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HDAC inhibitors dysregulate neural stem cell activity in the postnatal mouse brain

Stacey Beth Foti, Athena Chou, Andrew D. Moll, A. Jane Roskams*

Department of Zoology, Life Sciences Institute and Brain Research Centre, University of British Columbia, Vancouver, BC V6T 1Z3, Canada

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ABSTRACT

The mammalian central nervous system (CNS) undergoes significant expansion postnatally, producing astrocytes, oligodendrocytes and inhibitory neurons to modulate the activity of neural circuits. This is coincident in humans with the emergence of pediatric epilepsy, a condition commonly treated with valproate/valproic acid (VPA), a potent inhibitor of histone deacetylases (HDACs). The sequential activity of specific HDACs, however, may be essential for the differentiation of distinct subpopulations of neurons and glia. Here, we show that different subsets of CNS neural stem cells (NSCs) and progenitors switch expression of HDAC1 and HDAC2 as they commit to a neurogenic lineage in the subventricular zone (SVZ) and dentate gyrus (DG). The administration of VPA for only one week from P7–P14, combined with sequential injections of thymidine analogs reveals that VPA stimulates a significant and differential decrease in the production and differentiation of progeny of NSCs in the DG, rostral migratory stream (RMS), and olfactory bulb (OB). Cross-fostering VPA-treated mice revealed, however, that a postnatal failure to thrive induced by VPA treatment had a greater effect on DG neurogenesis than VPA action directly. By one month after VPA, OB interneuron genesis was significantly and differentially reduced in both periglomerular and granule neurons. Using neurosphere assays to test if VPA directly regulates NSC activity, we found that short term treatment with VPA *in vivo* reduced neurosphere numbers and size, a phenotype that was also obtained in neurospheres from control mice treated with VPA and an alternative HDAC inhibitor, Trichostatin A (TSA) at 0 and 3 days *in vitro* (DIV). Collectively, these data show that clinically used HDAC inhibitors like VPA and TSA can perturb postnatal neurogenesis; and their use should be carefully considered, especially in individuals whose brains are actively undergoing key postnatal time windows of development.

1. Introduction

1.1. Histone deacetylation is essential for neuro-glial development

Although it is primarily patterned during embryonic development, the mammalian central nervous system (CNS) expands postnatally, producing astrocyte and oligodendrocytes, and inhibitory interneurons that modulate the activity of newly active circuits (Finlay and Darlington, 1995; Cayre et al., 2009). The progressive differentiation of both neurons and glia is driven by a balance of transcriptional activation and changes in chromatin structure, as a result of epigenetic gene silencing. A final step in histone-mediated epigenetic gene silencing is catalyzed by histone deacetylases (HDACs) enzymes that remove the acetyl groups from histone protein tails and can act in a DNA methylation-dependent and independent manner. Histone deacetylation enhances histone–DNA binding, and compacts chromatin, thus restricting transcription factor access to regulatory regions. Histones can also be modified by methylation and phosphorylation – processes that have also been strongly implicated in modifying learning, synaptic plasticity and cognition (Day and Sweatt, 2011).

1.2. Histone deacetylases in the nervous system

There are 11 potential mammalian HDACs (1–11) organized into a superfamily of four classes (I–IV) based on function and DNA sequence similarity. Although the acetylation and compaction of chromatin is a common mechanism of Class I and II HDACs, some individual isoforms can also acetylate transcription factors

Abbreviations: HDACs, histone deacetylases; HDACi, HDAC inhibitors; SVZ, subventricular zone; DG, dentate gyrus; RMS, rostral migratory stream; OB, olfactory bulb; VPA, valproic acid; P, postnatal day; DIV, days *in vitro*; OE, olfactory epithelium; ORN, olfactory receptor neuron; NSCs, neural stem cells; IdU, iododeoxyuridine; CldU, chlorodeoxyuridine; BrdU, bromodeoxyuridine; TSA, Trichostatin A.

