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BRAIN RESEARCH

Research Report

Functional significance of aldehyde dehydrogenase ALDH1A1 to the nigrostriatal dopamine system

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ABSTRACT

Aldehyde dehydrogenase 1A1 (ALDH1A1) is a member of a superfamily of detoxification enzymes found in various tissues that participate in the oxidation of both aliphatic and aromatic aldehydes. In the brain, ALDH1A1 participates in the metabolism of catecholamines including dopamine (DA) and norepinephrine, but is uniquely expressed in a subset of dopaminergic (DAergic) neurons in the ventral mesencephalon where it converts 3,4-dihydroxyphenylacetaldehyde, a potentially toxic aldehyde, to 3,4-dihydroxyphenylacetic acid, a non toxic metabolite. Therefore, loss of ALDH1A1 expression could be predicted to alter DA metabolism and potentially increase neurotoxicity in ventral mesencephalic DA neurons. Recent reports of reduced levels of expression of both *Aldh1a1* mRNA and protein in the substantia nigra (SN) of Parkinson's disease patients suggest possible involvement of ALDH1A1 in this progressive neurodegenerative disease. The present study used an *Aldh1a1* null mouse to assess the influence of ALDH1A1 on the function and maintenance of the DAergic system. Results indicate that the absence of *Aldh1a1* did not negatively affect growth and development of SN DA neurons nor alter protein expression levels of tyrosine hydroxylase, the DA transporter or vesicular monoamine transporter 2. However, absence of *Aldh1a1* significantly increased basal extracellular DA levels, decreased KCl and amphetamine stimulated DA release and decreased DA re-uptake and resulted in more tyrosine hydroxylase expressing neurons in the SN than in wildtype animals. These data suggest that in young adult animals with deletion of the *Aldh1a1* gene there is altered DA metabolism and dysfunction of the DA transporter and DA release mechanisms.

1. Introduction

Aldehyde dehydrogenase 1 (ALDH1A1) and aldehyde dehydrogenase 2 (ALDH2) are members of a diverse family of enzymes that participates in the oxidation of a variety of aldehydes. In the brain, ALDH1A1 is significantly involved in the metabolism of biogenic aldehydes, norepinephrine, dopamine (DA) and gamma-aminobutyric acid (Maring et al., 1985). 3,4-Dihydroxyphenylacetaldehyde (DOPAL), a potentially toxic metabolite of DA is formed by the oxidative deamination of DA

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catalyzed by monoamine oxidases (Burke et al., 2003). DOPAL, an intermediate in the degradation of DA, is then oxidized to 3,4-dihydroxyphenylacetic acid (DOPAC) by ALDH1A1 or reduced to 3,4-dihydroxyphenylethanol (DOPET) by aldose/aldehyde reductase. Potentially damaging oxygen radicals are formed by this process (Adams et al., 2001). Although impaired DA transmission may influence ALDH activity and/or changes in ALDH-mediated metabolism may affect DA levels in nerve cell bodies and terminal fields in the basal ganglia and limbic system (Galter et al., 2003), data directly supporting this are lacking.

While ALDH1A1 is strongly and specifically expressed in ventral mesencephalic DA neurons in rodents (Westerlund et al., 2005) and man (Galter et al., 2003), its functional significance to the DA system and its possible relevance to Parkinson's disease (PD) is incompletely understood. mRNA encoding cytosolic ALDH1A1 is highly expressed in human substantia nigra (SN) and ventral tegmental area (VTA) dopaminergic (DAergic) neurons, with the virtual absence of this gene in neighboring non-DAergic cells (Galter et al., 2003). In brains from PD patients, there was a markedly lower expression of *Aldh1a1* mRNA in tyrosine hydroxylase (TH)-positive and DA transporter (DAT)-positive neurons in the SN, compared to non-PD controls. In contrast, *Aldh1a1* expression was not significantly decreased in TH- or DAT-positive neurons in the VTA in most PD patients (Galter et al., 2003). Based on these data, it was suggested that a down-regulation of *Aldh1a1* in SN DA neurons in PD might reflect a type of compensatory response of these cells to decrease the rate of DA degradation. However, reduced ALDH1A1 expression in the SN could also contribute to the development of PD rather than be a consequence of PD. With reduced ALDH1A1 expression, it is possible that the degradation of DA would be slowed at the level of DOPAL. An accumulation of DOPAL could be potentially toxic to DA neurons and could render these neurons more susceptible to aldehyde toxicity and degeneration.

