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Review

Roles of 17β-hydroxysteroid dehydrogenase type 10 in neurodegenerative disorders

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ABSTRACT

17β-Hydroxysteroid dehydrogenase type 10 (17β-HSD10) is encoded by the *HSD17B10* gene mapping at Xp11.2. This homotetrameric mitochondrial multifunctional enzyme catalyzes the oxidation of neuroactive steroids and the degradation of isoleucine. This enzyme is capable of binding to other peptides, such as estrogen receptor α, amyloid-β, and tRNA methyltransferase 10C. Missense mutations of the *HSD17B10* gene result in 17β-HSD10 deficiency, an infantile neurodegeneration characterized by progressive psychomotor regression and alteration of mitochondria morphology. 17β-HSD10 exhibits only a negligible alcohol dehydrogenase activity, and is not localized in the endoplasmic reticulum or plasma membrane. Its alternate name – Aβ binding alcohol dehydrogenase (ABAD) – is a misnomer predicated on the mistaken belief that this enzyme is an alcohol dehydrogenase. Misconceptions about the localization and function of 17β-HSD10 abound. 17β-HSD10's proven location and function must be accurately identified to properly assess this enzyme's important role in brain metabolism, especially the metabolism of allopregnanolone.

The brains of individuals with Alzheimer's disease (AD) and of animals in an AD mouse model exhibit abnormally elevated levels of 17β-HSD10. Abnormal expression, as well as mutations of the *HSD17B10* gene leads to impairment of the structure, function, and dynamics of mitochondria. This may underlie the pathogenesis of the synaptic and neuronal deficiency exhibited in 17β-HSD10 related diseases, including 17β-HSD10 deficiency and AD. Restoration of steroid homeostasis could be achieved by the supplementation of neuroactive steroids with a proper dosing and treatment regimen or by the adjustment of 17β-HSD10 activity to protect neurons. The discovery of this enzyme's true function has opened a new therapeutic avenue for treating AD.

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1. Introduction

Human 17β-hydroxysteroid dehydrogenase type 10 is a multifunctional protein encoded by the *HSD17B10* (formerly *HADH2*) gene (OMIM300256) [1]. Human HSD10 cDNA was cloned from brain (NM_004493), and the resulting protein, a homotetramer [2,3] (Fig. 1), was first characterized as a short chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) [2,4,5]. Active sites of this enzyme can accommodate different substrates; 17β-HSD10 is involved in the oxidation of isoleucine, branched-chain fatty acids, and xenobiotics as well as the metabolism of sex hormones and neuroactive steroids (Table 1) [1–15].

17β-HSD10 is the sole 17β-hydroxysteroid dehydrogenase, out of 14 different types, that is localized in the mitochondria [16,17]. Ever since the role of this mitochondrial enzyme in the metabolism of steroid hormones and neuroactive steroids was recognized [4,5,15], it has been designated as 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10) [18,19]. This enzyme also can form complexes with other proteins to generate mtRNase P activity [20]. When 17β-HSD10 binds to estrogen receptor alpha or amyloid β-peptides, various 17β-HSD10 functions are inhibited [21,22]. The molecular weight of 17β-HSD10/SCHAD that is composed of four identical subunits is 108 kDa; each subunit consists of 261 amino acid residues [2]. Although the endoplasmic reticulum-associated amyloid-β peptide binding protein (ERAB) was reported to be associated with the ER and to consist of 262 residues with a molecular weight of 27 kDa [23,24], ERAB is actually identical to 17β-HSD10/SCHAD [2] that is localized in mitochondria but not ER [4,5,15,18,19]. In experimental animals, rat 17β-HSD10 and mouse 17β-HSD10 encoded by the rat *Hsd17b10* gene and the mouse *Hsd17b10* gene, respectively, are also localized in the mitochondria [25,26]. Nevertheless, generalized alcohol dehydrogenase activity and triple intracellular localization was attributed to ERAB by publishing artifacts. On the basis of such misconceptions, ERAB [6,23] was renamed Aβ-binding alcohol dehydrogenase (ABAD) [6,27]. Later reports claimed that ABAD concentrates in mitochondria [28], yet made no reference to published characterizations of 17β-HSD10/SCHAD [2,4,5,15,18,19].

