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Review

Targeting astrocytes in bipolar disorder

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Abstract

Astrocytes are homeostatic cells of the central nervous system, which are critical for development and maintenance of synaptic transmission and hence of synaptically connected neuronal ensembles. Astrocytic densities are reduced in bipolar disorder, and therefore deficient astroglial function may contribute to overall disbalance in neurotransmission and to pathological evolution. Classical anti-bipolar drugs (lithium salts, valproic acid and carbamazepine) affect expression of astroglial genes and modify astroglial signalling and homeostatic cascades. Many effects of both antidepressant and anti-bipolar drugs are exerted through regulation of glutamate homeostasis and glutamatergic transmission, through K^+ buffering, through regulation of calcium-dependent phospholipase A_2 (that controls metabolism of arachidonic acid) or through Ca^{2+} homeostatic and signalling pathways. Sometimes anti-depressant and anti-bipolar drugs exert opposite effects, and some effects on gene expression in drug treated animals are opposite in neurones vs. astrocytes. Changes in the intracellular pH induced by anti-bipolar drugs affect uptake of *myo*-inositol and thereby signalling via inositoltrisphosphate ($InsP_3$), this being in accord with one of the main theories of mechanism of action for these drugs.

Key words: astrocytes; bipolar disorder; mechanism of drug action

1. Introduction: Astroglia in synaptic transmission

Synaptic connectivity lies at the very core of the functional nervous system, and impairments of synaptically connected neuronal networks underlie neuropathological evolution. Psychiatric diseases can be broadly regarded as pathological remodelling of synaptic connectivity and disbalance of neurotransmission in the brain [1-3]. This neurochemical disbalance affects not only transmission through excitatory (glutamatergic) and inhibitory (mostly GABAergic) synapses but modifies the action of other systems mediated by serotonin, dopamine or adenosine [4,5].

The human brain contains in excess of 15 trillions of chemical synapses, which come in many guises. Nonetheless the majority (60 to 70%) share the common architecture being composed of several cellular elements, and thus often being defined as a tri- or tetra- or multipartite synapses [6-10]. The cellular compartments forming the synapse are represented by (i) the presynaptic terminal, (ii) the postsynaptic neuronal part, (iii) the perisynaptic astroglial process, (iv) the microglial process that periodically contacts the synapse and (v) the extracellular matrix [8,10,11]. There is a clear distribution of functions between different components of the central synapse. In the presynaptic terminal the neurotransmitter vesicles concentrate, and specific protein machinery ascertains rapid vesicular release of these neurotransmitters following electrical excitation. The postsynaptic neuronal compartment (often associated with dendritic spines) is mainly concerned with reception of neurotransmitters (and hence bear appropriate neurotransmitter receptors) as well as with synaptic plasticity mediated by multiple enzymatic cascades that affect neurotransmitter receptors densities or control morphological postsynaptic remodelling [12,13]. Extracellular matrix present in the synaptic cleft and surrounding synaptic compartments regulates both pre- and postsynaptic elements [14]. Central synapses are regularly surveyed by microglial process that, if needed, prune synaptic contacts thus offering another level of plastic remodelling of neuronal networks [11]. Finally the multipartite synapses are closely enwrapped by astroglial perisynaptic processes, which form the "astroglial cradle" that controls all aspects of synaptic life, from synaptogenesis to synaptic maturation, maintenance and extinction [10,15,16].

Through multiple transporters densely populating perisynaptic astroglial membranes, astrocyte regulates all fundamental aspects of synaptic cleft homeostasis [10,17]. These transporters (many of which are regulated by astroglial Na^+ signalling [18]) control extracellular potassium, regulate extracellular pH, provide scavengers of reactive oxygen species and maintain the turnover and homeostasis of main neurotransmitters including glutamate, GABA and adenosine [19,20]. Most of glutamate (~ 80%), released in the course of synaptic transmission in the CNS is removed through accumulation into astrocytes; the latter being mediated by astroglia-specific plasmalemmal glutamate transporters EAAT1/SLC1A3 and EAAT2/SLC1A2 (excitatory amino acid transporters 1 and 2). These transporters are Na^+ dependent; transport of a single glutamate molecule is associated with an influx

of three Na^+ ions, one H^+ ion and efflux of one K^+ ion [21]. Part of glutamate accumulated by astrocytes is converted into glutamine by astroglia-specific enzyme glutamine synthetase, which is also fundamental for nitrogen metabolism [22,23]. Glutamine subsequently is transported back to neurones where it serves as an obligatory precursor for glutamate and GABA; this system generally known as glutamate-glutamine and GABA-glutamine shuttle. Thus astroglial compartment is critical for maintenance of glutamatergic and GABA-ergic transmissions and hence it defines the balance between excitation and inhibition in the brain; impairments of astroglial control over synaptic connectivity may therefore be fundamental for development of psychiatric diseases.

