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Research Paper

Targeting histone deacetylation for recovery of maternal deprivation-induced changes in BDNF and AKAP150 expression in the VTA

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ABSTRACT

Severe early life stressors increase the probability of developing psychiatric disorders later in life through modifications in neuronal circuits controlling brain monoaminergic signaling. Our previous work demonstrated that 24 h maternal deprivation (MD) in male Sprague Dawley rats modifies dopamine (DA) signaling from the ventral tegmental area (VTA) through changes at GABAergic synapses that were reversible by *in vitro* histone deacetylase (HDAC) inhibition which led to restoration of the scaffold A-kinase anchoring protein (AKAP150) signaling and subsequently recovered GABAergic plasticity (Authement et al., 2015). Using a combination of *in situ* hybridization, Western blots and immunohistochemistry, we confirmed that MD-induced epigenetic modifications at the level of histone acetylation were associated with an upregulation of HDAC2. MD also increased *Akap5* mRNA levels in the VTA. Western blot analysis of AKAP150 protein expression showed an increase in synaptic levels of AKAP150 protein in the VTA with an accompanying decrease in synaptic levels of protein kinase A (PKA). Moreover, the abundance of mature brain-derived neurotrophic factor (BDNF) protein of VTA tissues from MD rats was significantly lower than in control groups. *In vivo* systemic injection with a selective class I HDAC inhibitor (CI-994) was sufficient to reverse MD-induced histone hypoacetylation in the VTA for 24 h after the injection. Furthermore, HDAC inhibition normalized the levels of mBDNF and AKAP150 proteins at 24 h. Our data suggest that HDAC-mediated targeting of BDNF and AKAP-dependent local signaling within VTA could provide novel therapeutics for prevention of later-life psychopathology.

1. Introduction

Child abuse and neglect are shown to increase the risk of developing stress-related disorders and substance abuse. This increased vulnerability seems to be related to brain monoaminergic dysfunction which includes an altered dopamine (DA) signaling from the ventral tegmental area (VTA) (Authement et al., 2015; Heim and Nemeroff, 2002; Pena et al., 2017; Sinha, 2008; Strathearn, 2011). Early life stressors also have a significant impact on epigenome, including histone modifications that may underlie subsequent changes in gene expression, synaptic plasticity and behavior (Abel and Zukin, 2008; Heim and Binder, 2012; Klengel and Binder, 2015; Levine et al., 2012; Palmisano and Pandey, 2017; Tesone-Coelho et al., 2015). One of the robust long-lasting histone modifications associated with severe early life stress are histone deacetylase (HDAC)-mediated changes in histone acetylation (Adler and Schmauss, 2016; Levine et al., 2012; Tesone-Coelho et al., 2015; Xie et al., 2013). In general, histone deacetylation by HDACs is associated with chromatin condensation and gene repression. On the other hand, blocking histone deacetylation by HDAC inhibitors can increase histone acetylation to possibly promote gene expression through chromatic relaxation. HDAC inhibitors have shown great potential for treatment of age-associated cognitive and memory impairments by improving synaptic plasticity (Penney and Tsai, 2014). Moreover, HDAC inhibitors have also been shown to have antidepressant properties and ameliorate symptoms of post-traumatic stress disorder, depression, and addiction (Covington 3rd et al., 2009; Klengel

Abbreviations: Histone H3 acetylation at lysine 9, Ac-H3K9; A-kinase anchoring protein, AKAP; Calcineurin, CaN; Dopamine, DA; Histone deacetylase, HDAC; Long-term depression, LTD; Mature brain derived neurotrophic factor, mBDNF; Calcium permeable AMPARs, CP-AMPARs; Maternal deprivation, MD; Nucleus accumbens, NAc; NMDA receptor, NMDAR; Non-maternally deprived, non-MD; Prefrontal cortex, PFC; Protein kinase A, PKA; Spike-timing-dependent plasticity, STDP; Type II regulatory subunit of PKA, RII; Ventral tegmental area, VTA

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and Binder, 2015; Palmisano and Pandey, 2017).

We have also shown that acute morphine-induced synaptic plasticities in VTA DA neurons involved HDAC-mediated changes in histone acetylation and were also reversible by *in vitro* application of an HDAC inhibitor through increases in histone acetylation (Authement et al., 2016; Langlois and Nugent, 2017). We demonstrated that a 24 h early maternal deprivation (MD, an animal model of child abuse), on postnatal day 9 (P9) induces synaptic abnormalities at GABAergic synapses onto VTA DA neurons through disruption of AKAP79/150 (human 79/rodent 150; also known as AKAP5) signaling in juvenile rats that might also be targeted by HDACs during MD (Authement et al., 2015). AKAPs were first discovered as the scaffold proteins that principally mediate the crosstalk of cAMP/PKA signaling with other signaling pathways. Although AKAPs identified to bind to the type II regulatory subunit of PKA (RII); it is now known that AKAPs have several binding sites for other signaling molecules including protein kinase C, protein phosphatases including calcineurin (CaN), G-protein coupled receptors, adenylyl cyclases and phosphodiesterases. AKAPs tether these signaling enzymes with their substrates (for example, synaptic AMPA, NMDA and $GABA_A$ receptors and ion channels) within distinct subcellular compartments for specific spatial and temporal interplay of postsynaptic signaling molecules in synaptic plasticity and neuronal function, as well as in synaptic and neuronal dysfunction associated with disease (Esseltine and Scott, 2013; Wild and Dell'Acqua, 2018; Woolfrey and Dell'Acqua, 2015). Therefore, therapeutic targeting of AKAP-directed signaling has become an emerging and novel concept in selective normalization of dysfunctional signaling pathways assembled by AKAPs in neurological disorders (Wild and Dell'Acqua, 2018). We found that MD-induced GABAergic metaplasticity (an increased susceptibility of GABAergic synapses to induction of AKAP150-dependent long-term depression, LTD) and dysregulated AKAP signaling could be reversed with local *in vitro* HDAC inhibition in the VTA, suggesting the potential clinical benefits of targeting of AKAP signaling within the VTA by HDAC inhibitors soon after the stress. Given that MD-induced disruption of AKAP signaling was associated with significant increases in the levels of AKAP150 expression in VTA DA neurons (Authement et al., 2015), this suggested that MD may induce HDAC-mediated transcriptional changes in specific signaling molecules that directly interact with AKAPs or act upstream from AKAP signaling. In fact, activity-dependent alterations of brain-derived neurotrophic factor (BDNF) transcriptional levels and BDNF expression act upstream to regulate proteasome-dependent synapse remodeling and synaptic protein concentrations including synaptic levels of AKAP150 (Jia et al., 2008). Moreover, early life adversity results in epigenetic changes in BDNF gene expression and signaling that are critical for synaptic plasticity (Adler and Schmauss, 2016; Daskalakis et al., 2015; Palmisano and Pandey, 2017; Roth et al., 2009).