* Corresponding author at: Rm 3-309 Life Sciences Institute, University of British Columbia, 2350 Health Sciences Boulevard, Vancouver, BC V6T 1Z4, Canada. Tel.: +1 604 827 5080; fax: +1 604 827 5085.
E-mail address: roskams@zoology.ubc.ca (A.J. Roskams).

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and cytoplasmic proteins directly. HDAC1 and HDAC2 appear to be segregated to distinct stages of neuronal and glial lineages during CNS development (MacDonald and Roskams, 2008), and their expression patterns suggest that they may modulate distinct gene expression events at different developmental stages. Neural progenitors that maintain the expression of HDAC1 largely differentiate into glial cells, while those that lose HDAC1 and up-regulate HDAC2 differentiate into neuronal progenitors and neurons. HDAC1 is highly expressed in the corpus callosum during oligodendrocyte differentiation, and when HDACs are inhibited, oligodendrocytes fail to differentiate and cause hypomyelination in the corpus callosum of postnatal rats (Shen et al., 2005). In addition, an elegant body of work has suggested HDAC1 as a critical regulator of the production and differentiation of oligodendrocyte precursor cells (Marin-Husstege et al., 2002; Shen and Casaccia-Bonnefil, 2008). HDAC1 can also directly regulate stem cell proliferation, and HDAC1 null animals display a significant reduction in cell proliferation (Lagger et al., 2002). HDAC2, on the other hand, appears necessary to inhibit astrocyte differentiation, while HDAC1 is not (Humphrey et al., 2008), suggesting that HDAC2 may be involved in silencing glial gene expression, while HDAC1 likely silences neuronal genes.

1.3. Histone deacetylase inhibitors in the nervous system

HDAC activity can be inhibited with a variety of drugs – some of which are in widespread clinical or experimental use. HDAC inhibitors (HDACi) are classified into six groups based on their chemical structures, but most HDACi are broad-spectrum and affect multiple HDACs within the classical HDAC family (HDACs1–11). HDAC isoform-specific inhibitors have been difficult to design due to the high sequence homology within the catalytically active sites of HDACs (Bieliauskas and Pflum, 2008), but are now beginning to emerge experimentally. Valproic acid (sodium valproate, VPA) is one of the most commonly used anticonvulsants in treating pediatric epilepsy, in addition to being used as a mood stabilizer and for migraine headache prophylaxis (Aldenkamp et al., 2006). In children and adolescents, VPA is well tolerated with a low incidence of serious side effects, despite the fact that it impacts sodium channel and GABA activity, in addition to being a HDACi (DeVane, 2003). However, given that VPA is a potent teratogen associated with neural tube closure defects and is used in modeling autism in rodents (DiLiberti et al., 1984; Nau et al., 1991), VPA may also have additional detrimental effects on postnatal neural development, which have not been fully explored.

1.4. Histone deacetylases and neurogenesis

In the olfactory epithelium (OE) of mice (a site of continual postnatal neurogenesis) DNA methyltransferases (DNMTs), methyl-binding domain proteins (MBDs), and HDACs regulate distinct transitional stages of olfactory receptor neuron (ORN) differentiation (Macdonald et al., 2010). Furthermore, acute HDAC inhibition with VPA results in the dysregulation of adult OE neurogenesis, and causes precocious differentiation (concurrent with a loss of neural stem cell (NSC) activity) and compromised ORN survival (Macdonald et al., 2010). HDAC1-null embryonic stem (ES) cell-derived embryoid bodies undergo precocious differentiation and increase expression of neuronal-specific markers (Dovey et al., 2010). In addition, treatment with HDAC inhibitors such as VPA and TSA *in vitro* can increase neuronal production from embryonic NSCs (Hsieh and Gage, 2004; Balasubramaniyan et al., 2006; Shaked et al., 2008), and adult NSCs (Siebzehnrubl et al., 2007; Schneider et al., 2008; Zhou et al., 2011). However, we do not know if, as in the OE, this increase in neuron production is at the expense of NSC activity, or the switch from a glial to a neuronal lineage. In particular, we do not know if short or long-term HDACi activity may detrimentally impact the stability of the NSC niche, or the genomic stability and long-term function of the neurons produced. Because of this, here we test if HDAC1 and 2 may serve a similar role postnatally in different CNS neurogenic regions, and if neural stem cells (NSCs) and neural progenitors may be dysregulated by short-term treatment with VPA *in vivo*. In this, we identify distinct progenitors in the lateral ventricle subventricular zone (SVZ) and dentate gyrus subgranular zone (SGZ), using markers specific to their stage of commitment, as outlined in Fig. 1. In addition, we further test if VPA (a broad spectrum HDAC inhibitor) or Trichostatin A (a more specific HDAC inhibitor) may have direct effects on progenitor activity *in vitro*.