2. Astroglia in bipolar disorder

Glial cell numbers in the brain from patients of bipolar disorders are decreased [24-26]. This coincides with evidence for excessive activity of the glutamatergic system which has been well established in brains of bipolar patients by magnetic resonance imaging [25,27-29], and may be indicative of decreased glial glutamate homeostatic capacity. Administration of riluzole, a drug increasing expression and activity of glutamate transporters in astrocytes and hence increasing their glutamate clearance [30] modifies glutamine/glutamate metabolism, improves glutamatergic transmission in patients suffering from bipolar disorder [31] and ameliorates disease symptoms [32], again suggesting underlying astrocytic glutamatergic dysfunction. In mood disorders, serum levels of astroglia-specific biomarker S100 protein are increased, although it is more significant in major depression than bipolar disorder [33]. Serum levels of S100 protein are thought to reflect active secretion by astrocytes rather than their damage.

Three classical anti-bipolar drugs, lithium salts, valproic acid (VPA) and carbamazepine (CBZ) differ in their chemical structures and in acute drug effects. Lithium salt is effective in treating mania episode and bipolar depression, and in preventing relapses [34], whereas carbamazepine and valproic acids are used for treating mania episodes [35,36]. Of note, classic anti-bipolar drugs have to be administered for a couple of weeks before their therapeutic action becomes manifest. In studies of mechanisms(s) of action for anti-bipolar drugs it is therefore important to determine the effects that are not immediate but only appear after chronic administration.

3. Astroglial actions of anti-bipolar drugs

3.1. Inositol turnover

The *myo*-inositol is constantly degraded or released from cells, and a continuous resupply is needed, partly from the diet and slow transfer across the blood-brain barrier [37] and partly by *de novo* synthesis, which in the brain occurs in the

vasculature [38]. Although it is often assumed that *myo*-inositol in the brain is exclusively found in astrocytes [39,40], this may be an oversimplification. Treatment of cultured mouse astrocytes with 1 mM LiCl for 8 days inhibited uptake of 50 μ M inositol, whereas acute administration of lithium had no effect [41]. Chronic treatment of cultured rat astrocytes with any of the three anti-bipolar drugs (lithium, CBZ, and VPA) had a similar effect [42]. Suppression of the uptake was reflected by decreased inositol contents (measured as equilibrated [3 H]inositol spaces) in human astrocytoma cells [43]. The opposite effects at different *myo*-inositol concentrations, for example increase of uptake at lower *myo*-inositol concentrations but decrease at higher concentrations, might be explained on the basis of the presence of two inositol transporters in astrocytes, the high-affinity Na⁺-dependent *myo*-inositol transporter (SMIT), accounting for the most of the uptake at 25 μ M inositol, and the lower-affinity H⁺-dependent HMIT, accounting for the most of the uptake at 50 μ M inositol [44]. Since increased intracellular pH (pH_i) is known to stimulate SMIT but inhibit HMIT, the effect of anti-bipolar drugs might be explained by a gradually developing intracellular alkalinisation. This was confirmed later by experiments of [3 H]*myo*-inositol uptake in astrocytes at different *myo*-inositol concentrations and pH and by different *myo*-inositol transporters [45].

3.2. *InsP₃* signalling

Therapeutic effects of lithium in bipolar disorder are often ascribed to the depletion of *myo*-inositol following Li⁺-induced non-competitive inhibition of inositolphosphate hydrolysis (see above). The InsP₃ produced by PLC degrades to *myo*-inositol by inositol phosphatases, whereas DAG is converted to phosphatidate and condensed with cytidine triphosphate to form cytidine monophosphoryl-phosphatidate (CMP-PA), which together with *myo*-inositol re-generates PIP₂. Since Li⁺ inhibits formation of inositol from InsP₃, it decreases the regeneration of PIP₂, and thus occludes PLC pathway. The [Ca²⁺]_i response to noradrenaline in cultured astrocytes is, indeed, reduced by chronic, but not acute, exposure to Li⁺ [46]. Neither CBZ, nor VPA, however, inhibited inositolphosphate hydrolysis. This raises the question if they might reduce InsP₃ formation by other means; such as for example by reducing cellular uptake of inositol [43,44].

