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Research report

Age-related alterations in histone deacetylase expression in Purkinje neurons of ethanol-fed rats

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ABSTRACT

Ethanol and age-induced pathologies of the Purkinje neuron (PN) may result from histone deacetylases (HDACs), enzymes which repress transcription through coiling of the DNA. The purposes of this study were to investigate expression patterns of Class 1 and IIa HDACs in PN and the effects of aging and alcohol on the density of HDACs and histone acetylation in PN. Ninety, eight month old rats (30/diet) were fed a liquid ethanol, liquid control, or rat chow diet for 10, 20, or 40 weeks (30/treatment duration). Double immunocytochemical labeling on tissue sections from these rats used antibodies against HDAC isoforms or acetylated histones, and calbindin, a marker for PN. Fluorescent intensities were also measured. Results showed a significant age but not an alcohol-related decrease in the densities of HDACs 2, 3, and 7. In contrast, there were age related-increases in the densities of phosphorylated form of HDAC (4, 5, 7) PN and in PN nuclei expressing HDAC 7. There were also a trend towards ethanol-induced inhibition of acetylation as the density of AH2b PN nuclei and AH3 and AH2b fluorescent intensity was significantly lower in the EF compared to the PF rats. This study presents unique data concerning which HDACs are commonly expressed in PN and indicates that aging rather than lengthy alcohol expression alters expression of the HDACs studied here. These results also suggest that lengthy ethanol consumption may inhibit histone deacetylation in PN.

1. Introduction

The Purkinje neuron, the sole output neuron of the cerebellar cortex has been shown to be excessively sensitive to both aging (Zhang et al., 2010; Dlugos, 2015) and alcohol consumption (National Institute of Health, National Institute of Alcohol Abuse and Alcoholism, 2010; Shanmugarajah et al., 2016). Taken together, aging and alcohol effects on the cerebellum have important connotations for the health and the mobility of the large number of elderly humans in the United States who drink alcohol (45%), binge drink (12.2%), and drink heavily (3.25%) (The National Survey on Drugs and Health, 2002). Alcohol-induced changes to the cerebellum and the PN[1] are important factors in the predisposition of elderly drinkers to fall more than their non-drinking cohorts (Rigler, 2000) with the added concern that abstinent alcoholics retain permanent deficits in cerebellar motor coordination that become more pronounced with age (Sullivan et al., 2000; Deshmukh et al., 2002). Data collected from this laboratory show that chronic alcohol exposure for lengthy periods in aging adult rats results in structural, molecular, and behavioral alterations to PN which include regression of the extensive dendritic arbor (Pentney, 1995), decreased synapse number (Dlugos and Pentney, 1997), dilation of dendritic smooth endoplasmic reticulum (Dlugos, 2006a, Dlugos, 2006b), increases in dendritic degenerating bodies (Dlugos, 2008), decreases in levels of the sarco/endoplasmic reticulum calcium adenosine triphosphate pump and balancing ability (Cassidy et al., 2013), and increased accumulation of caspase 12 (Dlugos, 2014).

The mechanisms behind ethanol-induced changes to the rat PN are unknown although epigenetics, specifically removal of acetyl groups by HDACs[2], is a candidate. Histone deacetylation by HDACs results in removal of acetyl groups from lysines on histone tails resulting in DNA coiling, and repression of transcription (Shahbazian and Grunstein, 2007). HDACs deacetylate many other

Abbreviations: PN, Purkinje neuron; HDACs, histone deacetylases; HDAC, histone deacetylase; CREB, c-AMP-responsive element binding protein; BDNF, brain derived nerve factor; CF, Chow-Fed; PF, Pair-Fed; EF, Ethanol-Fed; AH2b, Acetylated histone 2b; AH3, Acetylated histone 3; CTCF, Corrected total cell fluorescence; AIN, American Institute of Nutrition.