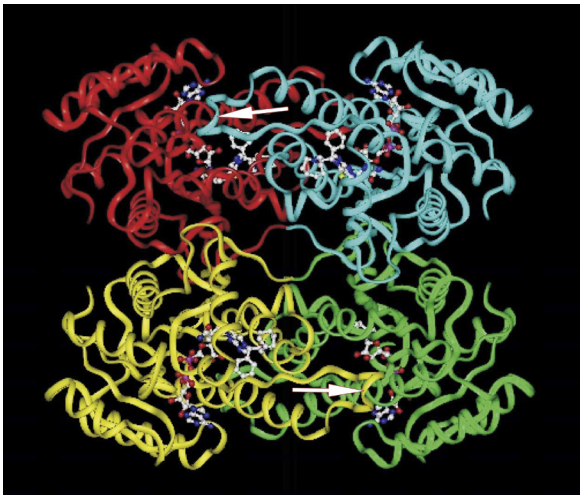


Fig. 1. Ribbon representation of human 17β-HSD10 in complex with cofactor NAD^+ and inhibitor AG18051. The tetramer is viewed down one of three mutually perpendicular 2-fold axes. Individual subunits are shown in red, green, blue and yellow. The bound NAD^+ and suicide inhibitor are shown in ball-and-stick representations. White arrows indicate the D loop close to the viewer. (Adapted from Fig. 3 of [3]).

2. 17β-HSD10 deficiency – a progressive infantile neurodegeneration

2.1. Mutation hotspot

The *HSD17B10* gene maps to a chromosomal region closely associated with X-linked mental disorders [29]. In addition to its enzymatic activities, the capability of 17β-HSD10 to bind to various peptides also is believed to play an important role in human health and disease [30]. Missense mutations in the *HSD17B10* gene result in a progressive infantile neurodegeneration [31], namely 17β-HSD10 deficiency (OMIM#300438). About 50% of such cases are caused by a c.388C>T transition due to a 5-methylcytosine hotspot, which was identified at the +2259 nucleotide from the initiation codon ATG of the *HSD17B10* gene [32].

2.2. Mimics a mitochondrial disease

Individuals with 17β-HSD10 deficiency may have microcephaly but no dysmorphism or organomegaly. One of the predominant clinical features of this disease is psychomotor regression whose onset often occurs at 6–36 months after birth [8,30–38]. Mimicking a mitochondrial disease [37], the cells of 17β-HSD10 deficient patients exhibit mitochondrial morphology that is severely altered such that the majority of mitochondria appear to be rounded with depleted cristae [38].

Table 1
Catalytic properties of human 17β-hydroxysteroid dehydrogenase type 10.

Substrate	Product	Km(μM)	kcat(min^{-1})	kcat/Km(min^{-1} mM^{-1})	Ref
Acetoacetyl-CoA	3-Hydroxy-butyryl-CoA	89 ± 5.4	2,220 ± 96	24,943.80	[2]
2-Methyl-3-hydroxybutyryl- CoA	2-Methylacetoacetyl-CoA	7.1 ± 1.1	398.96 ± 37.74	56,191.50	[8]
Methanol	Formaldehyde	–	Not detectable	–	[6]
Ethanol	aldehyde	–	Not detectable[a]	–	[5]
2-Propanol	Acetone	280,000 ± 33,000	2.16 ± 0.14	0.0077	[5]
Butanol	Butyl aldehyde	43,500 ± 8,300	0.50 ± 0.03	0.011	[9]
D-3-hydroxy-butyrate	Acetoacetate	4,500 ± 1,000	0.11 ± 0.001	0.024	[9]
17β-Estradiol	Estrone	43.0 ± 2.1	0.66 ± 0.01	15.3	[4,5,11]
Allopregnanolone	5α-Dihydroprogesterone	15.0 ± 1.7	6.40 ± 0.20	426.7	[12]
Allotetrahydrodeoxycorticosterone	5α-Dihydrodeoxycorticosterone	9.7 ± 1.3	13.40 ± 0.70	1,381.0	[12]
3α-androstanediol	5α-Dihydrotestosterone	34.0 ± 2.4	5.58 ± 0.17	164	[5,11,15]
5α-Dihydrotestosterone	3α-androstanediol	112.0 ± 18	1.94 ± 0.21	17.3	[11,15]
Androsterone	Androstanedione	45.0 ± 9.3	0.66 ± 0.08	14.7	[5,15]
Androstanedione	Androsterone	44.0 ± 2.5	0.23 ± 0.01	5.2	[15]

[a] The absorbance change (ΔA340) is within the instrumental error range of the UV spectrophotometer when 7% alcohol solution was used as substrate in the alcohol dehydrogenase assay. This alcohol concentration is about 100 times higher than the DWI (driving while intoxicated) limit.

