is noteworthy that 5 μ M PHT had no effect on the ratio of TUNEL-positive ADPs (Fig. 2E).

Effects of mood stabilizers on ADP differentiation

Retinoic acid (RA) is widely used as a potent inducer of neural differentiation by multipotent

cells of various types, such as neural stem cells, embryonal carcinoma cells, and embryonic stem cells *in vitro* (Takahashi et al., 1999; Soprano et al., 2007). Recent findings have shown that endogenous RA is involved in neural differentiation in adult hippocampus *in vivo* (Jacobs et al., 2006; McCaffery et al., 2006). We have already shown that RA induces ADP to both neuron and astrocyte (Boku et al., 2009). Moreover, ADP did not differentiate at all by only the depletion of bFGF, and all of Li, VPA, CBZ, and LTG had no effect on ADP differentiation without RA (data not shown). Therefore, we examined the effects of Li (1, 3, 10 mM), VPA (100, 300 μ M, 1 mM), CBZ (1, 3, 10, 30 μ M), and LTG (10, 30, 100, 300 nM) on ADP differentiation induced by RA. Results show that 1 μ M RA differentiated around 50% of ADP into a neuron-like cell (Tuj1-positive cell) and around 12% of ADP into an astrocyte-like cell, which is GFAP-positive and which has larger size and a more spread shape than that of ADP (Figs. 3A–3C). Both 1 mM Li and 3 μ M CBZ increased the ratio of neuron-like cells to around 70% and decreased the ratio of into astrocyte-like cells to around 5% (Figs. 3B–3C). Both 1 mM VPA and 30 nM LTG decreased the ratio of neuron-like cells to around 10% and increased the ratio of astrocyte-like cell to around 25% (Figs. 3B–3C). None of Li, VPA, CBZ, or LTG had any effect on the number of ADP in these doses (data not shown). None of these four mood stabilizers showed any effect on the ratio of Tuj1 or GFAP-positive cells or peeled off many cells from the bottom of the eight-well chamber at other doses (data not shown).

4. Discussion

Results show that four commonly used mood stabilizers—Li, VPA, CBZ, and LTG—have varying effects on ADPs (Table 1). Only four comparative studies have examined cells of other types, such as tumor-derived cell lines or primary neuron cultures. In addition, each study specifically addressed only a single phenomenon: neuronal differentiation or apoptosis (Li et al.,

2002; Mai et al., 2002; Williams et al., 2002; Daniel et al., 2005). Therefore, the present report describes is the first comparative study of the effects of four mood stabilizers in adult DG-derived neural precursor cells on three phenomena which constitute neurogenesis: proliferation, apoptosis and differentiation.

Our present results differ from past studies in many points. In the case of proliferation, some reports have described that Li and VPA increased the proliferation of neural precursor cells (Kim JS et al., 2004; Laeng et al., 2004; Wexler et al., 2008). In the case of differentiation, some reports have described that Li and VPA, but not CBZ, promote the differentiation of neural precursor cells into neurons (Hao et al., 2004; Hsieh et al., 2004; Kim JS et al., 2004; Laeng et al., 2004). In these studies, neural precursor cells are derived from entire adult hippocampi, partly including the subventricular zone, and embryos. In contrast, our ADP is derived from dissected DG from adult hippocampi. Moreover, the proliferation potency of neural stem cells decreases according to age (Molofsky et al., 2006). Although neural stem cells in the early developmental stage tend to be differentiated into neurons by LIF, those in the late developmental stage tend to be differentiated into astrocytes (Takizawa et al., 2001). Moreover, in contrast to ADP-derived dissected DG, adult hippocampal neural progenitors in past studies are derived from entire adult hippocampi. The culture condition of ADP also differs from that of adult hippocampal progenitors. Therefore, the reactivity to drugs of ADP might differ from those of embryonic neural stem cells and adult hippocampal neural progenitors. To confirm that point, some comparison is needed of the effects of mood stabilizers on proliferation, apoptosis and differentiation between ADP and neural precursor cells derived from other sources.