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targets within the cell besides histones (Yang and Grégoire, 2005) and are divided into zinc-dependent and nicotinamide adenine dinucleotide dependent groups. The zinc-dependent HDACs are subdivided into four families (Classes I, IIa, IIb and IV) and members of each family are found in the brain (Broide et al., 2007; Volmar and Wahlestedt, 2015). The class I family consists of HDACs 1, 2, 3, and 8 (Yang and Seto, 2008; Haberland et al., 2009) and the class II a family consists of HDACs 4, 5, 7, and 9 (Yang and Grégoire, 2005; Yang and Seto, 2008; Yao and Yang, 2011). The class IIb family consists of HDACs 6 and 10 whereas HDAC 11 is the sole member of the class IV family.

Members of the Class 1 and IIa HDACs have been extensively studied as they are essential to life (Lagger et al., 2002; Vega et al., 2004: Montgomery et al., 2007; Bhaskara et al., 2008; Knutson et al., 2008; Montgomery et al., 2009). In the class I family, HDACs 1 and 2 share similar sequences and nuclear localization but are functionally different as HDAC[3] 1 is expressed in glia and HDAC 2 in neurons (MacDonald and Roskams, 2008). HDAC 2 appears to be essential in neuronal function as overexpression results in reduced dendritic spine density and poor performance on memory tests (Guan et al., 2009). In contrast to other Class 1 HDACs, HDAC 3 is expressed in the nucleus but may translocate to the cytoplasm with substrates which include p300/CREB binding protein-associated factor (Blanco-Garcia et al., 2009) or cleavage products of HDAC 3 during apoptosis (Escaffit et al., 2007).

Class IIa HDACs normally shuttle between the nucleus and cytoplasm, providing multiple functions for the cell. For example, the export of HDACs 4, 5, and 7 from the nucleus is facilitated by a C-terminal nuclear export sequences and phosphorylation of highly conserved serine residues (Li et al., 2004). Cytoplasmic retention is maintained by anchoring the phosphorylated serines to 14-3-3 chaperone proteins (Annemieke et al., 2003; Li et al., 2004; Parra and Verdin, 2010; Yao and Yang, 2011). HDACs 4 and 7 appear to have a neuroprotective role that was demonstrated directly as mice lacking HDAC 4 lost PN (Majdzadeh et al., 2008). Class IIa HDACs also appear to have an anti-apoptotic effect on other neurons as forced expression of HDACs 4 (Majdzadeh et al., 2008) and 7 (Ma and D'Mello, 2011) reduced low potassium apoptosis in cerebellar granule neurons.

An early study using *in situ* hybridization revealed that, of all the 11 zinc-dependent HDACs, only messenger RNA for HDAC 11 was found in PN (Broide et al., 2007). Later studies with antibody staining showed nuclear localization of HDAC 2 in developing PN (Yoo et al., 2013). HDAC 4 was also reported in the PN cytoplasm of control mice and in the PN nucleus of mice with ataxia telangiectasia (Li et al., 2012). Another study demonstrated a more variable localization of HDAC 4 in normal PN ranging from nuclear to cytoplasmic and including some PN with HDAC 4 in both the nucleus and cytoplasm (Darcy et al., 2010). HDAC7 has not been identified specifically in PN rather in the cytoplasm and nucleus of neurons of mouse cerebellar cortex, cerebral cortex, and striatum (Benn et al., 2009). There is some evidence for the presence of HDAC 3 in PN as PN specific HDAC3 null mice displayed a loss of PN, a thin molecular layer, and decreased rotorod performance (Venkatraman et al., 2014). Similarly, the knockout of HDAC 3 in neuronal progenitor cells resulted in underdeveloped cerebellar folia, lower cell density, and lower staining intensity for a calbindin antibody in PN (Norwood et al., 2014).