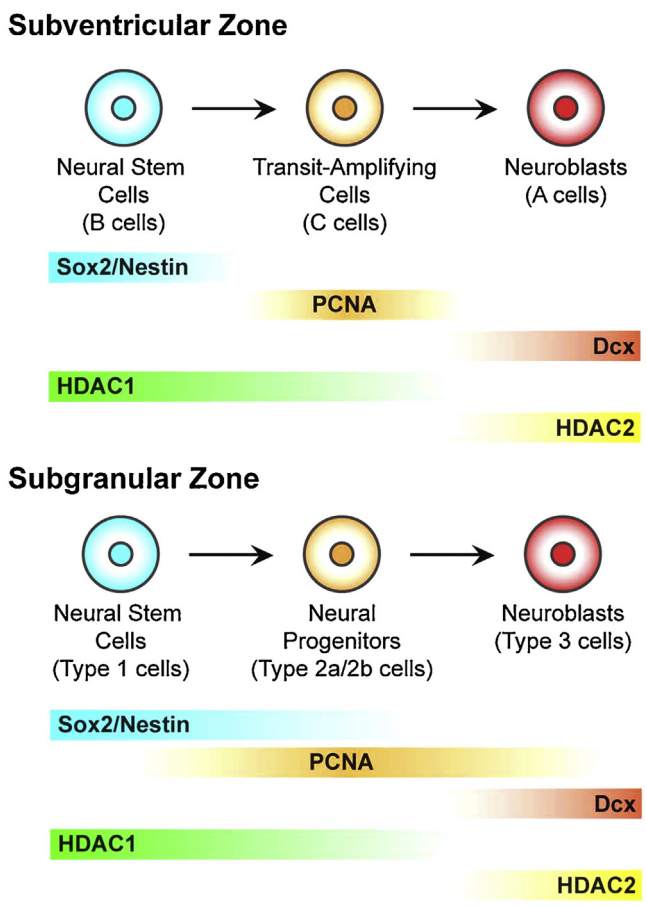

Fig. 1. *Stages of neurogenesis in the subventricular zone (SVZ) and the subgranular zone (SGZ).* When the neural stem cells commit to a neuronal lineage, they divide and become the transit-amplifying cells in the SVZ or Type 2a/2b neural progenitors in the SGZ. These cells then migrate as neuroblasts and differentiate into mature neurons. HDAC1 is highly expressed in early stem/progenitor cells in both neurogenic zones whereas HDAC2 appears to be induced at the commitment to neurogenesis.

2. Methods

2.1. Animals

All experiments were performed on CD-1 mice (Jackson Laboratories, Bar Harbor, ME) in accordance with the guidelines of the Canadian Council for Animal Care and the University of British Columbia Animal Care Committee.

2.2. Injections

Mice were injected with valproic acid, VPA (sodium salt; Sigma), saline, iododeoxyuridine (IdU; Sigma), and chlorodeoxyuridine (CldU; Sigma) either singly or in combinations at varying time points depending on the injection paradigm. All injections were intraperitoneal using the following concentrations: IdU 57.5 μg/g, CldU 42.5 μg/g and VPA 250 μg/g.