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BRAIN RESEARCH

Research Report

Valproic acid regulates catecholaminergic pathways by concentration-dependent threshold effects on TH mRNA synthesis and degradation

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ARTICLE INFO

Article history:
Accepted 24 September 2008
Available online 14 October 2008

Keywords:
Short chain fatty acids
Valproic acid
Butyrate
Neurotransmitter
Gene expression
Tyrosine hydroxylase

ABSTRACT

The spectrum of neurological conditions and psychiatric disorders affected by valproic acid (VPA) ranges from control of seizure and mood disorders to migraine, neuropathic pain, and even congenital malformations and autism. While widely used clinically, the mechanism(s) of action of VPA is not completely understood. Emerging evidence indicates that brain noradrenergic systems contribute to the symptoms of mood disorders and may involve regulation of tyrosine hydroxylase (TH) expression, the rate-limiting enzyme in the biosynthesis of dopamine, norepinephrine and epinephrine. We previously showed that the structurally related short chain fatty acid sodium butyrate (SB) induces TH transcription and alters TH mRNA stability in PC12 cells. The present study was undertaken to determine whether the branched short chain fatty acid VPA could also regulate TH gene expression in vitro. Similar to SB, VPA induced TH transcription at all concentrations tested. VPA-stimulated transcription was significantly attenuated by introducing point mutations in either the canonical cAMP- or in the butyrate-response elements of the TH promoter; or by co-expression of dominant-negative forms of CREB. As with SB, increasing concentrations of VPA demonstrated opposing effects on TH mRNA and protein abundance: elevation of both at low (0.1 mM) but attenuation at concentrations higher than 0.5 mM. This concentration-dependence is consistent with a novel and previously unrecognized cellular/molecular drug regulatory step at the level of TH mRNA stability. Thus, the therapeutic efficacy of VPA might be related to its ability to regulate TH mRNA and protein levels, and thereby central catecholaminergic-dependent behavioral pathways.

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Abbreviations: SCFA, short chain fatty acid; SB, sodium butyrate; VPA, valproic acid; TH, tyrosine hydroxylase; PC12, pheochromocytoma cell line; mRNA, messenger RNA; rRNA, ribosomal RNA; CREB, cAMP response element binding protein; AP-1, activator protein; DBH, dopamine beta hydroxylase; GABA, gamma aminobutyric acid; DMEM, Dulbecco's-Modified Eagle Medium; BRE, butyrate response element; CRE, cAMP response element; HDAC, histone deacetylase

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doi:10.1016/j.brainres.2008.09.088

1. Introduction

Dysregulation of central monoaminergic systems is a major unifying contributor to the underlying pathology of depression and bipolar mood disorders — two leading causes of psychiatric disability world wide (Schatzberg and Schieldkrautt, 1995). In postmortem studies, increased norepinephrine turnover occurs in cortical and thalamic areas of patients with bipolar disorders (Young et al., 1994). Moreover, in vivo measurements show lower levels of plasma norepinephrine in bipolar depressed subjects than in unipolar depressed patients. Bipolar patients also had higher norepinephrine plasma levels when manic than when depressed (Manji and Potter, 1997). These clinical and laboratory findings implicate an etiological role of the catecholaminergic system in each of these clinical syndromes. Understanding the cellular and molecular actions of drugs proven effective in the treatment of mood disorders is likely to provide insights into the pharmacological mechanisms through which those treatments exert therapeutic effects as well as help identify potential biomarkers and disease-related gene targets.

VPA is a drug with a broad spectrum of efficacy in mania, bipolar disorders, epilepsies and addictions (Peterson and Naunton, 2005). It is also used for the treatment of migraine and neuropathic pain (Johannessen and Johannessen, 2003; Finnerup et al., 2007). In spite of a wide range of neurological and behavioral clinical effects the molecular mechanisms underlying its therapeutic actions remain unclear. In addition, emerging evidence supports the notion that at therapeutic levels, VPA has neuroprotective properties in cellular and animal models. These include protection against glutamate-induced excitotoxicity (Kanai et al., 2004; Leng et al., 2008), lipopolysaccharide-induced dopaminergic neuronal death (Peng et al., 2005) and apoptotic cell death in neurons (Leng and Chuang, 2006). In a rat cerebral ischemia model, VPA treatment following the injury suppressed ischemia-induced brain damage as well as attendant neuronal deficits (Ren et al., 2004).