2.3. Clinical hallmark – a blockade of isoleucine degradation

Because 17β-HSD10 (formerly SCHAD [1,7,8,10] is a key enzyme in the isoleucine oxidation pathway (Fig. 2), all individuals with 17β-HSD10 deficiency have a blockade of isoleucine degradation [8,34,39]. Some patients who have a mild mental disability have normal levels of isoleucine metabolites in the blood because they carry a silent mutation, c.574C>A, in the *HSD17B10* gene [40]. They suffer from X-linked mental disabilities, choreoathetosis and abnormal behavior (MRXS10) [41] (OMIM#300220) – but not 17β-HSD10 deficiency. In some individuals with 17β-HSD10 deficiency, one biochemical finding of mitochondrial dysfunction is an increase in lactate levels [42]. No signs of abnormal ketone body metabolism are exhibited, and the oxidation of D-3-hydroxybutyrate catalyzed by 17β-HSD10, if any [27], is insignificant (Table 1). Although elevated levels of isoleucine metabolites in blood and their excretion in urine are a clinical hallmark of 17β-HSD10 deficiency, the accumulation of tiglylglycine and 2-methyl-3-hydroxybutyrate is perhaps only a pathophysiological background of this disease; an imbalance in neurosteroid metabolism could be a major cause of the observed neurological handicap [8].

A low-protein, high-energy dietary regimen with carnitine supplementation reduces the accumulation of isoleucine metabolites in blood and urine (Fig. 3), but it cannot ameliorate the patients' psychomotor regression [31–33].

2.4. Metabolism of allopregnanolone

Allopregnanolone (ALLOP) is a potent, positive allosteric modulator of $GABA_A$ receptors, and has a significant impact on the $GABA_A$ receptor plasticity [43–45]. ALLOP can be de novo synthesized in the central nervous system by the reduction of progesterone to 5α-dihydroprogesterone (5α-DHP) under the catalysis of 5α-reductase. 5α-DHP has no effect on $GABA_A$ receptors, but it can be further reduced to ALLOP by the oxidation of NADPH under the catalysis of 3α-hydroxysteroid dehydrogenase type 3 (3α-HSD3) [46]. Since the ratio of NADPH/$NADP^+$ is much greater than 10 in the cytosol [47] and since 3α-hydroxysteroid dehydrogenase type 3 (AKR1C2) itself prefers to form an E·NADPH binary complex, 3α-HSD3 acts predominantly as a 17-ketosteroid reductase (AKR1C2) to generate ALLOP. As a result, any serious discussion of the metabolism of ALLOP has to include a reliable mechanism that could effectively inactivate ALLOP.

It was reported [12,48] that mitochondrial 17β-HSD10, but not 3α-HSD3 (AKR1C2), should be responsible for the catalysis of the oxidative inactivation of ALLOP. Since NAD^+ is the coenzyme of 17β-HSD10 that is localized in mitochondria where the ratio of NADH/NAD^+ is about 0.1, ALLOP is converted back to 5α-DHP in mitochondria. Two distinct enzymes, i.e., 3α-HSD3 (AKR1C2) and 17β-HSD10, in two different compartments have a part to play in the metabolism of ALLOP (Fig. 4).

When neuronal progenitors migrate from the ventricular zone to the cortical plate region they become predominantly GABAergic [49]. A normal level of wild type 17β-HSD10 is, therefore, essential for brain development and cognitive function. The imbalance of neuroactive steroid metabolism, especially ALLOP metabolism, in brains of individuals with 17β-HSD10 deficiency remains to be studied. Such investigations would lay a foundation for the effective treatment of this kind of developmental intellectual disabilities.