That GSK-3 β and β -catenin/TCF pathways regulate cell proliferation is well known (Takahashi-Yanaga and Sasaguri, 2009). Regarding the effect of Li on ADP proliferation decreased by DEX, we have already shown part of its mechanism: DEX decreases ADP

proliferation through activation of GSK-3 β and following inhibition of β -catenin/TCF pathway; and Li reverses the inhibitory effect of DEX on ADP proliferation through inhibiting activated GSK-3 β and following activation of β -catenin/TCF pathway (Boku et al., 2009). In the present study, we showed that VPA recovered ADP proliferation decreased by DEX as in the case of Li. We also showed that the recovery effect of VPA on ADP proliferation decreased by DEX is reversed by quercetin, an inhibitor of β -catenin/TCF pathway, as in the case of Li (our unpublished data). In addition, some reports have described that VPA promotes β -catenin/TCF pathway through inhibition of GSK-3 β (Chen et al., 1999; Kim et al., 2005). These findings suggest that VPA also regulates ADP proliferation through GSK-3 β and the β -catenin/TCF pathway. However, the other reports have described that the activity of GSK-3 β is not inhibited by VPA (Williams et al., 2002; Kozlovsky et al., 2003; Ryves et al., 2005). Additionally, it has been shown that VPA actions are partly mediated by histone deacetylase (HDAC) inhibition (Phiel et al., 2001). Further investigation is necessary to elucidate how VPA recovers ADP proliferation decreased by DEX.

Our results of the effects of mood stabilizers on ADP differentiation induced by RA have shown that Li and CBZ increase neuronal differentiation and decrease astroglial differentiation and that VPA and LTG are vice versa. The opposite effects of Li/CBZ and VPA/LTG on ADP differentiation are so interesting that the investigation of the mechanism underlying these opposing effects might be expected to engender new aspects of the action mechanism of mood stabilizers. RA and brain-derived neurotrophic factor (BDNF) synergistically promote neuronal differentiation of neural precursor cells (Takahashi et al., 1999). In addition, RA and leukemia inhibitory factor (LIF) synergistically promotes astroglial differentiation of neural precursor cells (Asano et al., 2009). Therefore, Li/CBZ might affect BDNF pathway and VPA/LTG might affect the LIF pathway. This speculation is apparently interesting but has some problems. For example,

the pathways can not be merely separated from each other because some reports have described the existence of cross talk between BDNF and LIF pathways (Rajan et al., 1998; Lund et al., 2008; Yasuda et al., 2009). Although it has been shown that Li induces BDNF expression (Fukumoto et al., 2001), the effect of CBZ on BDNF pathway and the effects of VPA and LTG on LIF pathway remain poorly understood. However, our speculation might be worth further consideration. To confirm results of the effects of mood stabilizers on ADP differentiation induced by RA and validate our speculation of the mechanism underlying them, further investigation using other physiological inducers of neural and glial differentiation, such as BDNF and LIF, is needed.

Although the effects of mood stabilizers on ADP proliferation decreased by DEX and ADP differentiation induced by RA differ among them, all of these four mood stabilizers decreased ADP apoptosis induced by STS (Table 1). In addition, phenytoin, an anticonvulsant but not mood stabilizer, had no effect on it. Therefore, their anti-apoptotic effects on neural precursor cell might be involved in a part of common mood stabilizing effects. Results of some studies have suggested that internal pathway-related factors (e.g., GSK-3 β , Bcl-2, HSP70) might be candidates of the common anti-apoptotic effects of mood stabilizers (Chen et al., 1999; Li et al., 2002; Jope and Bijur, 2002; Ren et al., 2003; Pan et al., 2005). Furthermore, Williams et al. showed that prolyl oligopeptidase is a candidate factor of the common effects of mood stabilizers on the collapse of sensory neuron growth cones (Williams et al., 2002); prolyl oligopeptidase is reportedly involved in apoptosis (Okada et al., 2002). Therefore, we are going to investigate the mood stabilizing effects of these molecules in ADP, which might engender elucidating the molecular mechanism of mood stabilizing effects. However, staurosporine is not a physiological inducer of apoptosis. Therefore, further investigation using more physiological inducers of apoptosis, such as mitogen or insulin withdrawal, glucose deprivation, is necessary to confirm

our results.

