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Neuronal Peroxisome Proliferator-Activated Receptor γ Signaling: Regulation by Mood-Stabilizer Valproate

Martin J. Lan, Peixiong Yuan, Guang Chen, and Hussein K. Manji

Laboratory of Molecular Pathophysiology, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA

Abstract

Valproate (Depakote) remains an effective medication for the prevention and treatment of seizures in epilepsy and of mood symptoms in bipolar disorder. Both of these disorders are severe and debilitating, and both warrant further medication options as well as a better understanding of the side effects associated with their current treatments. Although a number of molecular and cellular processes have been found to be altered by valproate, the medication's therapeutic mechanism has not been fully elucidated. In this paper, peroxisome proliferator-activated receptor (PPAR) signaling was examined to determine valproate's effects on this transcriptional regulatory system in neuronal tissue. PPAR signaling has been found to affect a number of biochemical processes, including lipid metabolism, cellular differentiation, insulin sensitivity, and cell survival. When primary neuronal cultures were treated with valproate, a significant decrease in PPAR γ signaling was observed. This effect was demonstrated through a change in nuclear quantities of PPAR γ receptor and decreased DNA binding of the receptor. Valproate also caused gene expression changes and a change to the peroxisome biochemistry consistent with a decrease of PPAR γ signaling. These biochemical changes may have functional consequences for either valproate's therapeutic mechanism or for its neurological side effects and merit further investigation.

Keywords

Valproate; Bipolar disorder; Epilepsy; PPAR gamma; Metabolism

Introduction

Valproate (Depakote) is the salt of a short, branched chain fatty acid. It is widely used as a medication for the prevention and treatment of seizures in epilepsy and for mood symptoms in bipolar disorder (Belmaker 2004; Browne and Holmes 2001). Bipolar disorder is thought to affect 1% of the adult population, and epilepsy is thought to affect 0.6% of the population (Hauser 1990; Weissman et al. 1996). Both of these common disorders are debilitating and have a major public health impact. Seizures, for instance, can be life threatening and are a source of significant disruption in patients' lives (Browne and Holmes 2001). The mood episodes of depression and mania that comprise bipolar disorder impair patients' interpersonal and professional functioning, and are associated with markedly increased suicide risk (Belmaker 2004). Both disorders consequently hold significant financial costs to society (Begley et al. 2001; Begley and Beghi 2002). Several other medications besides valproate can be used to treat bipolar disorder, and several other anti-seizure medications exist. None of these

medications are universally effective, however, and all of them are associated with significant side effects. A wider selection of treatment options for both disorders, as well as a better understanding of the medications' side effects, are therefore greatly needed.

A number of molecular mechanisms have been suggested to explain valproate's mechanism of action (reviewed below), but its mechanism has not been fully elucidated. It is unknown if valproate's anti-seizure and anti-manic properties derive from the same phenomenon. Carbamazepine and lamotrigine, both of which are anti-epileptic medications, have also proven efficacious in the treatment of bipolar disorder, indicating a common therapeutic mechanism for the two disorders. Valproate, however, generally takes longer to affect behavior than to reduce epileptic activity, indicating that other processes may be involved in its efficacy in bipolar disorder (Phrolov et al. 2004).

Valproate has been found to affect a number of molecular and cellular phenomena, but none definitively explain its mechanism of action. It has been shown to alter gamma-aminobutyric acid (GABA) and glutamate neurotransmission (Perucca 2002), glucose metabolism (Gaillard et al. 1996; Johannessen et al. 2001), lipid metabolism (Bosetti et al. 2005), neurogenesis (Hao et al. 2004; Laeng et al. 2004), and arachidonic acid signaling (Bosetti et al. 2003) in the cell. It has also been shown to have neuroprotective effects, altering neurotrophic cascades (Einat et al. 2003) and anti-apoptotic bcl-2-related molecules (Chen et al. 1999b; Zhou et al. 2005). Because a clear pattern of neurotransmission has not been determined with valproate and because valproate affects such fundamental cellular properties as metabolism, basic cellular signaling cascades are currently being investigated to understand its effects on the brain. For example, valproate has been found to be a histone deacetylase (HDAC) inhibitor (Phiel et al. 2001). Histone deacetylases control the transcription of a large number of genes by altering chromosome packaging, and this inhibition may prove to be important in valproate's mechanism. Peroxisome proliferator-activated receptors (PPARs) are also particularly interesting in this regard because their activity alters a number of basic cellular functions.