2.5. Aβ undetectable in patient's cerebrospinal fluid

Whether the amyloid-β peptide is involved in the infantile neurodegeneration of 17β-HSD10 deficiency is of great interest. It was recently reported [50] that Aβ peptide is undetectable in the cerebrospinal fluid (CSF) of an individual with 17β-HSD10 deficiency. If this observation is confirmed in other affected individuals, the implication would be that a missense mutation in the *HSD17B10* gene could alter the metabolism or trafficking of the amyloid precursor protein (APP) [51]. Because APP is a known risk factor for AD, studies on 17β-HSD10 deficiency may lead to new avenues for the treatment of AD.

3. Misconceptions in 17β-HSD10 research

3.1. Is Aβ-binding alcohol dehydrogenase really an alcohol dehydrogenase?

3.1.1. The myth of generalized alcohol dehydrogenase activity

Literature references to Aβ-binding alcohol dehydrogenase (ABAD) as a recharacterization of ERAB [6,27,28] – itself a misnomer for 17β-HSD10 – do not account for the establishment of 17β-HSD10/SCHAD as a mitochondrial hydroxysteroid dehydrogenase [2–5,11,15,18,19]. The ABAD designation relied on a reported generalized alcohol dehydrogenase activity that purportedly catalyzed the oxidation of a variety of aliphatic alcohols in which the alkyl groups range from *n*-decanol (C10) to ethanol (C2). According to the published Experimental Procedure section of the key reference that attempted to establish ERAB as ABAD, the "[a]lcohol dehydrogenase assays employed ERAB/HADHII (20 μg/ml), a range of alcohol substrates and concentrations (methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, (±)-2-octanol, (+)-2-