3.3. Cytosolic pH

Chronic treatment with any of the three antibipolar drugs increased cytosolic pH (pH_i) in astrocytes measured by a fluorometric method [47,48]. The acid-controlling transporters that exists in almost all cell types, including neurones and astrocytes [49] are the Na⁺-H⁺ exchanger (NHE) and the Na⁺/bicarbonate co-transporter NBCe1. There are seven subtypes of HNE, NHE1 - NHE7. Cultured astrocytes from rat hippocampus [50] and from mouse cerebral cortex [47] express NHE1. Chronic treatment with the therapeutically relevant Li⁺ concentration of 1 mM for 2 or 3 weeks increased pH_i by ~ 0.10, whereas 0.5 mM was ineffective, and 2 mM caused an even

larger increase [47]. The alkalinisation resulted from acute stimulation of NHE by extracellular Li^+ , which was directly demonstrated after acid load with NH_4Cl in the absence of HCO_3^- in the incubation buffer in order to exclude the contribution of Na/HCO_3^- co-transporters (NBC). The changes in pH_i occurred only in chronic treatment, this being longer at the lower Li^+ concentrations.

The NBC co-transport Na^+ with HCO_3^- and functions either as acid-loader or acid-extruder, according to the $\text{Na}^+:\text{HCO}_3^-$ stoichiometry [49]. The NBCe1 subtype with a 1:2 $\text{Na}^+:\text{HCO}_3^-$ stoichiometry is the major transporter in astroglia. The activity of NBCe1 is HCO_3^- - and Na^+ -dependent. The increased pH_i in cultured astrocytes following chronic treatment with therapeutically relevant concentrations of CBZ or VPA results from an increase in the activity of NBCe1. Carbamazepine or valproate up-regulated expression of NBCe1 mRNA and protein in astrocytes as revealed by RT-PCR and Western blotting [48,51].

3.4. *GluK2 kainate receptors*

Reduced expression of GluK2 in the brains from bipolar patients, most of whom had received medication, has been described in the entorhinal cortex [52] and in the hippocampus [53]. Chronic treatment of primary cultures of astrocytes with Li^+ , VPA or CBZ at clinically relevant concentrations down-regulated gene expression of GluK2 (Fig. 1), but had no effect on other GluK receptors [54]. These effects are not associated with drug toxicity, since oxcarbazepine (OCBZ), a keto-congener of CBZ used in bipolar disorder [55] with less side effects than CBZ [56], has similar effect as CBZ, whereas topiramate, an anticonvulsant with no anti-bipolar effect, does not affect GluK2 expression [54]. Two weeks of intraperitoneal injection of CBZ, decreased expression of GRIK2 gene (the gene encoding GluK2 protein) in the brain *in vivo*. These observations may be of special interest, since the GRIK2 resides in a genetic linkage region (6q21) associated with bipolar disorder [57].

3.5. *Phospholipase A2 (cPLA₂)*

The Ca^{2+} -dependent phospholipase A_2 (cPLA₂ IVA) mobilises arachidonic acid, which is a precursor for a multiple physiologically active molecules, including prostaglandins and inflammatory agents [58]. Expression of IVA subtype of cPLA₂ (85 kD; cPLA_{2a}) mRNA and protein is increased in bipolar patients, without significant effects of anti-bipolar medication [59]. Conversely, chronic treatment with Li^+ for 6 weeks decreases the expression of cPLA₂ in the rat brain, whereas Li^+ has no effect on two other types of PLA₂, sPLA₂ and iPLA₂ [60-62]. This down-regulation is also specific for the IVA subtype of cPLA₂. Similar results were found in rat chronically treated with CBZ [63], whereas chronic treatment with VPA does not influence cPLA₂ [64].