The differentiation stages of proliferative neural precursor cells have been proposed for the adult rodent hippocampus *in vivo* (Kempermann, 2006; Gage et al., 2008): the first stage (Type-1 cell), the second stage (Type-2a cell), and the third stage (Type-2b cell). Type-1 cell corresponds to stem cells. Type-2a cell and Type-2b cell correspond to amplifying progenitor cells. In these developmental stages, our ADP might correspond to Type-2a cell (Boku et al., 2009). Fluoxetine, an antidepressant, increases Type-2a-like cells in adult DG (Encinas et al., 2006); electroconvulsive seizure mainly increases Type-1-like cells in adult DG (Segi-Nishida et al., 2008). Our present study has elucidated the effects of mood stabilizers on Type-2a cell, but not on Type-1 cells and Type-2b cells. Therefore, it might be important to investigate the effects of specific drugs on precursor cells of other types.

Finally, results show that the four common mood stabilizers—Li, VPA, CBZ, and LTG—exert various effects on the actions of ADP, type-2a like neural precursor cell in adult DG. However, *in vivo* functional significance of these results and mechanisms underlying them remain unclear. To investigate them might be beneficial to further the understanding of action mechanisms of mood stabilizers and the pathophysiology of mood disorders, which might engender the development of new therapeutic targets of mood disorder.

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Figure legends

Figure 1. Effects of mood stabilizers on ADP proliferation

Li (A) and VPA (B) recovered ADP proliferation decreased by DEX, but not CBZ (C) and LTG (D). Alamar Blue assay was performed 3 days after drug treatments for four independent cultures. Data are shown as means $\pm$ SEM. * $p < 0.05$, compared with control; ** $p < 0.05$, compared with 0 mM (A: Li), 0 μ M (B: VPA).

Figure 2. Effects of mood stabilizers on ADP apoptosis

All four mood stabilizers decreased ADP apoptosis induced by staurosporine (STS) (A–D). Phenytoin (PHT), an anticonvulsant drug, did not affect staurosporine-induced apoptosis (E).

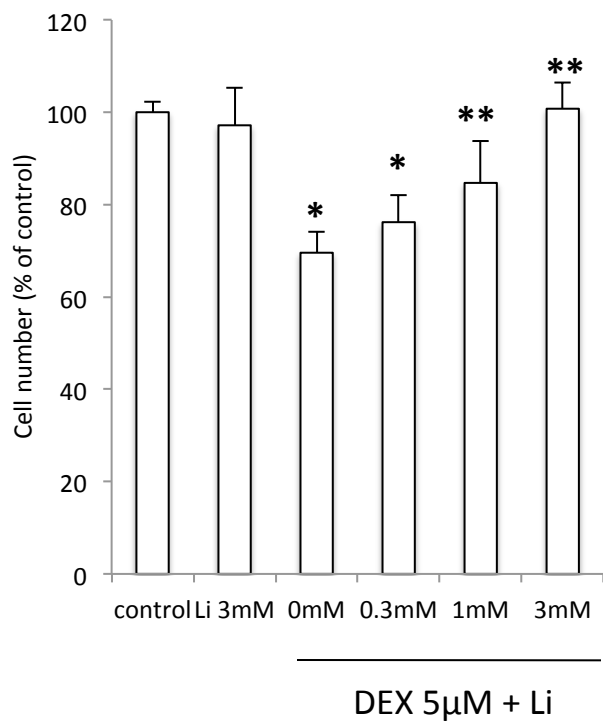
TUNEL assay was performed 2 days after drug treatments for four independent cultures. The percentage of TUNEL (+) / DAPI (+) ADPs with STS is expressed as control. Data are shown as means $\pm$ SEM. * p <0.05, compared with control.