The neuroprotection by VPA is associated with increased levels of acetylated histones, decreased cell damage and improved behavioral outcomes (Kanai et al., 2004; Jeong et al., 2003; Williams et al., 2006), an effect replicated by other structurally un-related histone deacetylase (HDAC) inhibitors like trichostatin A as well as the structurally related short chain fatty acid (SCFA) SB (Kim et al., 2007). Separate from broad neuroprotection effects, VPA also up-regulates selected genes such as TH in the rat locus coeruleus during either acute or chronic treatment paradigms (Sands et al., 2000). Since TH is the rate limiting enzyme in the biosynthetic pathway of dopamine, norepinephrine and epinephrine, these data suggest that VPA may create its pharmacological clinical actions through alterations in this transmitter system or through other neuroprotective mechanisms. Additional support for the first contention is provided by the attenuation of the preventive beneficial effects of acute VPA treatment on seizure susceptibility in dopamine beta-hydroxylase deficient mice (Schank et al., 2005). Other physiologically significant effects of VPA include increased GABA'ergic activity, reduction in excitatory neurotransmission, blockade of voltage-gated sodium channels and modulation of dopaminergic and serotoninergic neurotransmission (Perucca, 2002; Johannessen and Johannessen, 2003).

VPA (2-propylpentanoic acid) is a branched SCFA structurally similar to the naturally occurring SCFA butyrate (featuring a tetrahedral α-carbon connected to a free carboxyl group, hydrogen and two alkyl groups). We previously showed that the CH_3–CH_2–R motif of the 4 carbon SCFA butyrate is the active structural moiety responsible for its effects on TH gene expression in rat PC12 cells (Mally et al., 2004; Nankova et al., 2003). Similarly, VPA has two such motifs.

The present study was undertaken to investigate whether VPA alters the expression of TH gene in PC12 cells, the molecular mechanisms mediating its effects and the role of cis- and trans-acting factors in the process.

2. Results

2.1. *VPA induces the transcription of TH gene*

VPA was recently demonstrated to inhibit HDAC enzymes in several cell lines, associated with induction of differentiation, growth arrest, chromatin decondensation and changes in the transcription of a subset of genes (Gottlicher et al., 2001; Phiel et al., 2001); similar to its structurally related and well known HDAC inhibitor with specific-gene transcription regulation properties, the SCFA butyrate (Marks et al., 2000; Zhu and Otterson, 2003; Langley et al., 2005; Chen et al., 2003). Chromatin remodeling and the resulting long lasting epigenetic regulation of gene expression have been implicated in the stable neuronal adaptations in specific brain regions that might underlie complex behavioral changes in psychiatric disorders as well as changes in neurotransmitter-related gene systems (Tsankova et al., 2007).

We have previously shown (Nankova et al., 2003; Parab et al., 2007; Patel et al., 2005) that TH is among the 2% of all genes regulated by HDAC inhibitors like SB. To test whether VPA also causes induction of TH gene transcription we performed transient transfection experiments. Plasmid constructs with a rat TH promoter (−773/+27 bp) driving the expression of luciferase reporter gene were electroporated into PC12 cells pre-treated for one day with vehicle (C) or with increasing concentrations of VPA (0.1, 1 or 6 mM). The response to VPA was determined after additional 24 h of treatment. The data are summarized in Fig. 1. VPA induced TH promoter-driven reporter gene activity in a concentration-dependent manner consistent with transcriptional activation of the gene.

2.2. *CRE and BRE promoter elements mediate the transcriptional effects of VPA*

We have shown previously that two regions of the TH promoter are important for enabling butyrate-dependent responses (Patel et al., 2005). One region involves the canonical cAMP response element (CRE, 5′..TGACGTCA; −45 to −38 bp upstream of the TH start site). The other involves a novel butyrate response element (BRE, 5′…GCCTGG; −509 to −504 bp upstream of the TH start site). To determine whether the same promoter elements mediate the transcriptional response to VPA we used plasmids containing a single nucleotide change