Here, we investigated whether reversible HDAC-mediated histone modifications were associated with MD in the VTA and tested the effects of a single *in vivo* injection of a cell permeable potent selective class I HDAC inhibitor (CI-994, also called *N*-acetyldinaline or tacedinaline) on MD-induced changes at the level of histone acetylation, BDNF protein levels and AKAP gene expression within the VTA. We found that MD indeed increased HDAC2 (a class I HDAC) expression specifically in VTA DA neurons and is associated with a reduction of histone H3 acetylation at lysine 9 (Ac-H3K9). MD also reduced levels of BDNF protein while increased synaptic levels of AKAP150 protein in the VTA, and these changes were reversible by the *in vivo* HDAC inhibition 24 h after the injection (see our model in Fig. 7). Taken together, our results suggest that a single *in vivo* HDAC inhibition soon after the stress may be sufficient to epigenetically ameliorate MD-induced changes in BDNF signaling that may act upstream to regulate subcellular organization of AKAP150 complexes in VTA synapses.

2. Materials and methods

All experiments were carried out in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Uniformed Services University Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

2.1. Maternal deprivation procedure

Half of the male pups in litters of Sprague–Dawley rats (Taconic Farms) at P9 were isolated at 10:00 a.m. from the dam and their siblings for 24 h (MD group). The isolated rats were placed in a separate quiet room and kept on a heating pad (34 °C) and not disturbed until being returned to their home cage 24 h later. The remaining non-separated male rat pups received the same amount of handling but were kept with the dam serving as the non-maternally deprived control group (non-MD group). Rats were maintained on a 12 h light/dark cycle and provided food and water *ad libitum*. The animals were taken for study during the light period, between 3 and 5 h after light was turned on. Each day, two MD and non-MD rats (age-matched) from the same litter were sacrificed over days P14–21 for electrophysiology recordings, immunohistochemistry and Western blotting. We blindly performed the analysis with respect to the treatment of the rats to reduce the potential for investigator bias.

2.2. Slice preparation for Western blotting

Rats were anesthetized with isoflurane and immediately decapitated. The brains were quickly dissected and placed into ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 21.4 $NaHCO_3$, 2.5 KCl, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 1.00 MgSO4, 11.1 glucose, 0.4 ascorbic acid, saturated with 95% O_2–5% CO_2. Horizontal slices were cut at 300 μm for Western blot experiments.

2.3. HDAC inhibitor treatment

Sprague–Dawley male rats (P14-P21) (non-MD or MD) received either one intraperitoneal (i.p.) injection of CI-994 (10 mg/kg) dissolved in 1% Tween80 (vehicle) or an injection of comparable volumes of 1% Tween80 (vehicle) 24 h prior to sacrifice for immunohistochemical or Western blot studies.

2.4. Western blotting

The VTA was dissected bilaterally from horizontal slices (300 μm) of non-MD or MD rats in ACSF and then snap frozen in liquid nitrogen and stored at −80 °C. Tissues were thawed, washed in ice-cold PBS and lysed in RIPA buffer containing protease inhibitors (Sigma). Samples were then sonicated, incubated on ice for 30 min and centrifuged at 10,000*g* for 20 min at 4 °C. Protein concentration in the supernatant was determined by Pierce BCA Protein Assay Kit (Life Technologies). Equal amounts of protein (20 μg) were combined with loading buffer, boiled for 5 min, and loaded onto 4–20% precast polyacrylamide gel (Bio-Rad Laboratories). Separated proteins were transferred onto nitrocellulose membranes, blocked with casein-based blocking reagent (I-Block, Life Technologies) for 60 min at room temperature and then incubated overnight at 4 °C with antibodies recognizing HH3 (1:10,000, Abcam ab1791), antibody against PKA regulatory β2 subunit (1:5000, Abcam, ab75993), antibody against PSD95 (1:500, Cell signaling 362,333), antibody against ac-H3K9 (1:1000 cell signaling 3649), antibody against calcineurin subunit A (1:2000, Abcam ab3673), antibody against AKAP150 (1:500, Santa Cruz Sc-6445), antibody against mature BDNF(mBDNF, 1:1000 ab108319), antibody against vinculin (1:1000, Abcam ab129002) and antibody against β-actin (1:10,000, Abcam, ab6276). Secondary antibodies used were HRP-linked specific for rabbit