Previous studies have suggested that valproate may alter PPAR signaling in non-neuronal tissue (discussed below). PPARs are members of the nuclear hormone receptor superfamily. Upon activation, these receptors bind to their DNA response elements and change the transcriptional level of a number of genes. As its name suggests, the PPAR signaling system specifically regulates both the molecular content and the number of peroxisomes in the cell (Latruffe et al. 2000; Wanders and Waterham 2006). It has also been shown to have a wide variety of cellular effects, including the regulation of insulin sensitivity, lipid metabolism, cellular differentiation, and cell survival (Feige et al. 2006). Three isoforms of PPARs have been characterized: α , β/δ , and γ . Each isoform has its own transcriptional profile and its own tissue distribution, and all three have been found to be present in both the brain and primary neuron cell culture (Cimini et al. 2005; Cristiano et al. 2001; Cullingford et al. 1998; Kainu et al. 1994; Moreno et al. 2004).

The function of PPAR signaling in the brain has not been fully characterized and is under active investigation. PPAR agonists are known to alter cellular insulin sensitivity and lipid metabolism. These processes are also likely to be regulated by PPAR γ signaling within brain tissue. PPAR agonists have been found to alter midbrain neuronal differentiation (Park et al. 2004) as well as neural stem cell migration and differentiation (Cimini et al. 2005; Wada et al. 2006), suggesting that they may be involved with neurogenesis in the same way that they affect development in other tissues. The behavioral effects of PPAR signaling in the brain remain largely unknown. One recent study reported that a PPAR γ agonist had behavioral effects on amphetamine sensitization, an animal model of mania (Maeda et al. 2006). PPAR signaling in the brain may therefore prove to have behavioral consequences. PPAR signaling may also affect neuroprotection. Several PPAR agonists have been shown to be neuroprotective (Chen

et al. 2006; Luo et al. 2006; Ou et al. 2006; Shimazu et al. 2005; Sundararajan et al. 2005; Victor et al. 2006; Zhao et al. 2006b), although PPAR signaling was not proven to be responsible for these effects.

As mentioned previously, valproate may alter PPAR signaling in non-neuronal tissue. In the liver and kidney, valproate treatment has been found to increase the activity of peroxisomal enzymes (Horie and Suga 1985; Van den Branden and Roels 1985) and alter the ultrastructure of the peroxisomes (Ponchaut et al. 1991), indicating possible stimulation of PPAR signaling in those tissues. Experiments using reporter expression plasmids in several cell lines have also indicated that valproate may activate PPAR signaling (Lampen et al. 2001; Lampen et al. 2005; Werling et al. 2001). Moreover, an interaction of valproate with the PPAR system is also intuitive biochemically. Valproate is itself a branched chain fatty acid, and PPAR activity alters in response to the lipid environment of the cell. For these reasons, many PPAR agonists—such as poly-unsaturated fatty acids (PUFAs) or eicosanoids—are themselves lipids (Forman et al. 1997).

The effects of valproate on PPAR signaling have never been characterized in neuronal tissue. We conducted several experiments to investigate this biochemical interaction.

Materials and Methods

Reagents

Valproic acid and troglitazone were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Acyl coA oxidase (ACOX) antibody was a generous gift from Alfred Volkl (Beier et al. 1988). Antibodies to glyceraldehyde-3-phosphate dehydrogenase, catalase, and PPAR γ were purchased from Abcam (Cambridge, MA, USA); actin antibody was purchased from Chemicon (Temecula, CA, USA); and PMP-70 antibody was purchased from Invitrogen (Carlsbad, CA, USA). All cell culture solutions were purchased from Invitrogen. Scriptaid, a well-characterized HDAC inhibitor, was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Primary Neuron Culture

Cortical neurons were prepared from dissected cortex from embryonic day 18 (E18) rat embryos and cultured in a humidified atmosphere of 95% air–5% CO₂ at 37°C as previously described (Hao et al. 2004). Cells were incubated overnight in Dulbecco's modified Eagle's medium + 10% FBS + antibiotics, and the medium was changed to neurobasal medium plus B27 supplement with antibiotics for 10 days (changing medium at three-day intervals). At the end of the tenth day, the medium was changed to neurobasal without B27 supplement. This withdrawal eliminates the confounding effects of B27 supplement on valproate signaling. The next morning, drug treatment was administered for times and doses indicated for each experiment. This protocol has been used routinely in our laboratory to study the mechanism of mood stabilizer medications (Chen et al. 1999b; Zhou et al. 2005). Any cells with nuclear blebbing or inclusion bodies, indicating cell damage or death, were discarded and not used in the analysis.

