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Regulation of the dopaminergic system in a murine model of aromatic L-amino acid decarboxylase deficiency

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ABSTRACT

Aromatic L-amino acid decarboxylase (AADC) is responsible for the syntheses of dopamine and serotonin. Children with AADC deficiency exhibit compromised development, particularly with regard to their motor functions. Currently, no animal model of AADC deficiency exists. We inserted an AADC gene mutation (IVS6+4A>T) and a neomycin-resistance gene into intron 6 of the mouse AADC (*Ddc*) gene. In the brains of homozygous knock-in (KI) mice ($Ddc^{IVS6/IVS6}$), AADC mRNA lacked exon 6, and AADC activity was <0.3% of that in wild-type mice. Half of the KI mice were born alive but grew poorly and exhibited severe dyskinesia and hindlimb clasping after birth. Two-thirds of the live-born KI mice survived the weaning period, with subsequent improvements in their growth and motor functions; however, these mice still displayed cardiovascular dysfunction and behavioral problems due to serotonin deficiencies. The brain dopamine levels in the KI mice increased from 9.39% of the levels in wild-type mice at 2 weeks of age to 37.86% of the levels in wild-type mice at 8 weeks of age. Adult KI mice also exhibited an exaggerated response to apomorphine and an elevation of striatal c-Fos expression, suggesting post-synaptic adaptations. Therefore, we generated an AADC deficient mouse model, in which compensatory regulation allowed the mice to survive to adulthood. This mouse model will be useful both for developing gene therapies for AADC deficiency and for designing treatments for diseases associated with neurotransmitter deficiency.

Introduction

Aromatic L-amino acid decarboxylase (AADC, EC 4.1.1.28) is a homodimeric pyridoxal phosphate-dependent enzyme involved in the metabolic pathways responsible for the syntheses of two monoamine neurotransmitters, dopamine and serotonin (Hamosh and McKusick, 2011). AADC decarboxylates L-3,4-dihydroxyphenylalanine (L-DOPA) to dopamine, L-5-hydroxytryptophan to serotonin, and L-tryptophan to tryptamine (Consortium, 2011; Hamosh and McKusick, 2011). Dopamine is the precursor of catecholaminergic hormones and is also itself a neurotransmitter in the basal ganglia. Dopamine is responsible for reward-driven learning, voluntary movement control, feeding, neuroendocrine secretion, cognition, and behavior (Crittenden and Graybiel, 2011; Hamosh and McKusick, 2011). Serotonin has important neuromodulatory actions in cognitive, emotional, impulse control, circadian rhythm, sleep–wake cycle, pain, respiratory, and cardiovascular functions (Benarroch, 2009). AADC also mediates the syntheses of trace amines (Berry, 2004).

Defects in the AADC gene result in a deficiency of dopamine and serotonin and their downstream metabolites. AADC deficiency (MIM #608643) is an autosomal recessive inborn error of metabolism and was first identified in 1990 by Hyland and Clayton (Hyland and Clayton, 1990; Hyland et al., 1992). Approximately eighty cases were reported worldwide between 1990 and 2010 (Abeling et al., 1998; Brautigam et al., 2000; Brun et al., 2010; Fiumara et al., 2002; Korenke et al., 1997; Maller et al., 1997; Pons et al., 2004; Swoboda et al., 1999; Swoboda et al., 2003; Tay et al., 2007). Clinical manifestations of AADC deficiency include hypotonia, hypokinesia, oculogyric crises, and signs of autonomic dysfunction, beginning early in life (Brun et al., 2010).

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AADC deficiency is comparatively less rare in Taiwan relative to other countries because of a splice site mutation (IVS6+4A>T) in the Taiwanese population (Brun et al., 2010). This mutation interrupts the donor consensus sequence of intron 6, which causes aberrant splicing of the gene (Lee et al., 2009; Tay et al., 2007).

