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Research Paper

Reduced vesicular monoamine transport disrupts serotonin signaling but does not cause serotonergic degeneration

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ABSTRACT

We previously demonstrated that mice with reduced expression of the vesicular monoamine transporter 2 (VMAT2 LO) undergo age-related degeneration of the catecholamine-producing neurons of the substantia nigra pars compacta and locus ceruleus and exhibit motor disturbances and depressive-like behavior. In this work, we investigated the effects of reduced vesicular transport on the function and viability of serotonin neurons in these mice. Adult (4–6 months of age), VMAT2 LO mice exhibit dramatically reduced (90%) serotonin release capacity, as measured by fast scan cyclic voltammetry. We observed changes in serotonin receptor responsivity in in vivo pharmacological assays. Aged (months) VMAT2 LO mice exhibited abolished 5-HT1A autoreceptor sensitivity, as determined by 8-OH-DPAT (0.1 mg/kg) induction of hypothermia. When challenged with the 5HT2 agonist, 2,5-dimethoxy-4-iodoamphetamine (1 mg/kg), VMAT2 LO mice exhibited a marked increase (50%) in head twitch responses. We observed sparing of serotonergic terminals in aged mice (18–24 months) throughout the forebrain by SERT immunohistochemistry and [^{3}H]-paroxetine binding in striatal homogenates of aged VMAT2 LO mice. In contrast to their loss of catecholamine neurons of the substantia nigra and locus ceruleus, aged VMAT2 LO mice do not exhibit a change in the number of serotonergic (TPH2 +) neurons within the dorsal raphe, as measured by unbiased stereology at 26–30 months. Collectively, these data indicate that reduced vesicular monoamine transport significantly disrupts serotonergic signaling, but does not drive degeneration of serotonin neurons.

1. Introduction

Parkinson's disease (PD) consistently features disruption and preferential degeneration of catecholamine neurotransmitter systems, including the destruction of the nigrostriatal dopamine (DA) system and the noradrenergic locus ceruleus (Manaye et al., 1992; Mann and Yates, 1983). Alterations in the serotonin (5-HT) system are less consistent. The serotonergic raphe nuclei also degenerate in PD (Halliday et al., 1990), though this pathology does not correlate with progression of motor symptoms (Politis et al., 2010), but rather is most pronounced in PD patients with depression (Jellinger and Paulus, 1992; Paulus and Jellinger, 1991). In contrast to striatal dopamine system, serotonergic innervation in the striatum is often preserved through later stages of PD (Bédard et al., 2011). Despite this resilience, reduced levels of 5-HT (Kish et al., 2008) and disruptions in serotonergic signaling likely contribute to the high incidence of comorbid depression and other psychiatric symptoms in PD (Fahn, 2003), and have been hypothesized to underlie the pathophysiology of L-DOPA induced dyskinesia (Cenci and Lundblad, 2006).

The basis for the shared vulnerability of monoaminergic neurons in PD may lie in their common molecular processes, which include the synthesis, storage, and metabolism of reactive monoamine neurotransmitters, all of which are influenced by the vesicular monoamine transporter 2 (VMAT2). Following synthesis or plasmalemmal uptake of monoamines, VMAT2 sequesters these transmitters from the cytosol into acidic synaptic vesicles for stable storage and exocytic release. Epidemiological studies have shown that *Slc18a2* promoter haplotypes that confer increased expression or function of VMAT2 are protective against PD (Brighina et al., 2013; Glatt et al., 2006), while reduced *Slc18a2* mRNA expression has been detected in the platelets of PD patients (Sala et al., 2010). Immunohistochemical analyses of postmortem brain tissue show that there is substantially less VMAT2 in caudate and putamen of PD cases than would be expected from degenerative loss (Miller et al., 1999a,b, 1997). This was recently confirmed to have a functional effect, when Pifl and colleagues demonstrated that vesicular transport of dopamine via VMAT2 is reduced in synaptic vesicles

Abbreviations: 5-HT, 5-hydroxytryptamine serotonin; DA, dopamine; NE, norepinephrine; SERT, serotonin transporter; TPH2, tryptophan hydroxylase 2; VMAT2, vesicular monoamine transporter 2.

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isolated from the striata of PD patients in comparison to control cases (Pifl et al., 2014).

While there is strong support for the role of reduced vesicular function in dopaminergic degeneration in PD, there has been less direct investigation into the relationship between vesicular function and the vulnerability of noradrenergic and serotonergic neurons in PD. However, these neurotransmitters are subject to similar cytosolic degradation pathways, which led us to hypothesize that reduced vesicular function contributes to their dysfunction and degeneration as well. In settings of reduced vesicular function, monoamines accumulate in the alkaline conditions of the cytosol, where they are subject to breakdown by spontaneous and enzymatic oxidative processes. Excessive cytosolic breakdown of DA and NE by either fate (enzymatic deamination or autoxidation) has been demonstrated to be neurotoxic (Caudle et al, 2007; Crino et al., 1989; Hastings et al., 1996; Mosharov et al., 2009; Taylor et al., 2014; Ulusoy et al., 2012). Less is known about the neurotoxic potential of cytosolic 5-HT, though mice with conditional knockout of VMAT2 in 5-HT neurons exhibit normal initial development of the raphe cytoarchitecture (Narboux-Neme et al., 2011).

Mice with globally reduced (~95%) expression of VMAT2 (VMAT2 LO) exhibit neurochemical depletion and increased cytosolic breakdown of DA (~95%), NE (90%), and 5-HT (80%). These mice develop normally, but undergo age-related degeneration of the SNpc and LC, and display motor and nonmotor symptoms (including depressive behavior) of PD (Caudle et al., 2007; Taylor et al., 2014, 2009). In this work, we used VMAT2 LO mice to investigate the effects of disrupted vesicular monoamine transport on the synaptic release of 5-HT. We then evaluated physiological consequences specific to the 5-HT system in tests of 5-HT1A and 5-HT2 receptor responsivity. Lastly, we evaluated the integrity of serotonergic innervation and the neuronal population of the dorsal raphe to determine if reduced vesicular 5-HT storage can cause neurodegeneration.