Animal and Drug Treatments

All experiments were approved by the National Institutes of Health guidelines on the care and use of animals. Male Wistar Kyoto rats (150–250 g) were housed three to four per cage with access to food and water ad libitum and were maintained under a 12 h light/dark cycle. After a 1-week acclimatization period, animals were fed either regular chow ($n = 8$), lithium carbonate chow (2.4 g/kg; $n = 8$), or valproate chow (20 g/kg; $n = 8$) for 28 days. These doses have previously been shown to produce serum levels of the medications similar to the human therapeutic range (Chen et al. 1999b). In addition to tap water, all animals had a bottle of saline

available to minimize electrochemical imbalances from any diuretic properties of the medications. At the end of the treatment, rats were euthanized by decapitation between 9:00 A.M. and 11:59 A.M., and trunk blood was collected to determine drug serum concentration. Aliquots of the serum were sent to commercial toxicological laboratory (MedTox, St. Paul, MN, USA) for analysis of drug concentrations. This service measures serum levels of lithium with atomic absorption and inductively coupled plasma/mass spectrometry and valproate levels using an immunoassay. Serum analysis yielded lithium levels of 0.66 ± 0.14 mM and valproate levels of 49.18 ± 27.4 μ g/ml (mean $\pm$ standard deviation), which are within the human therapeutic range. Brain tissue was removed, and the cortex, hippocampus, and striatum samples were dissected on ice and immediately frozen in dry ice and stored at -80°C .

Immunoblot Analysis

Cell culture samples were washed twice with ice-cold phosphate buffer saline (PBS), and lysis buffer was added to each well. Samples were scraped into solution and sonicated. Brain tissue was homogenized into lysis buffer (100 mM potassium phosphate, pH 7.0 with 1 mM ethylenediaminetetraacetic acid) by passing tissue through a syringe with an 18-gauge needle. Samples were centrifuged at $14,000 \times g$ for 10 min, and supernatants were further analyzed. Protein concentrations of both cell culture and brain lysates were determined using Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). Equal quantities of samples were loaded into 4–20% polyacrylamide gels and transferred to nitrocellulose membranes. Western blot protocol was conducted as previously described (Chen et al. 1999b). The antibodies were diluted according to the manufacturer's recommendations, and the primary–secondary immunocomplex was subsequently detected with an enhanced chemiluminescence reaction (Amersham ECL Plus Western Blotting Detection System). Densitometric scanning of exposed film was conducted using Kodak Image Analysis (Eastman Kodak, Rochester, NY, USA).

Nuclear PPAR Assays

Cells were prepared as above. Nuclear and cytosolic extracts were isolated using Active Motif nuclear extract kit. Protein concentrations of the extracts were determined using the Bio-Rad method, and equal amounts of nuclear protein were analyzed using an enzyme-linked immunosorbent assay (ELISA) kit that assesses isoform-specific PPAR DNA binding to the PPAR response element (PPRE; Panomics, Fremont, CA, USA). Nuclear and cytosolic extracts were also run on polyacrylamide gels (4–20%) and transferred to nitrocellulose membranes. Immunoblotting was then performed as described above.

Complementary DNA Synthesis

Cells were prepared as above, washed twice in ice-cold PBS, and Trizol was added to dishes. After incubation for 5 min at room temperature, cells were scraped into solution. RNA was extracted from the reagent using chloroform as per Trizol protocol (Invitrogen). RNA was resuspended in diethylpyrocarbonate-treated water and RNA concentrations were determined using Nanodrop ND-100 spectrophotometer. One microgram of RNA was used for reverse transcription using Superscript III First-Strand Synthesis System as per company protocol (Invitrogen).

qPCR Analysis

Taqman assays were ordered that used primer and probe sequences that spanned the exon–intron boundary for each gene. This design ensured that the data obtained were unlikely to be due to genomic DNA contamination. Taqman assays were performed as per Applied Biosystems (Foster City, CA, USA) protocol. The reactions were run on Applied Biosystems 7900. All data were normalized to actin house-keeping gene signal and run in triplicate. The probes used were ACTB (beta-actin) endogenous control (assay ID Rn00560930_m1), acyl-

coenzyme A oxidase (Acox1; assay ID Rn01645311_g1), catalase (assay ID Rn01512559_m1), and PPAR γ (assay ID Rn01492270_m1). For each probe on each plate, a standard curve was generated using a pooled complementary DNA sample, and the standard curve was used to convert Ct values to real expression data.