L-DOPA is an effective drug for Parkinson's disease and tyrosine hydroxylase (TH) deficiency. However, L-DOPA cannot be converted to dopamine in cases of AADC deficiency, and dopamine does not cross the blood–brain barrier. Patients with AADC deficiency only partially respond to dopamine agonists, and many patients die during childhood (Pons et al., 2004). Recently, gene therapy was tested in patients with AADC deficiencies (Hwu et al., 2012). The treatment employed an adeno-associated virus vector that was injected into the bilateral putamen of patients to replenish AADC levels. Gene therapy improves the motor function of patients and decreases the severity of oculogyric crisis. However, the development of a treatment for AADC deficiency is still hampered by the lack of an animal model of the disease.

Knockout of the mouse AADC (*Ddc*) gene results in death in utero (unpublished observation by H.I.). Therefore, in this study, we employed a knock-in (KI) strategy by inserting an IVS6+4A>T mutation into the mouse AADC gene. Homozygous KI mice were able to survive into adulthood and exhibited biochemical and movement abnormalities similar to those observed in patients with AADC deficiencies.

Materials and methods

Generating the KI mice

An artificial mouse bacterial chromosome containing exon 6 and intron 6 of the AADC gene was obtained from a 129/SvJ genomic library. The KI vector was constructed by the Transgenic Mouse Model Core Facility (Liu et al., 2003), and Southern blot analyses were performed to select KI embryonic stem (ES) cells, as previously described (Su et al., 2007). The AADC genotype was determined by polymerase chain reaction (PCR) using the primers 5′-AGGCGCATTCCTCTGTAGAA and 5′-CCCAAATAGTGCCAACACCT, followed by direct sequencing. Heterozygous ES cell clones ($Ddc^{+/IVS6}$) were microinjected into 129/B6 blastocysts and then transferred into pseudopregnant females to generate chimeras. The chimeric mice were mated with B6 mice to generate heterozygous F1 mice. Mice were housed under standard conditions with artificial 12-h dark–light cycles. Behavioral tests, tissue sampling, blood sampling, and imaging analyses were performed on homozygous ($Ddc^{IVS6/IVS6}$) KI mice at 2, 4, 8, and 12 weeks of age. Either wild-type or heterozygous littermates were used as controls. These animal studies were approved and performed in accordance with the guidelines of the National Taiwan University College of Medicine and College of Public Health Institutional Animal Care and Use Committee (IACUC no. 2110134).

RNA analyses

Mice were sedated with avertin (0.3–0.4 μg/g body weight) before euthanasia. Coronal slices of the brain containing either the striatum or the substantia nigra were homogenized with Trizol® for RNA studies. Total RNA was extracted using the Trizol® reagent according to the manufacturer's protocol. The reverse transcription (RT) reaction mixture included 2 μg of RNA and 0.5 μg of oligo(dT). The PCR reactions either included exon 6 using the primers 5′-ACTGGCTGCTCGGACTAAAG and 5′-CCCACTTCCAGGAGATTGTC or were specific to exon 6 using the primers 5′-CATGAGAGCTTCTGCCCTTC (located on exon 6) and 5′-GCAAACTCCACACCATTCAG; the latter was used for mRNA quantitation. The genes amplified and the PCR primers used are as follows: mouse dopamine active transporter (DAT; *Slc6a3*), 5′-TGCTGGTCATTGTTCTGCTC and 5′-TATGCTCTGATGCCATCCAT; vesicular monoamine transporter 2 (VMAT2; *Slc18a2*), 5′-CAAGCTGATCCTGTTCATCG and 5′-GGAAGTGGAGGCTGTGAGC; TH (*Th*), 5′-CGTCATGCCTCCTCACCTAT and 5′-CCCAGAGATGCAAGTCCAAT; catechol-*O*-methyltransferase (COMT; *comt*), 5′-CTGACTACGCTGCCATCACC and 5′-TAGCGGTCTTTCCAGTGGTC; c-Fos (c-*fos*), 5′-GAATGGTGAAGACCGTGTCA and 5′-GCAGCCATCTTATTCCGTTC; substance P (protachykinin-1; *Tac1*), 5′-GGATGCTGATTCCTCAGTTG and 5′-TAGTTCTGCATCGCGCTTCT; dynorphin (prodynorphin; *Pdyn*), 5′-TGCAGTGAGGATTCAGGATG and 5′-GCAACCTCATCTTCCAAGTCA; enkephalin (preproenkephalin; *Penk*), 5′-CCTGCCTCCTGGCTACAGT and 5′-GCAGGAGATCCTTGCAGGT; and *β-actin*, 5′-GCTACAGCTTCACCACCACA and 5′-TCTCCAGGGAGGAAGAGGAT. Quantitative PCR results were expressed as $2^{-\Delta\Delta CT}$, representing the quantity of PCR products of KI mice relative to that of wild-type mice after normalization to the quantity of *β-actin* (Livak and Schmittgen, 2001).