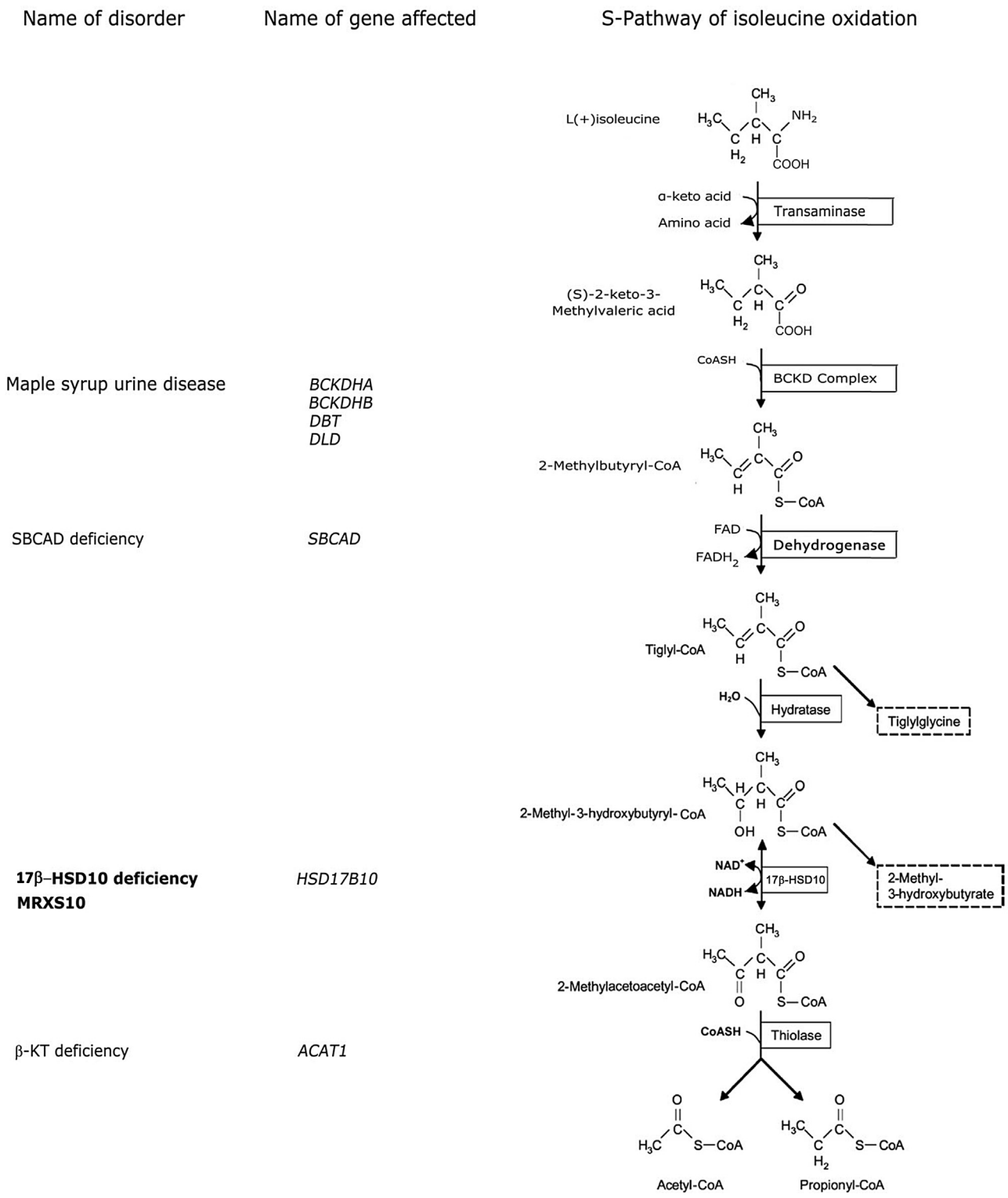


Fig. 2. Inborn errors in isoleucine metabolism. Dashed line boxes indicate the compounds excreted in the urine of 17β-HSD10 deficiency patients. A minor R pathway of isoleucine oxidation where the breakdown of 2-ethylhydracrylic acid is very slow [104] is not shown here. BCKD, branched-chain α-keto acid dehydrogenase; DBT, dihydrolipoamide branched-chain transacylase; DLD, dihydrolipoamide dehydrogenase; SBCAD, short branched-chain acyl-CoA dehydrogenase; β-KT, β-ketothiolase; ACAT1, acetyl-CoA acetyltransferase 1. (Adapted from Fig. 1 of [39]).

octanol, (−)-2-octanol, and *n*-decanol; Sigma), and NAD^+ (7.5 mM) in 22 mM sodium pyrophosphate, 0.3 mM sodium phosphate (pH 8.8). The reaction was run for 2 h at 25 °C, and the absorbance at 340 nm was monitored every 5 min as described above." [6]. These conditions raise very serious concerns about the reliability of the results as described below.

As is well known, *n*-decanol and 2-octanol are immiscible with water. For example, the saturating concentration of 2-octanol is no more than 7 mM [52]; immiscibility has no exceptions among enantiomers: (±)-2-octanol, (+)-2-octanol, or (−)-2-octanol. 160 mM or even 210 mM (−)-2-octanol was reportedly used as substrates for the alcohol dehydrogenase assays [6] (Fig. 5).