Figure 3. Effects of mood stabilizers on ADP differentiation

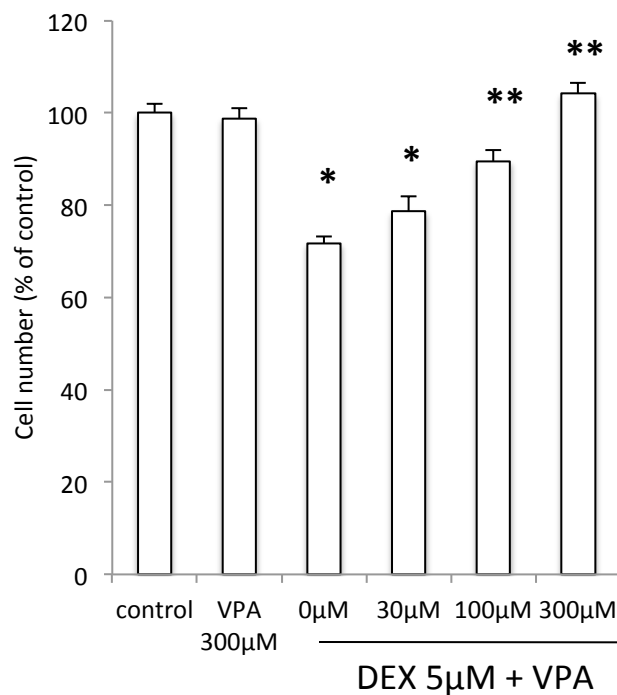
Li and CBZ increased the ratio of neuron-like cell induced by retinoic acid (RA). However, VPA and LTG decreased it and increased the ratio of astrocyte-like cell. Immunocytochemistry was performed 7 days after drug treatment for four independent cultures. A: Scale bar = 120 μ m. B, C: Concentrations of mood stabilizers are as follows: Li, 1 mM; VPA, 1 mM; CBZ, 3 μ M; LTG, 30 nM. Data are shown as means $\pm$ SEM. * p <0.05, compared with control.

