Progress in Neuro-Psychopharmacology & Biological Psychiatry 37 (2012) 111–120

Contents lists available at SciVerse ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Valproic acid mediates the synaptic excitatory/inhibitory balance through astrocytes — A preliminary study

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ARTICLE INFO

Article history:
Received 12 October 2011
Received in revised form 31 January 2012
Accepted 31 January 2012
Available online 7 February 2012

Keywords:
Astrocyte
Cell adhesion molecules
Extracellular matrix
Histone deacetylase inhibitor
Synaptic excitatory/inhibitory balance
Valproic acid

ABSTRACT

Valproic acid (VPA) is one of the most widely used anticonvulsant and mood-stabilizing agents for the treatment of epilepsy and bipolar disorder. However, the underlying therapeutic mechanisms of the treatment of each disease remain unclear. Recently, the anti-epileptic effect of VPA has been found to lead to modulation of the synaptic excitatory/inhibitory balance. In addition, the therapeutic action of VPA has been linked to its effect on astrocytes by regulating gene expression at the molecular level, perhaps through an epigenetic mechanism as a histone deacetylase (HDAC) inhibitor. To provide insight into the mechanisms underlying the actions of VPA, this study investigated whether the synaptic excitatory/inhibitory (E/I) balance could be mediated by VPA through astrocytes. First, using the primary rat neuronal, astroglial, and neuro-glial mixed culture systems, we demonstrated that VPA treatment could regulate the mRNA levels of two post-synaptic cell adhesion molecules(neuroligin-1 and neuregulin-1) and two extracellular matrices (neuronal pentraxin-1and thrombospondin-3) in primary rat astrocyte cultures in a time- and concentration-dependent manner. Moreover, the up-regulation effect of VPA was noted in astrocytes, but not in neurons. In addition, these regulatory effects could be mimicked by sodium butyrate, a HDAC inhibitor, but not by lithium or two other glycogen synthase kinase-3 beta inhibitors. With the known role of these four proteins in regulating the synaptic E/I balance, we further demonstrated that VPA increased excitatory post-synaptic protein (postsynaptic density 95) and inhibitory post-synaptic protein (Gephyrin) in cortical neuro-glial mixed cultures. Our results suggested that VPA might affect the synaptic excitatory/inhibitory balance through its effect on astrocytes. This work provides the basis for future evaluation of the role of astroglial cell adhesion molecules and the extracellular matrix on the control of excitatory and inhibitory synapse formation.

1. Introduction

Valproic acid (VPA, 2-propylpentanoic acid) is widely used as a broad-spectrum antiepileptic drug and also for the treatment of bipolar disorders (Chronicle and Mulleners, 2004). Its mechanism of action was initially found to be primarily related to neurotransmissions (Monti et al., 2009). In particular, its antiepileptic effect has been found to be related to the reduction of neuronal excitability (Loscher, 1999). Both an increase in GABAergic activity (Loscher and Siemes, 1984; Ticku and Davis, 1981; Zhang et al., 1996) and a decrease in N-methyl-D-aspartate (NMDA) receptor-mediated glutamatergic excitatory activity (Gean et al., 1994; Gobbi and Janiri, 2006; Ko et al., 1997; Zeise et al., 1991) have been reported. These reports suggested that the regulatory effects of VPA on the glutamate/GABA neurotransmitters system might modulate the synaptic excitatory/inhibitory (E/I) balance. However, whether VPA affects the synaptic excitatory/inhibitory balance through its effect on neurons or glial cells remains unclear.

Recent studies have shown that astrocytes are involved in controlling the timing, location, and number of the tripartite synapses (Allen and Barres, 2009; Christopherson et al., 2005; Mauch et al., 2001; Ullian et al., 2001). The tripartite synapse model proposes that in addition to the presynapse and the postsynaptic membranes, apposed processes of astrocytes constitute an integral part of the synapse. It was also proposed that all parts of the tripartite synapse interact, either directly or through soluble signaling molecules, with the synaptic cell adhesion molecules (CAMs) and extracellular matrices (ECMs)

Abbreviations: BDNF, brain derived neurotrophic factor; CAM, cell adhesion molecules; E/I, excitatory/inhibitory; EAAT2, excitatory amino acid transporter 2; ECM, extracellular matrix; GDNF, glial derived neurotrophic factor; GSK, glycogen synthase kinase; HDAC, histone deacetylase; NL-1, neuroligin-1; NMDA, N-methyl-D-aspartate; NP-1, neuronal pentraxin-1; NRG-1, neuregulin-1; PSD95, postsynaptic density 95; SAHA, suberoylanilidehydroxamic acid; SB, sodium butyrate; TSP-3, thrombospondin-3; VGAT, vesicular GABA transporter; VPA, Valproic acid.