There is a precedent for examining alcohol-induced alterations to the histone deacetylases and histone acetylation. Microarray analysis has correlated expression patterns of HDACs 1, 2, and 11 with ethanol intake in the nucleus accumbens of C57BL/6NCrl mice (Wolstenholme et al., 2011). There has been a recent escalation of studies which examine the histone deacetylases in alcohol sensitive areas such as the amygdala (Berkel and Pandey, 2017). Some studies indicate that HDACs are upregulated with ethanol whereas others show downregulation with ethanol. These differences are dependent on the regions studied, the ethanol paradigm used, and the particular isoform selected. For example, HDAC 3 was upregulated in the liver following binge alcohol exposure whereas HDACs 1, 7, 9, 10, and 11 were downregulated (Kirpich et al., 2013). Similarly, acute treatment with alcohol resulted in increased expression of HDAC 2 (Agudelo et al., 2011) and HDACs 1 and 3 (Agudelo et al., 2012) in a human neuroblastoma cell line. Ethanol-related upregulation of HDACs would explain the reported reduction in acetylated histones 3 and 4 in the perinatal rat (third trimester equivalent) cerebellar cortex (Guo et al., 2011). In addition, despite the fact that one binge episode was shown to reduce expression of HDACs 1–10 in the peripheral blood of alcohol naïve rats, repeated binges increased expression of HDACs 1 and 7 in the heart and HDACs 1, 2, and 5 in the amygdala (López-Moreno et al., 2015). HDAC downregulation with alcohol treatment has also been reported. For example, acute alcohol treatment resulted in downregulation of HDACs 1, 2, and 3 in the caudate and putamen of wild-type mice (Caputi et al., 2015) and also in decreases in messenger RNA for HDACs 1 and 4 in hippocampal slices (Zou and Crews, 2014).

Ethanol-induced decreases in HDACs might be responsible for increased acetylation of histone 3 in rat liver cultures (Park et al., 2003) or global hyperacetylation of liver proteins such as P53 tumor suppressor protein and α-tubulin (Shepard and Tuma, 2009). Intermittent ethanol consumption during adolescence resulted in increased acetylation of histones 3 and 4 in the prefrontal cortex and nucleus accumbens, decreased histone acetylation in the striatum, but showed no effect on the hippocampus. (Pascual et al., 2009). Withdrawal from ethanol also appears to downregulate HDAC expression as increased acetylation of histones 3 and 4 has been reported in adolescent (Pascual et al., 2012) and young adult rats (Jung and Metzger, 2015).

Extensive work in the amygdala reports that acute ethanol consumption reduced HDAC expression and induced levels of CREB[4] and neuropeptide Y binding whereas withdrawal from chronic treatment resulted in increased HDAC activity, decreased histone acetylation, and decreased levels of CREB and neuropeptide Y (Pandey et al., 2008). Ethanol-induced increases in HDACs have also been shown to be a factor in rapid tolerance to the anxiolytic effects of alcohol and histone acetylation in the amygdala (Sakharkar et al., 2012). In addition, alcohol preferring rats have higher HDAC 2 protein levels compared to the alcohol non-preferring strain. Acute ethanol treatment decreased these levels and increased global and gene specific acetylation of factors, such as BDNF[5], related to synaptic function (Moonat et al., 2013). A subsequent study in the amygdala showed that the HDAC inhibitor, trichostatin A, promoted histone acetylation and neuropeptide Y expression in the alcohol preferring rats as well as normalizing the low levels of neuropeptide Y promotor in these animals (Sakharkar et al., 2014). The use of a HDAC inhibitor during withdrawal from chronic ethanol also reduced anxiety and increased spine density in the amygdala with concomitant increases in the expression of two proteins related to synaptic function, BDNF and activity-regulated cytoskeleton-associated protein (You et al., 2014). In an adolescent intermittent alcohol model, withdrawal from chronic alcohol resulted in similar increases in HDAC 2 in the amygdala accompanied by decreases in global acetylation, BDNF expression, and dendritic spine density, deficits that persisted to adulthood (Pandey et al., 2015).

Aging is another factor that may influence HDAC activity enhancing or reducing alcohol-induced effects. The HDACs appear to play a major role during aging. In the lung, HDAC 2 has been shown to protect against cellular senescence and may be a regulating factor in the development of pulmonary disease (Yao and Rahman, 2012). In the brain, HDAC 2 has been deemed an