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BRAIN RESEARCH

Research Report

Aromatic L-amino acid decarboxylase expression profiling and isoform detection in the developing porcine brain

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ABSTRACT

Aromatic L-amino acid decarboxylase (AADC) enzymatic activity is essential for the biosynthesis of the serotonin and dopamine neurotransmitters, and AADC activity is functionally associated with a number of human neuronal disorders. Here we describe the molecular characterization of AADC from the pig. Pig AADC shows a high degree of similarity to human and rodent AADC at the cDNA and protein level. Exon position shuffling has exchanged the location of the stop codon in pig AADC to the last exon 15 instead for the exon 14 position in the human, the rat, and the mouse AADC. Several pig AADC isoforms were identified, including the also in human described extraneuronal and neuronal isoforms generated by alternative splicing and alternative promoter usage. The AADC expression in the developing pig brain is highly expressed in the basal ganglia and the brain stem regions, and also significantly expressed in the cortex, the hippocampus and the cerebellum. Moreover, we observe that both the neuronal and the extraneuronal AADC mRNA isoforms were present at early brain developmental stages in the brain stem and the basal ganglia. This presents the first evidence that the non-neuronal AADC isoform also is expressed in the brain. Together our results propose that the porcine model is useful for future functional delineations of the AADC gene at the molecular level.

1. Introduction

Aromatic L-amino acid decarboxylase (AADC) is a pyridoxal 5′-phosphate (PLP)-dependent enzyme that catalyzes the irreversible decarboxylation of aromatic L-amino acids, as dopa (3,4-dihydroxyphenylalanine), m-tyrosine, p-tyrosine, phenylalanine, 5-hydroxytryptophan, and tryptophan, and thereby generates important neurotransmitters (OMIM *107930) (Zhu and Juorio, 1995; Misu et al., 2002). Tyrosine hydroxylase catalyses the hydroxylation of tyrosine to generate L-DOPA which is decarboxylated by AADC to form dopamine. Dopamine is found in various areas of the brain but is also produced

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Abbreviations: AADC, Aromatic l-amino acid decarboxylase; Brd1, bromo domain protein 1; BSA, bovin serum albumin; Dopa, 3,4-dihydroxyphenylalanine; E, embryonic day; EST, expressed sequence tag; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; kDa, kilodalton; mRNA, messenger RNA; NF-Y, nuclear factor Y; ORF, open reading frame; POU3F2, POU class 3 homeobox 2; RT-PCR, reverse transcriptase polymerase chain reaction; SNP, single nucleotide polymorphism; SP1, specificity protein 1; THY-1, T-cell antigen 1; UTR, untranslated region

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by AADC in the sympathetic nervous system and is the precursor of the catecholaminergic hormones, noradrenaline and adrenaline in the adrenal medulla. Another neurotransmitter, serotonin, is also generated by AADC. In the nervous system, tryptophan hydroxylase produces 5-OH tryptophan, which is decarboxylated by AADC to generate serotonin. AADC expression is well characterized and is abundant mainly in catecholamine and 5-hydroxytryptophan containing neurons in the central nervous system. Expression of AADC in neurons in other parts of the central nervous system has also been observed, as well as expression in extraneuronal tissues (Lloyd and Hornykiewicz, 1970; Jaeger et al., 1984; Jaeger, 1986; Eaton et al., 1993). This suggests either an additional function of AADC as a decarboxylating enzyme with yet undetermined substrates or that AADC may have additional cellular functions (Zhu and Juorio, 1995; Wafa et al., 2003).

It is clear that AADC has major importance for the physiology and pathophysiology of neurotransmission. Deficiency of AADC is associated with severe developmental delay, oculogyric crises, and autonomic dysfunction (Pons et al., 2004). Patients with AADC deficiency develop symptoms in the first year of life, and approximately 50% are symptomatic during the neonatal period. Treatment with dopamine agonists is beneficial. Early initiation of treatment may lead to better prognosis because appropriate stimulation of dopamine and serotonin receptors is likely to be crucial for normal motor and cognitive development (Pons et al., 2004). AADC has also been implicated in the pathogenesis of Parkinson's disease and, in addition, AADC is a candidate gene for predisposition to a variety of neurological disorders including bipolar affective disorder and schizophrenia (Zhu and Juorio, 1995; Borglum et al., 1999; Jahnes et al., 2002; Tehranian et al., 2006).