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doi:10.1016/j.pnpbp.2012.01.017

(Dityatev and Schachner, 2003; Faissner et al., 2010; Gundelfinger et al., 2010).

Evidence has revealed that CAMs and ECMs are involved in the formation and maturation of synapses (Barrow et al., 2009; Dalva et al., 2007; Dityatev and Rusakov, 2011; Dityatev et al., 2010). In addition, it has also been reported that these CAMs and ECMs can regulate the synaptic E/I balance (Dityatev and Fellin, 2008; Dityatev et al., 2006; Scheiffele et al., 2000). For example, neuoligin-1 (NL-1; a cell adhesion molecule) together with beta-neurexin could trigger postsynaptic and presynaptic differentiation in a developing glutamatergic synapse (Banovic et al., 2010; Barrow et al., 2009; Craig and Kang, 2007; Scheiffele et al., 2000; Siddiqui et al., 2010). Neuregulin-1 (NRG-1) could bind to erbB4 receptor tyrosine kinase to control glutamatergic synapse maturation and plasticity (Li et al., 2007). Neuronal pentraxin-1 (NP-1) was noted to act transsynaptically to cluster AMPA receptors at postsynaptic sites and also promote excitatory synaptogenesis (Koch and Ullian, 2010; Xu et al., 2003). Thrombospondins (TSPs) are large oligomeric ECM proteins that mediate cell–cell and cell–matrix interactions by binding with other ECM proteins and have the ability to induce synaptogenesis (Christopherson et al., 2005; Ehlers, 2005; Freeman, 2005; Iruela-Arispe et al., 1993; Lu and Kipnis, 2010).

Our previous reports using a primary rat neuro-glial mixed culture showed that VPA and other histone deacetylase (HDAC) inhibitors could induce brain derived neurotrophic factor (BDNF) and glial derived neurotrophic factor (GDNF) transcription/expression in astrocytes to provide a consequent neuroprotective effect in vitro (Chen et al., 2006; Wu et al., 2008). These findings suggested that astrocytes could be important targets for VPA to achieve its therapeutic effect and histone modification could be the underlying molecular mechanism. To further analyze the importance of specific astroglial synaptic CAMs and ECMs modulated by VPA, in the current study we investigated the effect of VPA on the mRNA levels of two excitatory postsynaptic CAMs, neuroligin-1 (NL-1) and neuregulin-1 (NRG-1), and two ECMs, neuronal pentraxin-1 (NP-1), and thrombospondin-3 (TSP-3), in primary astrocyte cultures. Moreover, we explored the possible underlying mechanism and its impact on the synaptic E/I balance.

2. Materials and methods

2.1. Animals

Timed-pregnant SD (Sprague–Dawley) rats were obtained from the animal center at *National Cheng Kung University* (NCKU), Taiwan. Housing and breeding of the animals were performed in strict accordance with the National Institutes of Health guidelines.