Figure 1

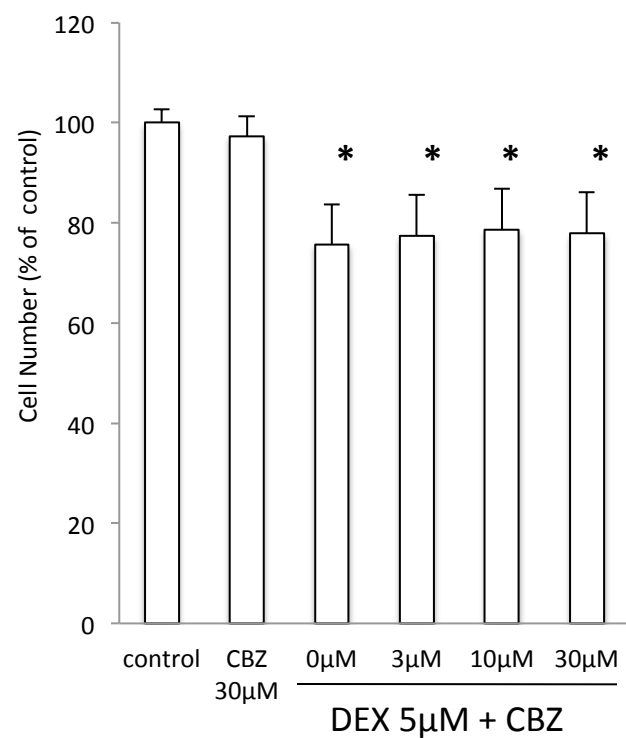
A



B



C



D

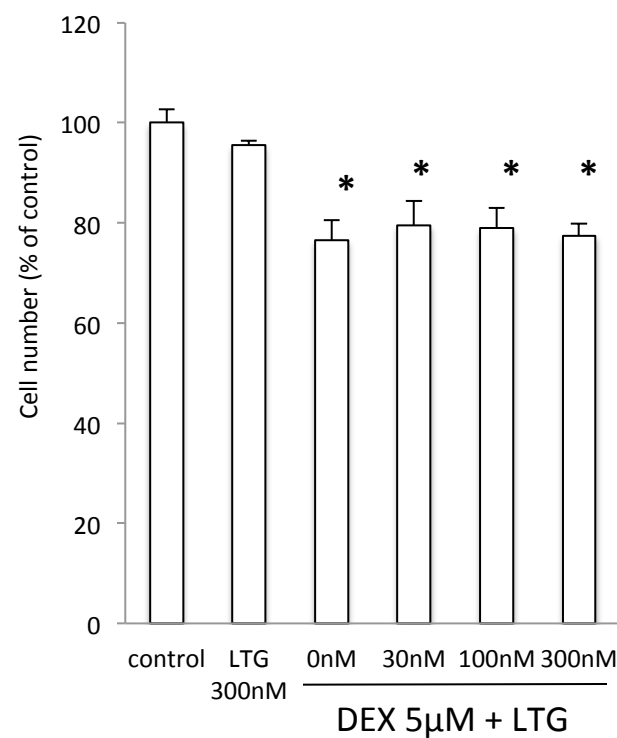
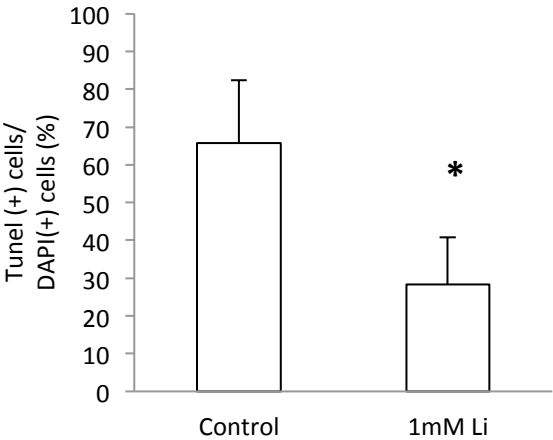
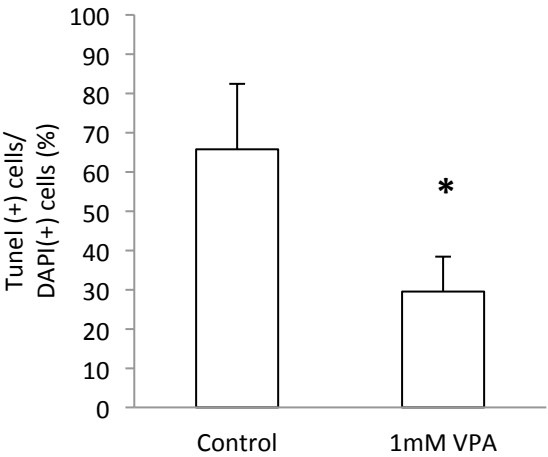