2. Materials and methods

2.1. Chemicals and reagents

(R)-(+)-8-OH-DPAT (#ab1210507) was purchased from Abcam (Cambridge, MA), DOI ((R)-(−)-2,5-dimethoxy-4-iodoamphetamine; #D153), levodopa (D150), benserazide (B7283), and fluoxetine (#F132) were purchased from Sigma-Aldrich (St. Louis, MO), and [^{3}H]-paroxetine (#Art177) was purchased from American Radiolabeled Chemical Inc. (St. Louis, MO). Antibodies and their sources are described below. All other chemicals were purchased from Thermo Fisher Scientific (Waltham, MA).

2.2. Mice

Male and female VMAT2 LO mice (expressing 5% of wildtype VMAT2 levels) were generated as previously described (Caudle et al., 2007). Mice of both genders were used in all experiments. All procedures were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Emory University.

2.3. Ages of mice used in experiments

From staging of catecholaminergic neurodegeneration in previous work (Caudle et al., 2007; Taylor et al., 2014), VMAT2 LO mice exhibit DAT and NET loss at 12 months, LC loss at 12 months, and nigral degeneration at 18–24 months. We have used aged mice as follows:

Young adult mice (between 4 and 6 months of age) were used in voltammetry experiments and in the DOI head-twitch response assay to determine the phenotypic, baseline effect of reduced VMAT2 expression on synaptic 5-HT release and postsynaptic signaling. Aged mice (between 18 and 24 months) for paroxetine-binding and SERT immunohistochemistry to capture a time point during which DAT and NET loss had already occurred (and LC and nigral degeneration had begun). 18–24 month old mice were also used in the 8-OH-DPAT assay. Advance-aged mice (26–30 months) were used the test for levodopa induced dyskinesia and for dorsal raphe stereology to capture a most neurodegenerative state.

2.4. Immunohistochemistry

Mice were transcardially perfused with phosphate-buffered saline and 4% paraformaldehyde. Their brains were then removed and stored in 4% paraformaldehyde for 24 h, and cryopreserved in 30% sucrose for 48 h. Brains were then sliced on a freezing microtome at 40 μm and collected into cryopreservative. Immunohistochemistry was performed as previously described (Caudle et al., 2007; Taylor et al., 2014). Following tissue slicing, free-floating sections were incubated with a polyclonal rabbit antibody against either TPH2 (1,5:000; Novus NB100-74555, Littleton, CO) overnight at 4°, or SERT for 48 h at room temperature (1:10,000; Immunostar 24,330, Hudson, WI). Secondary incubations were performed with biotinylated polyclonal goat anti-rabbit antibody (1:200; Jackson 111-065-003, Hudson, WI).

2.5. Stereology of the dorsal raphe nucleus

Stereology was performed to determine the number of serotonergic (TPH2+) neurons in the dorsal raphe nucleus of aged (26–30 months) mice. Following immunohistochemistry against TPH2, tissue slices were mounted on microscope slides, processed, and counterstained with cresyl violet to identify cell nuclei as previously described (Taylor et al., 2014). Stereological sampling (West et al., 1991) was performed using Stereo Investigator software (MicroBrightField, Colchester, VT). Dorsal raphe contours (comprising the dorsal, ventral, and intrafascicular regions of the nucleus) were outlined as defined by the atlas of Paxinos and Franklin (2001), using a 4× objective. Final tissue thickness was 24 μm; guard zones of 2 μm were used to exclude lost profiles on the top and bottom of tissue sections. Cells were counted with a 40× objective (1.3× numerical aperture), using a counting frame of 50 × 50 μm, and sampling grid of 80 × 100 μm, at an evaluation interval of three sections (yielding 6–7 sections per animal). A serotonergic neuron was defined as an in-focus TPH2-immunoreactive cell body with a TPH2-negative nucleus. Nissl+ cells were defined as having a cresyl-violet positive nucleus or TPH2+ cell body. The Gundersen ($m = 1$) coefficient of error was less than 0.1 for each animal.

2.6. Fast scan cyclic voltammetry in substantia nigra pars reticulata

Fast scan cyclic voltammetry was performed as previously described (Lohr et al., 2014), with modification to measure 5-HT release in the substantia nigra pars reticulata (SNr). Adult wildtype and VMAT2 LO mice (aged 4–6 months, $n = 3$) were decapitated and their brains quickly transferred to ice cold oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (aCSF). Brains were mounted in aCSF onto the stage of a vibratome (Leica VT100S) and sliced at 300 μm. Midbrain slices were collected and maintained in oxygenated aCSF at room temperature. After 30 min, slices were placed in a slice perfusion chamber. Stimulating and carbon-fiber recording electrodes were placed in the lateral region of the SNr, as defined by Paxinos. A four-recording survey of three different sites within the SNr was taken for each animal with a 5-min rest interval between each stimulation (20 pulses, 100 Hz, 350 μA, 4 ms, monophasic). Neurochemical identity of 5-HT was confirmed with a 5-HT-specific waveform and by observing increased signal in the presence of 10 μM fluoxetine. Synaptic clearance of 5-HT was assessed by measuring the rate constant *tau*, which is a measure of the time required for the 5-HT signal to decay to 1/3 of its peak height.

Carbon-fiber microelectrodes were calibrated with 5-HT standards using a flow-cell injection system. Kinetic constants were extracted