The human AADC gene is located on chromosome 7p12.2, consists of 15 exons, and encodes a protein of 480 amino acids (Ichinose et al., 1989). Multiple alternative splicing events have been described for the human and the rodent AADC (Krieger et al., 1991; Albert et al., 1992; Ichinose et al., 1992; O'Malley et al., 1995; Vassilacopoulou et al., 2004). Neuronal and extraneuronal expression is directed from distinct promoters resulting in mutually exclusive used first exons (Krieger et al., 1991; Albert et al., 1992; Ichinose et al., 1992; Le Van Thai et al., 1993; Aguanno et al., 1995; Chatelin et al., 2001). These alternative promoter usages and splicing events however results in the production of mRNA with identical AADC protein encoding open reading frames. Alternative splicing has also been described in the coding region and in particular exclusion of exon 3 is in humans found to be abundant, generating an AADC protein isoform with an internal deletion and without enzymatic activity (O'Malley et al., 1995; Vassilacopoulou et al., 2004).

Purified pig pancreas AADC is the reference enzyme for analysis of AADC activity. The entire cDNA has not been described and recombinant expression of pig AADC is based on synthetic constructs deduced from the amino acid sequence determined from the purified pig AADC protein (Moore et al., 1996). Moreover, the expression pattern within the developing pig brain for this key enzyme has not been described. To substantiate the basic knowledge of pig AADC and its expression during brain development and to explore the possibility of using pigs as an available source for also the biological analysis of AADC, we here present a primary sequence and expression characterization of pig AADC. Our presented data show that pig AADC at the genomic level and in expression patterns resembles human AADC but also points at important novelties. The detailed molecular and functional analysis possible in a pig model can be an important future tool to delineate the physiology and pathophysiology of AADC mediated neurotransmission during fetal brain development.

2. Results

2.1. *Characterization of porcine AADC DNA*

To identify the sequence of the entire pig AADC cDNA we utilized a combination of *in silico* cloning and reverse transcriptase polymerase chain reaction (RT-PCR) approaches. For a schematic illustration of the AADC gene see Fig. 1A. PCR primers were designed according to AADC regions conserved between the human and the mouse within the 15 AADC exons and through available porcine expressed sequence tag (EST) sequences (www.ncbi.nlm.nih.gov/genome/seq/SscBlast.html). Liver and brain cDNA from a newborn pig was used as template to amplify and sequence the AADC transcript (Fig. 1B). The entire open reading frame (ORF) encoded by exons 2–14 showed 89% identity and 95% homology to human AADC, 86% identity and 94% homology to rat AADC, and 87% identity and 95% homology to mouse AADC. The high degree of evolutionary conservation of the AADC protein is indicated from the 77% identity and 87% homology to zebrafish AADC and 59% identity and 78% homology to drosophila AADC. The pig AADC protein consists of 486 amino acids and has a deduced molecular weight of 54 kDa. Western blotting of pig brain and liver extracts showed the presence of a protein of the expected size (Fig. 1C).

To examine for common polymorphisms in pig AADC we extracted genomic DNA from the breeds, Hampshire, Duroc, Landrace, Yorkshire, and Goettingen minipigs and sequence analyzed PCR products spanning the AADC exons. Twelve alleles were examined for each breed. Several polymorphisms were detected. At position 1423 a G/A single nucleotide

Fig. 1 – Primary characterization of the pig AADC. (A) Schematic representation of the pig AADC gene and determined splicing events. AADC486 is the isoform containing all exons from exon 2 to exon 15. AADC438 is the isoform lacking exon 5 and AADC393 the isoform lacking exon 5 and exon 6 due to alternative splicing. The different 5′ alternative promoter usages and alternative splicing are indicated by lines. The position of translational start and stop codons are indicated. (B) Sequence of pig AADC cDNA corresponding to exon 2 to exon 15. The encoded AADC protein is shown below the sequence. The alternative exon 4 is indicated in bold and exon 5 sequences in italic. The polyadenylation consensus signal is underlined. (C) Western blot analysis of pig AADC in brain and liver. Whole cell extracts were processed for western blot analysis with an anti AADC antibody. Molecular weights in kDa are indicated to the left.