Neurochemistry International 90 (2015) 173–184

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Mammalian CSAD and GADL1 have distinct biochemical properties and patterns of brain expression

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ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form
30 July 2015
Accepted 22 August 2015
Available online 1 September 2015

Keywords:
Taurine
Cysteine sulfinic acid decarboxylase
Aspartate
Brain
Pyridoxal-phosphate
Lithium

ABSTRACT

Variants in the gene encoding the enzyme glutamic acid decarboxylase like 1 (GADL1) have been associated with response to lithium therapy. Both GADL1 and the related enzyme cysteine sulfinic acid decarboxylase (CSAD) have been proposed to be involved in the pyridoxal-5′-phosphate (PLP)-dependent biosynthesis of taurine. In the present study, we compared the catalytic properties, inhibitor sensitivity and expression profiles of GADL1 and CSAD in brain tissue. In mouse and human brain we observed distinct patterns of expression of the PLP-dependent decarboxylases CSAD, GADL1 and glutamic acid decarboxylase 67 (GAD67). CSAD levels were highest during prenatal and early postnatal development; GADL1 peaked early in prenatal development, while GAD67 increased rapidly after birth. Both CSAD and GADL1 are being expressed in neurons, whereas only CSAD mRNA was detected in astrocytes. Cysteine sulfinic acid was the preferred substrate for both mouse CSAD and GADL1, although both enzymes also decarboxylated cysteic acid and aspartate. *In silico* screening and molecular docking using the crystal structure of CSAD and *in vitro* assays led to the discovery of eight new enzyme inhibitors with partial selectivity for either CSAD or GADL1. Lithium had minimal effect on their enzyme activities. In conclusion, taurine biosynthesis in vertebrates involves two structurally related PLP-dependent decarboxylases (CSAD and GADL1) that have partially overlapping catalytic properties but different tissue distribution, indicating divergent physiological roles. Development of selective enzyme inhibitors targeting these enzymes is important to further dissect their (patho)physiological roles.

1. Introduction

Lithium salts are among the most effective pharmacological agents used in psychiatry. Although several molecular targets have been identified, including protein kinases and enzymes involved in phosphoinositide metabolism, lithium's mode of action at the cellular and molecular level is still being debated (Malhi et al., 2013). A strong genetic association between variants in the human glutamic acid decarboxylase like 1 (GADL1) gene and the response to lithium therapy in bipolar patients was recently reported (Chen et al., 2014). Although these findings were not replicated in other clinical samples (Cristiana et al., 2015), these results are intriguing and warrants a biochemical investigation on GADL1 and the effects of lithium on this enzyme.

Human GADL1 was recently found to function as a cysteine sulfinic acid decarboxylase and postulated to be involved in taurine and possibly also β-alanine and carnosine production *in vivo* (Liu et al., 2012). The sulfur amino acid taurine (2-amino-ethanesulfonic acid) is abundant in mammalian tissues and has been implicated in many physiological functions. Taurine has a regulatory role in maintenance of osmotic pressure and preservation of structural integrity of biological membranes (Hoffmann and

Abbreviations: AADC, Aromatic amino acid decarboxylase; ADC, Aspartate decarboxylase; APS-1, Autoimmune polyendocrine syndrome type 1; BSA, Bovine serum albumin; CA, Cysteic acid; CDO, Cysteine oxidase; CSA, Cysteine sulfinic acid; CSAD, CSA decarboxylase; GABA, Gamma-amino-butanoic acid; GAD, Glutamate decarboxylase; GADL1, Glutamate decarboxylase like 1; HDC, Histidine decarboxylase; ITT, *in vitro* transcription/translation system; OPA, *o*-phtaldialdehyde; MBP, Maltose-binding protein; PCW, Post conception week; PLP, Pyridoxal-5′-phosphate; TEV, Tobacco Etch Virus protease.

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http://dx.doi.org/10.1016/j.neuint.2015.08.013
0197-0186/

Pedersen, 2006; Schaffer et al., 2010). In the nervous system, taurine may modulate protein phosphorylation (Lombardini, 1994), serve as a trophic factor (Hernandez-Benitez et al., 2010; Pasantes-Morales and Hernandez-Benitez, 2010), or act as a neurotransmitter/neuromodulator (Jia et al., 2008). In several species, taurine deficiency has been linked to specific disease states (Schaffer et al., 2010) and also in humans dietary intake of taurine in the form of energy drinks or vitamin supplements is widespread, although with unclear health implications (Bigard, 2010).

In mammalian tissues taurine is mainly synthesized from cysteine in a three step sequential pathway, involving oxidation by cysteine dioxidase (CDO, E.C. 1.13.11.20), decarboxylation by cysteine sulfinic acid decarboxylase (CSAD, E.C. 4.1.1.29) and finally oxidation of hypotaurine to taurine. Alternatively, taurine may be formed from cysteamine by cysteamine dioxygenase (E.C. 1.13.11.19). The tissue distribution of the various enzymes involved in cysteine metabolism seems to reflect different metabolic demands of these tissues (Stipanuk et al., 2006). Thus, the protein levels of liver CDO, which is rate limiting in the degradation of the potentially toxic amino acid cysteine are tightly regulated in response to cysteine load (Stipanuk et al., 2009).