Western blot, immunohistochemistry (IHC), and immunofluorescence (IF) analyses

Mouse brain coronal slices were snap frozen in liquid nitrogen and stored at −80 °C for western blot analyses using TH (1:1000, Millipore, Billerica, MA, USA), AADC (1:1500, Abcam, Cambridge, UK), or tubulin (1:1500, MDBio, Taipei, Taiwan) antibodies. Mouse striatum was isolated for western blot analyses using dopamine D1 receptor (1:500, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), D2 receptor (1:500, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), and MAOA (1:500, Proteintech) antibodies. For IHC and IF studies, mice were perfused with 4% paraformaldehyde. The brains were removed, post-fixed overnight at 4 °C, cryoprotected with 30% sucrose in phosphate-buffered saline (PBS) for 48 h, mounted in OCT embedding compound, and frozen. Coronal sections (40 μm in thickness) were cut on a cryostat and collected in PBS. IHC was performed by incubation overnight with TH (1:50,000) (Nagatsu et al., 1979) or AADC (1:20,000) antibodies (Nagatsu et al., 1988). The slides were washed, incubated for 2 h with a biotinylated anti-rabbit IgG secondary antibody (1:200, Vector Laboratories, Burlingame, CA, USA), and visualized using the VECTASTAIN Elite ABC Kit and Peroxidase Substrate DAB Kit (both from Vector Laboratories, Burlingame, CA, USA). Dual IF staining for TH and AADC was performed by incubation with a mixture of TH (1:800, ImmunoStar, Hudson, WI, USA) and AADC (1:5000) antibodies, followed by Alexa Fluor 594-labeled anti-rabbit IgG and Alexa Fluor 488-labeled anti-mouse IgG secondary antibodies (both from Life Technologies, Grand Island, NY, USA). The sections were viewed and photographed with a confocal laser scanning microscope (FV10i, Olympus, Tokyo, Japan). Total numbers of AADC- and TH-stained neurons throughout the entire substantia nigra were counted stereologically in a blinded fashion with Stereo Investigator software (MBF Bioscience, Williston, VT, USA) using the Optical Fractionator Probe module. Coronal sections (10 μm in thickness) were also cut for IHC with c-Fos (1:300, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and serotonin reuptake transporter (SERT, 1:500, Millipore, Billerica, MA, USA) antibodies. For c-Fos staining, adult mice (8 weeks of age) were injected with apomorphine subcutaneously 2 h before sacrifice. The number of c-Fos-positive nuclei in the striatum was quantified under a light microscope. Three serial sections were stained, counted, and averaged for each striatum.

Positron emission tomography (PET)

PET was performed as previously reported (Lin et al., 2010; Vuckovic et al., 2010). For 6-[^{18}F]fluoro-L-DOPA (FDOPA) PET, mice were treated with carbidopa (25 mg/kg, i.p.) and entacapone (25 mg/kg, i.p.) 30 min before the injection of FDOPA (250 μCi for 2-week-old mice and 500 μCi for 8-week-old mice, i.p.). Thirty min after FDOPA injection, mice were anesthetized with isoflurane, and images were obtained using a small animal PET/CT scanner (eXplore Vista DR, GE Healthcare, Fairfield, CT, USA).