2.2. Chemicals and antibodies

Sodium salt of valproic acid (cat. P4543), sodium butyrate (SB, cat. B5887), and TZDZ-8 (cat. T8325) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Suberoylanilidehydroxamic acid (SAHA) was purchased from Alexis Biochemical (San Diego, CA, USA). SB216763 (3-[2, 4-dichlorophenyl]-4-[1-methyl-1H-indol-3-yl]-1H-pyrrole-2, 5-Dione) was purchased from Tocris Biosciences (Ellisville, MO, USA). Chemicals were dissolved in H_2O (VPA and SB) or 100% dimethylsulphoxide (DMSO) for SAHA and SB216763. DMEM/F12 media (Cat. 11330–032), non-essential amino acid (Cat. 11140–050), sodium-pyruvate (Cat. 11360–070), L-glutamine (Cat. 25030–081), penicillin/streptomycin (Cat. 15140–122), ultrapure DEPC-treated water (Cat. 750023), fetal bovine serum (Cat. 10091–148), and TRIzol (Cat. 15596–018) were purchased from Invitrogen (Carlsbad, CA, USA).TaqMan® Universal PCR Master Mix (Cat. 4304437), Neuroligin-1 probe (Rn01642900_m1), Neuregulin-1 probe (Rn00580917_m1), Neuronal Pentraxin-1 probe (Rn00596666_m1), Thrombospondin-3 probe (Hs00200157_m1), and GAPDH probe (Rn99999916_s1) were purchased from ABI. dNTP Mix (Cat. U1515), ImProm-II™ Reverse Transcriptase (Cat. A3802), recombinant RNasinRibonuclease Inhibitor (Cat. N2511), random Primers (Cat. C1181) were obtained from Promega (Madison, WI, USA). Mouse anti-GFAP antibody was purchased from Chemicon. Rabbit-anti-neuroligin-1, neuregulin-1, and -thrombospondin-3, and goat-anti-neuronal pentraxin-1 antibodies were purchased from Santa Cruz (Santa Cruz, CA, USA). Secondary fluorescence antibodies were purchased from Invitrogen (Carlsbad, CA, USA).

2.3. Rat astrocyte cultures

Mixed glial cultures were prepared from the brains of 1-day-old rat pups. Briefly, mechanically-dissociated brain cells (5×10^7) were seeded in 150-cm^2 culture flasks in Dulbecco's modified Eagle's medium/nutrient mixture F12 mixture (DMEM/F12) containing 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 μM non-essential amino acids, 50 U/ml penicillin, and 50 μg/ml streptomycin. The cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air, and the medium was replenished 4 days after the initial seeding. Upon reaching confluence (12–14 days), microglia were separated from astrocytes by shaking the flasks for 5 h at 180 rpm Astrocytes were then detached with trypsin–EDTA and seeded in the same culture medium. After at least five consecutive passages, cells were seeded (10^5/well) into 24-well plates for experiments.

2.4. Primary cortical neuron cultures

Cultures of rat primary cortex neurons were prepared from embryonic day 18 Sprague–Dawley (BioLASCO Taiwan Co., Ltd) rat embryos. The neocortexes of rat embryos were dissected and placed in cold BME/Hank's balanced salt solution (HBSS). After removal of meninges, the tissues were minced and incubated at 37 °C for 15 min in Ca^{2+}/Mg^{2+}-free HBSS containing 0.25% trypsin and 0.2 mg/ml DNase I, and the cell suspensions were centrifuged (300 rpm for 5 min). The resulting pellets were resuspended in a1:1 mixture of DMEM and F12 supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were plated into 6-well poly-D-lysine coated dishes at a density of 1.5×10^5/ml. Cytosine arabinoside (10 μM) was added to the culture 24 h after plating to prevent non-neuronal cell proliferation. The cultures were incubated at 37 °C in 5% CO_2. After 48 h, the medium was replaced with neurobasal medium supplemented with B-27 and penicillin (without-Glu, Gibco). Only mature cultures (7 days in vitro) were used for experiments.

2.5. Neuro-glial mixed culture

Neuro-glial mixed cultures were prepared from the cortical neuron tissues of embryonic day-17–18 Sprague–Dawley (BioLASCO Taiwan Co., Ltd) rats. In brief, the neocortexes of rat embryos were dissected and placed in cold Minimal Essential Medium (MEM). After removal of meninges, the tissues were dissociated into single cells by mechanical trituration and then seeded at a density of 5×10^5 cells/well in 6-well or 3.5-cm poly-D-lysine-coated plates or dishes. Cells were maintained in Minimal Essential Medium supplemented with 5% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. After 72 h, the medium was replaced. Seven-day-old cultures were used for treatment.

2.6. Quantitative real-time PCR

Quantitative RT-PCR analysis of total RNA was performed on cells extracted by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and