In contrast to the findings *in vivo*, each of the three anti-bipolar drugs induced both

up- and down-regulation of cPLA₂ gene expression in astrocytes in primary culture. Two weeks of treatment with Li⁺ at a high concentration (2 mM) caused an up-regulation of protein expression of cPLA₂ in astrocytes, whereas 1 week of treatment had no effect and treatment for 3 or 4 weeks down-regulated expression. At lower concentrations (0.5 and 0.25 mM), Li⁺ increased expression of cPLA₂ protein. Similarly, CBZ or VPA transiently increased cPLA₂ expression; this effect occurred more rapidly at higher concentrations (Fig. 2). This effect is specific for anti-bipolar drug, since topiramate was ineffective. In freshly isolated and FACS-sorted cells from mice expressing neurone or astrocyte-specific markers two weeks of treatment with CBZ up-regulated cPLA₂ in astrocytes but down-regulated it in neurones [51].

3.6. TRPC1 channels

Astrocytes are electrically non-excitabile cells, which employ transient fluctuations of intracellular concentration of cations, Ca²⁺, Na⁺ and possibly K⁺ as a substrate for their excitability [65-69]. Astroglial calcium signalling is mainly mediated by activation of inositol-1,4,5-trisphosphate receptors (InsP₃Rs) localised in the membrane of the endoplasmic reticulum with subsequent activation of store-operated Ca²⁺ entry [67,68]. In astrocytes the store operated Ca²⁺ influx provided by TRPC1/4/5 channels [67]. Chronic treatment with any of the 3 conventional anti-bipolar drugs Li⁺, carbamazepine or valproic acid decreases mRNA and protein expression of the TRPC1 (Fig. 3), which results in the inhibition of store-operated Ca²⁺ entry [70]. This is consistent with observations in platelets and lymphocytes from bipolar patients as well as with outcome of chronic treatment of lymphoblast cell lines derived from such patients with Li⁺. The store-operated Ca²⁺ entry activated following thapsigargin-induced depletion of intracellular Ca²⁺ was increased in platelets and lymphocytes from patients suffering from bipolar disorder [71], as well as in lymphoblast cell lines derived from bipolar patients [72], treatment of these cells Li⁺ treatment for 7 days reduced store-operated Ca²⁺ influx [73].

3.7. Glutamate release

Astrocytes produce and secrete numerous neuroactive agents, that include classical neurotransmitters (glutamate, ATP, or GABA). Release of neuroactive substances from astrocytes is mediated by several molecular pathways that include diffusion through plasmalemmal channels, translocation by membrane transporters and exocytotic vesicular release [74]. The latter is regulated by cytosolic Ca²⁺ signals. Chronic treatment Li⁺, carbamazepine and valproic acid decreased glutamate release from astrocytes induced by transient exposure to ATP (which activated purinoceptors) or to 45 mM K⁺ (which depolarised cell membrane to ~-30 mV). This suppression of glutamate release which may alleviate the hyperactivity of the glutamatergic transmission in the brain of patients with bipolar disorders and thus contribute the underlying mechanism of drug action [75].

3.8. *Na/K-ATPase (NKA)*

The role for NKA dysfunction in bipolar disorder was suggested almost 50 years ago [76,77]. The $\alpha 1$ subunit of NKA is expressed in both neurones and glia, the $\alpha 2$ subunit is primarily expressed in astrocytes, whereas the $\alpha 3$ subunit is expressed only in neurones [78]. A significantly lower NKA density has been found in the brain tissue from bipolar disorder patients compared to patients who suffered from major depression or schizophrenia, with no difference in α subtype expression in synaptosomal fractions [79], although there were indication for a decrease of the $\alpha 2$ gene expression [80]. Using transgenic mice carrying neurone or astrocyte-specific fluorescent markers (FVB/NTg(GFAP-GFP)14Mes/J or B6.Cg-Tg(Thy1-YFPH)2Jrs/J animals) from which neurones and astrocytes were isolated and FACS-sorted, demonstrated that $\alpha 2$ expression was up-regulated in astrocytes and neurones, the $\alpha 1$ was selectively increased in neurones, whereas $\alpha 3$ remained unchanged after administration of CBZ by intraperitoneal injection for 2 weeks [81]. This suggests, again, altered K^+ homeostasis in mood disorders.