Statistical Analyses

Data are presented as means $\pm$ standard errors of means. When more than two variables were tested, ANOVA was followed by a Tukey's post hoc test. When two groups were compared, a two-tailed Student's *t* test was used. Differences were considered statistically significant at $p < 0.05$.

Results

PPAR Activity Assay

An ELISA assay was used to test whether valproate treatment affected PPAR activity in primary neuronal cultures. In this assay, nuclear extracts of the treated cells were prepared, and binding of the α , β/δ , and γ PPAR isoforms within the extracts to the PPAR DNA response element sequence was measured. This binding reflected the activity of the receptors in the cells. PPAR γ showed significantly decreased binding to its DNA response element after 48 h of treatment with valproate (Fig. 1a). The PPAR α assay, however, showed no detectable signal, and the PPAR β/δ system showed no change with valproate treatment (Fig. 1b).

PPAR γ Nuclear Quantification

To further examine this effect on PPAR γ , the protein levels of PPAR γ in the nuclear extracts were measured. After 48 h of valproate treatment (1.0 mM), a significant downregulation of PPAR γ in the nucleus was noted as determined by Western blot (Fig. 1c and d). This effect was not found after treatment with scriptaid, a potent HDAC inhibitor. The levels of PPAR γ in the cytosol were undetectable by Western blot (data not shown). Western blot analysis of the nuclear extracts also showed minimal GAPDH signal in the nuclear extracts when compared to the cytosol fraction, indicating significant enrichment of the nuclear fraction (data not shown).

Gene Expression Data

The messenger RNA (mRNA) levels of two genes known to be regulated by the PPAR system—ACOX and catalase (Kane et al. 2006; Sohlenius et al. 1995; Zhao et al. 2006a)—were determined with and without valproate treatment. Both were found to be significantly downregulated in the cells with valproate (Fig. 2a and b). The transcriptional levels of PPAR γ mRNA, however, were found to be unchanged with valproate treatment.

ACOX and Catalase Levels in Primary Neuronal Cultures

Western blot analysis was applied to the lysates of primary neuronal cultures after treatment with valproate. After valproate treatment for 48 h, ACOX and catalase levels were found to be decreased in a dose-dependent manner in the cells, indicating an alteration to the biochemistry in the peroxisome. However, the peroxisome membrane protein PMP-70 did not change, indicating a peroxisomal matrix-specific alteration. These decreases were consistent with the transcriptional changes described above (Fig. 3a–c).

ACOX and Catalase Levels in Animal Brain Tissue

To test whether the biochemical effects of valproate on the peroxisome occur in intact animal brain tissue, rats were treated with valproate and lithium for 4 weeks in regimens similar to those in human patients. In animals chronically treated with valproate, frontal cortex brain

lysates showed a downregulation of both catalase and ACOX protein levels (Fig. 4a–c). Lithium, however, did not alter the level of either enzyme in the frontal cortex.

Discussion

The data presented in this study suggest that valproate decreases PPAR γ signaling in primary neuronal cultures. Valproate decreased both the DNA binding of the PPAR γ and the amount of the receptor within the nuclei of cultured neurons, indicating decreased signaling after valproate treatment. Valproate also elicited the decreased expression of two genes that are known to be regulated by PPAR signaling: ACOX and catalase (Kane et al. 2006; Sohlenius et al. 1995; Zhao et al. 2006a). ACOX and catalase are both located primarily in the matrix of the peroxisome, and they have both been found to contain PPREs in their gene promoters (Girnun et al. 2002; Varanasi et al. 1996). The levels of the proteins encoded by these genes were also significantly diminished after valproate treatment. These protein changes indicate a shift in the peroxisomal biochemistry, as would be expected with a change in PPAR signaling. The change in ACOX and catalase levels was also noted in rat frontal cortex after chronic animal treatment with valproate and did not occur with lithium treatment.