Figure 2

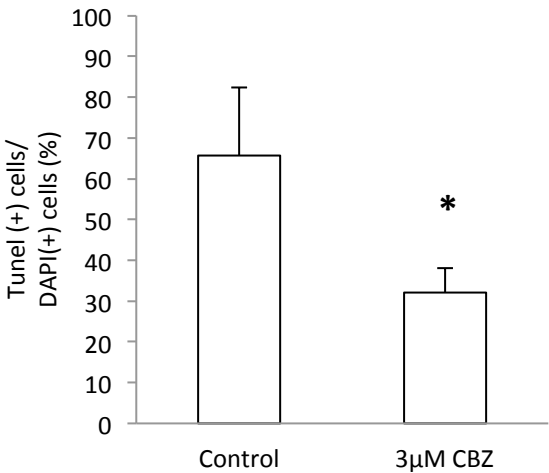
A



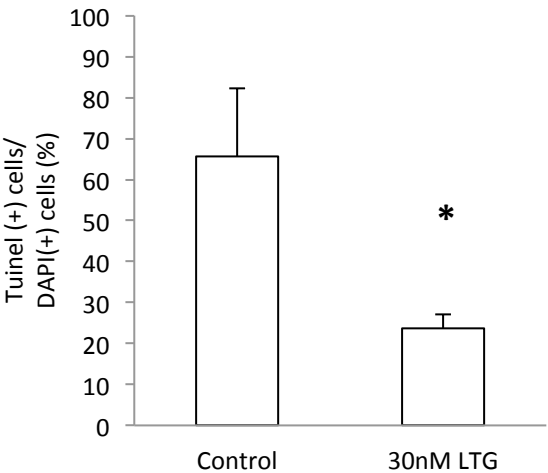
B



C



D



E

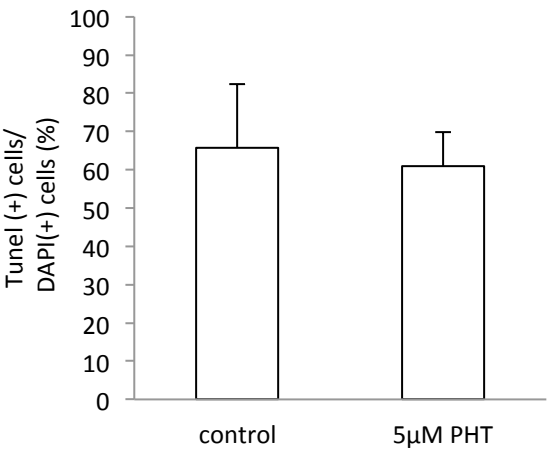
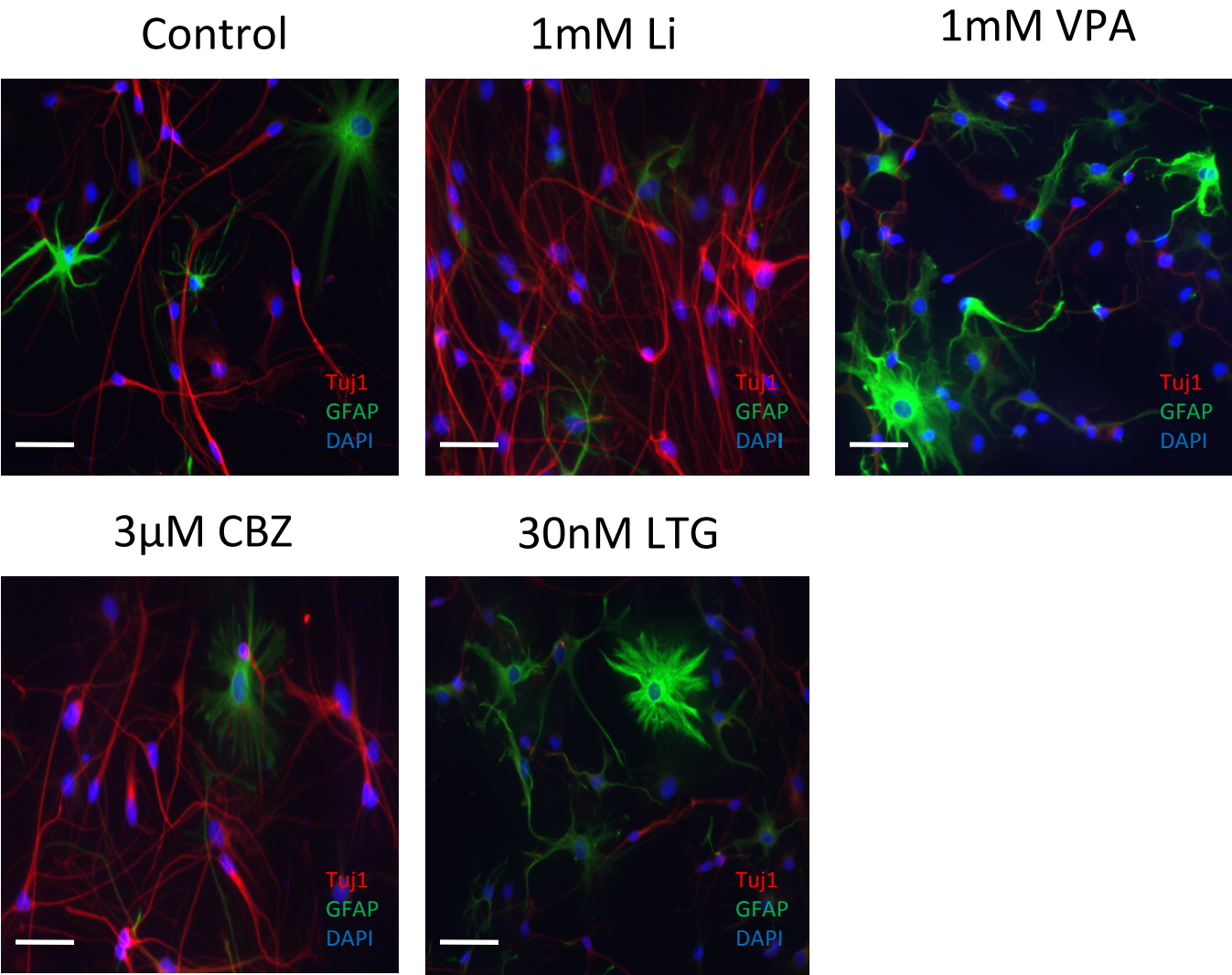
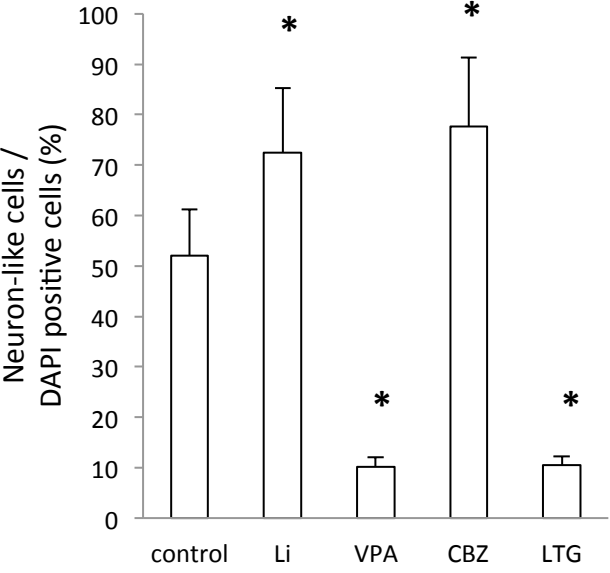