3.9. *Elongation protein zeta 1 (FEZ1)*

The FEZ1 is a mammalian ortholog of UNC-76, a protein initially found in the nematode *Caenorhabditis elegans*. The UNC-76 is associated with neuronal development and could contribute to various neuropathologies [82]. In mammals, the FEZ1 is expressed exclusively in the brain, in neurones and astroglia, and is found in the cytoplasm as well as in the nucleus. The FEZ1 interacts with various proteins related to cytoskeleton network, transport of cargoes, regulation of the cell cycle, and retrovirus propagation. In the neuropsychiatric context the FEZ1 is known as an interacting partner of the schizophrenia-related protein disrupted in schizophrenia 1 (DISC1), and FEZ1-deficient mice presented abnormal behaviours [82]. In human astrocyte-derived cell line U-87 MG, four anti-bipolar drugs, lithium, valproic acid, carbamazepine, and lamotrigine up-regulated FEZ1 mRNA and protein expression [83].

3.10. *Other receptors, enzymes and proteins*

An increase in expression of adenosine A_1 receptor was suggested to have potential neuroprotective effect in the mechanisms of the antidepressive effects of chronic treatment with carbamazepine [84]. In primary astrocyte cultures from different regions of the rat brain, CBZ treatment for 10 days had no effect on adenosine A_1 -receptor mRNA expression in cultures with high basal A_1 -receptor mRNA levels, but increased A_1 -receptor mRNA in cultures exhibiting low basal A_1 -receptor mRNA levels [85].

The glycogen synthase kinase 3 (GSK-3) is an enzyme that was first discovered to deactivate glycogen synthase, which converts glucose to glycogen; subsequently it

was found that GSK-3 mediates multiple signalling pathways, including AKT and Wnt signalling [86]. It is well established that lithium is an inhibitor of GSK-3 [86]. In astrocytes, lithium inhibits phosphoglucomutase (PGM) thus decreasing glycogen synthesis [87] and reduced the lipopolysaccharide (LPS)-induced secretion of pro-inflammatory cytokines [88]. Chronic treatment with lithium also increased expression of B cell CLL/lymphoma 2 protein, an anti-apoptotic factor bcl-2, vascular endothelial growth factor (VEGF), brain-derived growth factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) in astrocytes *in vitro* [89-91]. In the *in vivo* brain, lithium treatment significantly increased GFAP expression in rat hippocampus [92].

Treatment with VPA up-regulated the mRNA expression of two post-synaptic cell adhesion molecules (neuroligin-1 and neuregulin-1) and two extracellular matrices (neuronal pentraxin-1 and thrombospondin-3) in primary rat astrocyte cultures in a time- and concentration-dependent manner [93], suggesting that VPA might affect the synaptic excitatory/inhibitory balance through its effect on astrocytes. In cultured astrocytes VPA (but not carbamazepine or lamotrigine) evoked the strongest global chromatin modifications, including hyperacetylation of histone H3/H4, 2MeH3K9 and DNA demethylation that were reflected at the glutamate transporter GLT-1 promoter and enhanced transcription of this astroglia-specific gene [94]. In addition, VPA (but not lithium or carbamazepine) inhibits substance P-induced activation of protein kinase C epsilon and expression of the substance P receptor in astrocytes; of note the substance P was suggested to contribute to the etiopathology of affective disorders [95].

4. EXPERT COMMENTARY

Astrocytes, due to their unique position as central homeostatic elements in the brain, are fundamental for proper function of synaptically connected neuronal networks. Decrease in astroglial synaptic coverage due to the reduced astroglial density, which is observed in bipolar disorder, may be a critical factor underlying deregulation of neurotransmission responsible for this type of psychiatric pathology. The mechanism of action of anti-bipolar drugs was always associated with neurones. Nonetheless, all three major anti-bipolar drugs, lithium salts, valproic acid and carbamazepine affect astroglial receptors, signalling cascades and homeostatic function. In particular the anti-bipolar drugs affect glutamate and potassium homeostasis associated with astrocytes. It remains unclear how these astro-centric mechanisms contribute to overall therapeutic action, although further investigations of astroglial cells as targets in the context of bipolar disease may lead to a development of new drugs and therapeutic strategies.