As noted in the introduction, PPAR γ signaling has been found to affect a broad range of biochemical functions in the cell. The effects on neuronal tissue and the brain, however, have yet to be fully characterized. For this reason, the functional correlates of the change in PPAR γ signaling described in this paper are not straightforward, and further research into the PPAR γ system will likely better elucidate its consequence for valproate's mechanism. The alteration may prove to be involved with either valproate's therapeutic mechanism or its toxicity profile and may ultimately prove useful to our basic understanding of PPAR signaling in neuronal tissue. Below, we discuss the potential functional consequences of this alteration based on current knowledge of PPAR γ signaling.

Lipid Metabolism

PPAR signaling is known to affect lipid metabolism within the cell (Keller et al. 1993). ACOX, the gene expression found altered here, is one of the many lipid-metabolizing genes regulated by PPARs. ACOX metabolizes long-chain and very-long-chain fatty acids in the peroxisome; in the process, it releases hydrogen peroxide (H₂O₂) that in turn is broken down by catalase. A number of other lipid metabolic enzymes, including mitochondrial lipid metabolizing enzymes, lipid synthesizing enzymes, fatty acid transport enzymes, and enzymes that release fatty acids from transport molecules are also known to be controlled by PPAR signaling (Fredenrich and Grimaldi 2005; Mandard et al. 2004; Martin et al. 1997). Fibrates are PPAR γ agonists that are used to treat dyslipidemia due to this property. Fatty acids are known to regulate PPAR signaling, and PPARs act as a response element to the fatty acid milieu in the cell, regulating fatty acid metabolism in response to the levels of fats in the cell.

Studies suggest that valproate may affect lipid metabolism within the brain. In a recent gene expression micro-array study, a number of lipid metabolism genes were identified as differentially expressed with valproate treatment in the brain tissue (Bosetti et al. 2005). Chronic valproate was also found to alter the levels of the precursors to lipid biosynthesis in the brain—phosphocholine, phosphoethanolamine, and sterols—and it has been shown to affect the composition of the cellular membrane (Bazinet et al. 2005; Roberti et al. 1989). Valproate has also been found to alter lipid-based signaling processes in the brain, including arachidonic acid breakdown and signaling (Bosetti et al. 2003; Chang et al. 2001; Szupera et al. 2000). Notably, the effects of valproate on lipid metabolism and signaling described above may prove to be significantly regulated by the alteration of the PPAR system in neuronal tissue noted in this study.

Development/Differentiation

The PPAR system has been shown to affect development and differentiation in a number of cell types, although the effects in the brain are less well understood. PPAR agonists have been found to alter both midbrain neuronal differentiation (Park et al. 2004) and neural stem cell migration and differentiation (Cimini et al. 2005; Wada et al. 2006). Valproate has also been reported to affect cell differentiation in the brain (Hsieh et al. 2004; Laeng et al. 2004). The molecular change in the PPAR γ system reported in this paper may therefore help to explain its effects on brain differentiation.

Other Biochemical Correlates

PPAR γ agonists have been found to significantly regulate insulin sensitivity. Thiazolidinediones, which are PPAR γ agonists, are thought to be an effective treatment for diabetes type 2 due to this property (Patel et al. 2006; Rosenson 2007). It is therefore possible that valproate's effects on the PPAR γ system in neurons noted in this study reflect its effect on the brain's insulin sensitivity and, therefore, its carbohydrate metabolism.

A number of drugs that are PPAR signaling system agonists also affect cell survival in the brain in vivo and in neurons in vitro. The consequences of the decreased PPAR γ activity shown in this study for cell survival may therefore prove to be important either to valproate's toxicity or its neuroprotective abilities.

Mechanism of PPAR γ Alteration

The mechanism by which valproate causes the downregulation of PPAR γ in primary neurons was not determined by these studies. Nevertheless, the data presented here do suggest that this mechanism is not a downstream effect of HDAC inhibition because scriptaid, a potent HDAC inhibitor, did not downregulate PPAR γ nuclear quantitation. Valproate has been found previously to inhibit HDAC, likely affecting the transcription of a large number of genes (Phiel et al. 2001). It has also previously been found to inhibit *N*-methyl-D-aspartate-evoked transient depolarizations, increasing GABA turn-over and potentiating GABAergic function (Gobbi and Janiri 2006; Loscher 1999), as well as attenuate glycogen synthase kinase-3 (GSK-3) activity (Chen et al. 1999a; Gould et al. 2006). The effect on PPAR γ may prove to be downstream from changes in GABA signaling, GSK-3 activity, or neurotrophic signaling.