In comparison, the physiological role and regulation of CSAD and GADL1 are less understood. Mammalian CSAD has been isolated from liver, kidney and brain where it exists as a dimer with a reported subunit molecular mass of approx. 43–55 kDa (Heinamaki et al., 1982; Tang et al., 1996; Tappaz et al., 1998). GADL1 is expressed in muscle and kidney tissue (Liu et al., 2012). However, its pattern of expression in other tissues, including brain is not known. CSAD and GADL1 belong to a large family of pyridoxal-5′-phosphate (PLP)-dependent enzymes that catalyze a range of different reactions, such as decarboxylation, transamination, racemization or eliminations using amino acids or related substrates (Toney, 2011). Crystal structures of many PLP-dependent enzymes, including CSAD, have been published (http://www.rcsb.org/pdb/explore/explore.do?structureId=2JIS).

In the brain, CSAD has mainly been detected in astrocytes in cerebellum and hippocampus (Reymond et al., 1996a), although there are also reports of CSAD being found in neurons (Chan-Palay et al., 1982). Based on the different tissue distribution of CDO and CSAD, it was proposed that the taurine synthesis pathway is initiated in neurons and completed in astrocytes (Dominy et al., 2004). More recently, taurine biosynthesis from cysteine in murine neurons and astrocytes was reported, indicating that the complete enzymatic machinery for taurine synthesis is present in both cell types (Vitvitsky et al., 2011). However, the identity of the enzymes involved in the synthesis in the two cells types is not known.

A CSAD knockout mouse was recently described (Park et al., 2014). The plasma levels of taurine were reduced by 83% in CSAD−/− mice and most offspring from 2nd generation CSAD−/− mice died shortly after birth, indicating an important physiological role of CSAD.

The aims of our study were (i) to determine the effects of lithium on GADL1 and CSAD, (ii) to compare the substrate specificities of these enzymes, to use this knowledge to find inhibitors of the enzymes and (iii) to study their cellular, regional and temporal patterns of expression in the mammalian brain.

2. Experimental procedure

2.1. Source of materials

Chromatography materials for enzyme purification were purchased from GE Healthcare Life Sciences (Uppsala, Sweden), unless otherwise indicated, and all other reagents were from Sigma (St Louis, MO, USA).

2.2. Molecular modeling/docking of substrates in GADL1 and CSAD

To determine the structural relationships of GADL1, CSAD, and other decarboxylases, we created a homology model of GADL1. The sequence of GADL1 was aligned with that of CSAD in DeepView (Guex and Peitsch, 1997) and submitted to the Swiss-Model server (Schwede et al., 2003) to prepare a homology model of GADL1 (see Fig. 2). A virtual library of 8 million commercially available compounds was obtained from the ZINC database (Irwin et al., 2012) and docked into the active site of CSAD with the Glide software (Friesner et al., 2004) from Schrodinger®. A grid centered on the PLP cofactor in the CSAD binding site was defined with dimensions 17 Å in all three dimensions. The compounds were initially docked following the high throughput virtual screening protocol. The top 100,000 compounds were redocked following the standard precision protocol. Finally, the 10,000 top scoring compounds from this procedure were docked into the active site with the extra precision (Friesner et al., 2006) protocol.

2.3. Expression vectors

Multiple mRNA transcripts of CSAD and GADL1 have been described, probably due to alternative initiation codons and splicing events (Tappaz et al., 1999). The UniProt database (http://www.uniprot.org/uniprot/) lists three human CSAD sequences with 346–520 amino acids, two human GADL1 isoforms with 418–521 amino acids and two mouse GADL1 isoforms with 526–550 amino acids. We obtained cDNA clones corresponding to the 550 amino acids (62 kDa; Q80WP8-2) isoform of mouse GADL1

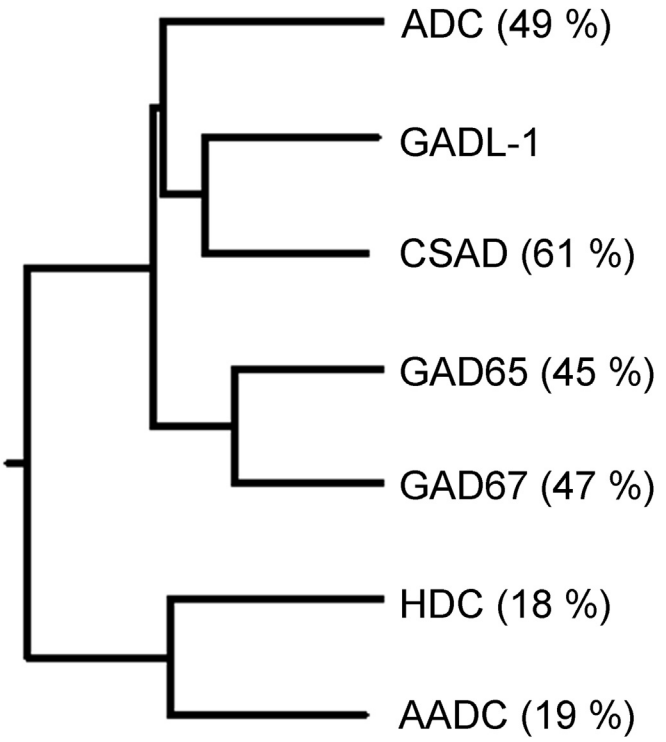

Fig. 1. Phylogenetic tree of PLP-dependent decarboxylases. Amino acid sequences of PLP-dependent decarboxylases were aligned using ClustalW version 2 software (2007) (Larkin et al., 2007). The phylogenetic tree was constructed by the neighbor-joining method based on alignment using the data base accession numbers: Q80WP8 (GADL1), Q9DBE0 (CSAD), P48318 (GAD1), P48320 (GAD2), O88533 (AADC), P23738 (HDC), A7U8C7 (ADC). The percentages of the amino acid sequence similarity to GADL1 are given in parenthesis. All the sequences are from *Mus musculus*, except for ADC, which is from *Tribolium castaneum*.