5. FIVE YEAR VIEW

The pathological potential of astrocytes, and specifically the role of astroglial cells in

neuropsychiatric disorders, received attention of scientific community only very recently [25,26,96,97]. Experiments performed mostly in astrocytes *in vitro*, in primary cultures, have demonstrated that classic anti-bipolar drugs regulate expression of multiple genes in astrocytes and affect astroglia-associated homeostatic systems. Identification of astroglial systems linked to bipolar disorders and recognition of molecular targets of anti-bipolar agents represents the next fundamental step towards positioning astrocytes within the context of neuropsychiatric pathology. Of particular interest are the astroglial effects of lithium salts. Another important development is the translation of the data obtained in laboratory animals towards human pathology, which require introduction of new disease models both *in vitro* and *in vivo*. These fundamental studies open new horizons in development of astroglia-specific therapies.

6. KEY ISSUES

Astrocytes are central elements of the synaptic transmission and neurochemical balance in the brain

In bipolar disorders astrocytes show signs of atrophy and their number decreases which may affect brain homeostasis and underlie deregulation of neurotransmission.

Classical anti-bipolar drugs affect astroglial receptors, signalling cascades and homeostatic functions.

Specific targeting of astroglia may represent a novel strategy in developing anti-bipolar therapies.

DECLARATION OF INTEREST

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Reference annotations

* Of interest

** Of considerable interest

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Figure legend

Figure 1. Effect of chronic treatment with CBZ on the expression of astroglial GluK2 receptors.

A: A representative experiment showing Western blots for GluK2 and for β -actin (a house-keeping protein), in control cultures and cultures treated with CBZ for 1 day, 3 days, 1 week, 2 weeks, 3 weeks, and 4 weeks.

B: Average (means $\pm$ SEM, three samples from three independent experiments) changes in expression ratio GluK2 and β -actin for control cultures, and cultures treated with 25 or 50 μ M CBZ; * $P < 0.05$ vs. control cultures from the same batch and treatment period.

Protein expression was measured by immunoblotting of GluK2 in primary cultures of mouse astrocytes treated for up to 4 weeks with 25 or 50 μ M CBZ. Primary cultures of astrocytes were prepared from the neopallia of the cerebral hemispheres of newborn CD-1 mice, and grown in Dulbecco's Minimum Essential Medium (DMEM). After 2 weeks *in vitro*, 0.25 mM dibutyryl cyclic AMP (dBcAMP) was included in the medium, which led to a morphological and functional differentiation. These cultures are highly enriched in astrocytes (>95% purity of glial fibrillary protein- and glutamine synthetase-expressing astrocytes). After 3 weeks *in vitro*, cells exposed to 0 (Control), 25 or 50 μ M CBZ; this treatment continued for another 1 and 3 days, 1, 2, 3 or 4 weeks. Specific binding was detected by horseradish peroxidase-conjugated secondary antibody.

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Fig.1

Figure 2. Effect of chronic treatment with CBZ on the expression of astroglial GluK2 phospholipase A_{2a}.

A: A representative experiment showing Western blots for cPLA_{2a} and for β -actin (a house-keeping protein), in control cultures and cultures treated with CBZ for 1, 2, 3, or 4 weeks.

B: Average (means $\pm$ SEM, four samples from four independent experiments) changes in expression ratio cPLA_{2a} and β -actin for control cultures, and cultures treated with 25 or 50 μ M CBZ; * $P < 0.05$ vs. control cultures from the same batch and treatment period.

Protein expression was measured by immunoblotting of cPLA_{2a} in primary cultures of mouse astrocytes treated for 1 to 4 week with 25 or 50 μ M CBZ. Primary cultures of astrocytes were prepared as described in the legend to Fig. 1. After 3 weeks in vitro, cells were exposed to 0 (Control), 25 or 50 μ M CBZ; this treatment continued for another 1, 2, 3, or 4 weeks. Specific binding was detected by horseradish peroxidase-conjugated secondary antibody.

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Fig.2

Figure. 3. Effect of chronic treatment with antibipolar drugs on the expression of astroglial TRPC1 channels.

A: A representative experiment showing Western blots for TRPC1 and for β -actin (a house-keeping protein), in control cultures and cultures treated with Li^+ , CBZ- or VPA.

B: Average (means $\pm$ SEM, three samples from three independent experiments) changes in expression ratio TRPC1 and β -actin for control cultures, and cultures treated with Li^+ , CBZ- or VPA; * $P < 0.05$ vs. control cultures from the same batch and treatment period.

Protein expression was measured by immunoblotting of TRPC1 in primary cultures of mouse astrocytes treated for 2 weeks with 1 mM Li^+ , 50 μM CBZ or 1 mM VPA.

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Fig.3