The activity of PPAR γ is regulated by a number of corepressors, and it is possible that these cofactors play a role in the downregulation of PPAR γ with valproate treatment shown here. Because valproate is itself a branched chain fatty acid, it also may directly interact with the PPAR γ receptor, inducing this decrease in activity.

Although the data presented in this paper demonstrate that valproate downregulates PPAR γ signaling in baseline neuronal tissue, it is possible that valproate may affect the PPAR γ system differently when the brain tissue is under duress, for instance, while experiencing seizures or in a manic or depressive state. Thus, future studies may be undertaken to determine PPAR γ signaling in the brain with and without valproate treatment under such conditions.

In summary, the findings of this study contribute to our basic understanding of PPAR γ signaling, and they also suggest a possible avenue to explain valproate's therapeutic profile. Elucidating the mechanism whereby this medication exerts its therapeutic effects has obvious benefits for treating the millions of individuals devastated by both epilepsy and bipolar disorder. Further studies are clearly warranted.

Abbreviations

ACOX	acyl-coA oxidase
bcl-2	B-cell CLL/lymphoma 2
BAG-1	B-cell CLL/lymphoma 2 associated athanogene 1
ERK	extracellular signal-regulated kinase
FeCl ₃	iron chloride
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GSK-3	glycogen synthase kinase-3
H ₂ O ₂	hydrogen peroxide
HDAC	histone deacetylase
PBS	phosphate buffer saline
PMP-70	peroxisome membrane protein-70
PPAR	peroxisome proliferators-activated receptor
PPRE	PPAR-response element
qPCR	quantitative PCR
SDS	sodium dodecal sulfate

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Figure 1.

Nuclear extracts were prepared from in vitro-cultured cortical primary neurons. ELISA assays were performed on the activity of PPAR receptor isoforms. After valproate treatment, DNA binding was decreased in PPAR γ (a), PPAR β/δ showed no change (b), PPAR α displayed no detectable signal. Western blots were also run on the nuclear extracts. Valproate treatment significantly decreased the amount of PPAR γ receptor in the nucleus, but this decrease was not caused by troglitazone—a known PPAR γ agonist—or by scriptaid, a known HDAC inhibitor (c). A representative Western blot is shown in (d). Data are mean $\pm$ SE of five (a, b) and seven (c) independent experiments, expressed as percentage change with respect to the control. Statistical differences were assessed using ANOVA with Tukey's post hoc test. * $p < 0.05$, signifies statistical significance

Figure 2.

RNA was isolated from in vitro-cultured cortical primary neurons after valproate treatment. Valproate treatment caused a significant downregulation of catalase mRNA (**a**) and ACOX mRNA (**b**). mRNA levels of PPAR γ receptor did not change with valproate treatment (**c**). Each assay was normalized to the transcriptional levels of actin. Data are mean $\pm$ SE of six independent experiments, expressed as percentage change with respect to the control (non-valproate-treated cells). Statistical differences were assessed using two-tailed Student's *t* test when compared to the control samples. **p* < 0.05, signifies statistical significance

Figure 3.

Cortical primary neurons were cultured in vitro and treated with valproate for 48 h. The treatment caused a dose-dependent downregulation of catalase (a) and ACOX (b). Representative Western blots of these changes are shown in (c). Actin was used as a loading control. PMP-70 is a peroxisomal membrane protein that was not altered with valproate treatment. Data are mean $\pm$ SE of seven independent experiments, expressed as percentage change with respect to the control. Statistical differences were assessed using two-tailed Student's *t* test when compared to the control samples. **p* < 0.05, signifies statistical significance

Figure 4.

Ex vivo rat cortex tissue was evaluated after chronic treatment with lithium and valproate. Western blot analysis revealed that valproate-treated tissue had decreased protein levels of catalase (**a**) and ACOX (**b**). Representative Western blots of ACOX and catalase levels after mood stabilizer treatment are shown in (**c**). GAPDH was used as a loading control. Data are mean $\pm$ SE of eight independent experiments, expressed as percentage change with respect to the control. Statistical differences were assessed using ANOVA with Tukey's post hoc test. * $p < 0.05$, signifies statistical significance