Figure 3

A



B



C

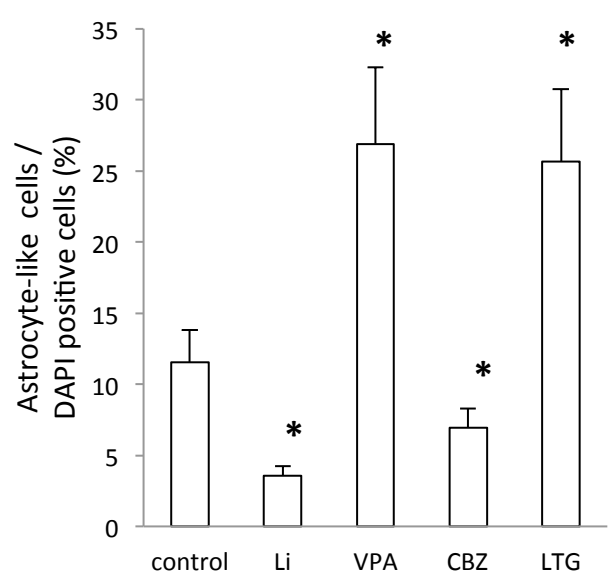


Table 1

The summary of effects of mood stabilizers on ADPs

	Lithium	Valproate	Carbamazepine	Lamotrigine
DEX-decreased Proliferation	recover	recover	no effect	no effect
STS-induced Apoptosis	recover	recover	recover	recover
RA-induced Differentiation	neuron  astrocyte 	neuron  astrocyte 	neuron  astrocyte 	neuron  astrocyte 