Although current evidence suggests that ALDH1A1 is important for DA neuron function, there is little detail available on this, primarily due to absence of an appropriate animal model for study. The present investigation characterizes the influence of ALDH1A1 on DAergic function using an *Aldh1a1* (*Raldh1*) null mouse model (Fan et al., 2003) utilizing *in vivo* microdialysis and post-mortem evaluations of pre-and post-synaptic DAergic markers.

2. Results

All mice used were genotyped by PCR to confirm the presence of the Neo cassette which deleted the expression of the ALDH1A1 protein in the *Aldh1a1*$^{-/-}$ animals (Fig. 1A). Western blotting was used to visualize the effective deletion of ALDH1A1 protein in the striatum (Fig. 1B) and immunohistochemistry verified the lack of ALDH1A1 expression in neurons in the SN in *Aldh1a1*$^{-/-}$ animals (Fig. 1C).

2.1. Microdialysis

Basal extracellular fluid (ECF) DA levels in the striatum in awake *Aldh1a1*$^{-/-}$ mice (23.39±4.43 nM) were significantly higher than those observed in wildtype animals (7.59±0.86 nM; $t(12)=3.023$, $p=0.0106$) (Fig. 2A). Perfusion of the probe with 120 mM KCl resulted in significant release of DA in both *Aldh1a1*$^{-/-}$ animals (402.0±71.49 nM) and wildtype control animals (383.4±100.5 nM). However, *Aldh1a1*$^{-/-}$ animals had significantly less KCl-stimulated DA release (67%) than did wildtype animals ($t(12)=2.814$, $p=0.0156$), relative to basal ECF DA levels (Fig. 2B). Perfusion of the probe with 50 μM D-amphetamine also resulted in significant release of DA in both *Aldh1a1*$^{-/-}$ animals (232.7±75.85 nM) and wildtype control animals (218.4±48.05 nM). There was significantly less (68%) amphetamine-stimulated DA release in *Aldh1a1*$^{-/-}$ animals compared to wildtype animals ($t(12)=2.857$, $p=0.0144$), relative to basal ECF DA levels (Fig. 2C).

2.2. Tissue DA levels

There were no statistically significant differences in the levels of striatal DA measured in post-mortem tissue from *Aldh1a1*$^{-/-}$ or wildtype animals (Fig. 3A). *Aldh1a1*$^{-/-}$ animals had a significantly lower level of striatal DOPAC when compared to wildtype animals ($t(13)=2.257$, $p=0.0419$; Fig. 3B). However, an index of DA turnover (DOPAC/DA ratio) showed no statistically significant differences between *Aldh1a1*$^{-/-}$ animals and wildtype controls (Fig. 3C).

2.3. Tyrosine hydroxylase positive neuron number

Stereological estimates of the number of tyrosine hydroxylase-immunoreactive (TH-IR) neurons in the substantia nigra pars

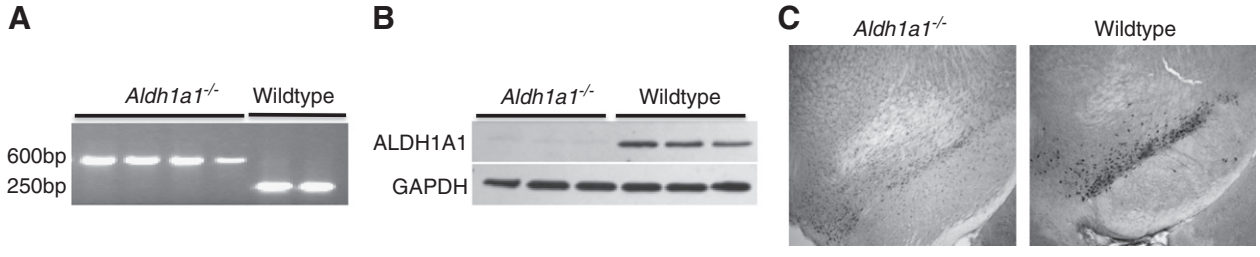

Fig. 1 – Expression of *Aldh1a1* mRNA and protein in *Aldh1a1*$^{-/-}$ and wildtype (WT) mice. (A) Representative genotyping gel. *Aldh1a1*$^{-/-}$ animals did not contain a functional copy of the *Aldh1a1* gene, but were positive for the Neo insert used to disrupt gene expression (NEO cassette, 600 bp; *Aldh1a1*, 250 bp). (B) Western blot analysis of ALDH1A1 protein in the striatum showed a significant loss of ALDH1A1 expression in the *Aldh1a1*$^{-/-}$ mice compared to normal expression levels in WT mice. Loading controls (using GAPDH) for all lanes are shown. (C) Immunohistochemical analysis of ALDH1A1 expression in the substantia nigra pars compacta showed significant loss ALDH1A1-immunoreactivity when compared to WT animals.