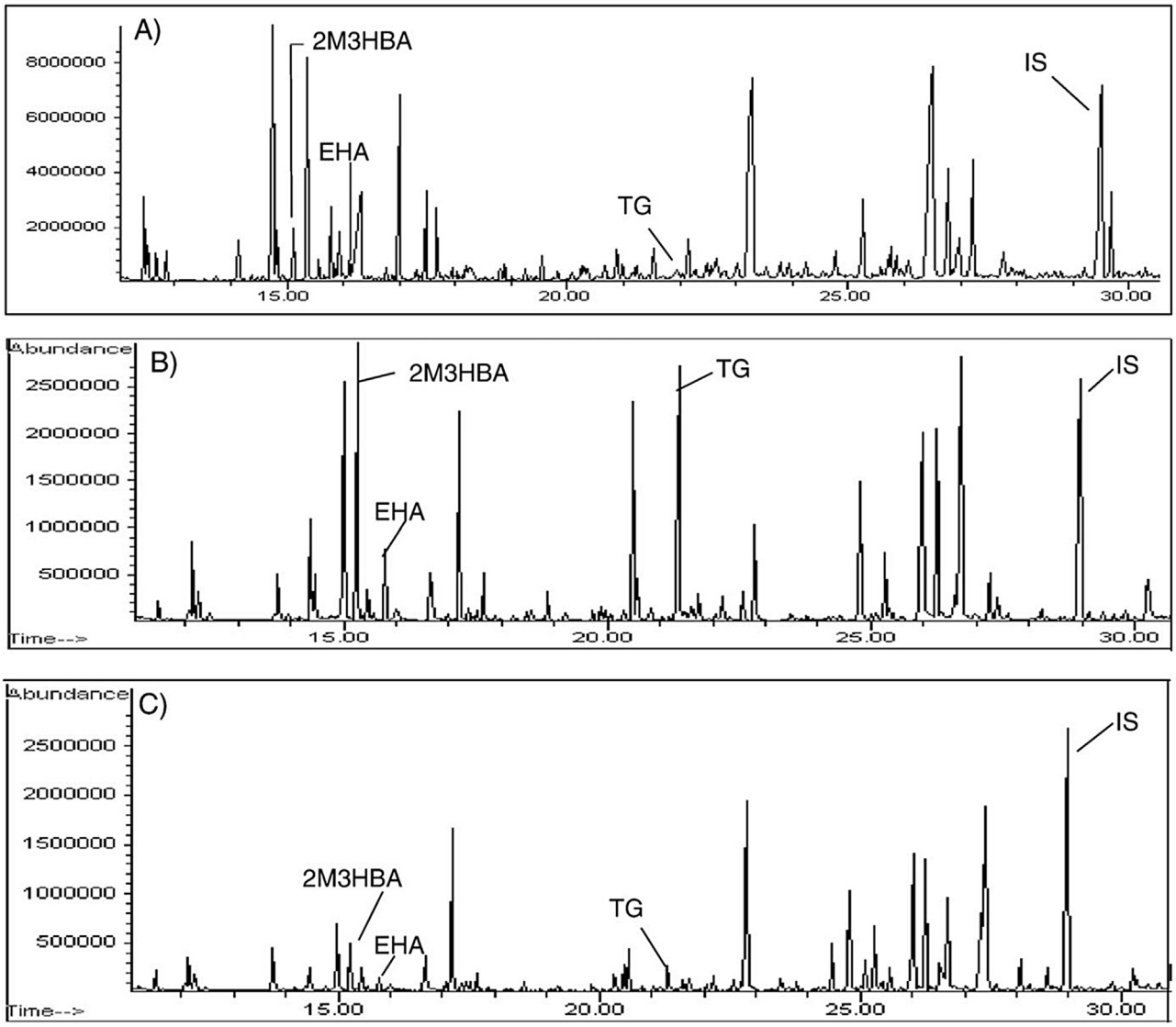


Fig. 3. Changes of isoleucine metabolite levels in 17β-HSD10 deficiency patient's urine due to the low-protein dietary regimen. Urine organic acids were analyzed as trimethylsilyl (TMS)-derivatives by gas chromatography-mass spectrometry. Urine organic acid profiles: (A) normal control; (B) 17β-HSD10 deficiency patient; (C) 17β-HSD10 deficiency patient under low protein dietary regimen. 2MHBA, 2-methyl-3-hydroxybutyrate; EHA, 2-ethylhydracrylic acid; TG, tiglylglycine; IS, internal standard. (Adapted from Fig. 1 of [33]).

Because no solubilization method was ever reported, it is not possible to reproduce the assays that purportedly establish ERAB/ABAD's generalized alcohol dehydrogenase activity. In addition, the catalytic rates of ABAD/ERAB reported for other substrates, such as 17β-estradiol and acetoacetyl-CoA, appear to have been exaggerated by three orders of magnitude and by five-fold, respectively [5].

3.1.2. Negligible alcohol dehydrogenase activity of 17β-HSD10

Although ethanol is an alcohol miscible with water, the reported turnover rate (k_{cat}) catalyzed by ABAD/ERAB was 0.5 per second when a 1.21 M ethanol solution was used as substrate [6]. The catalytic efficiencies of this dehydrogenase (k_{cat}/K_m) reported for aliphatic alcohols, e.g., ethanol (0.05 min^{-1} mM^{-1}) [6], 2-propanol or "*n*-isopropanol" as stated in ref. 27 (0.01 min^{-1} mM^{-1}) [5] and *n*-butanol (0.01 min^{-1} mM^{-1}) [9] are negligible, as compared with those for steroids such as allopregnanolone (432 min^{-1} mM^{-1}) and allotetrahydrodeoxycorticosterone (1381 min^{-1} mM^{-1}) [12]. Therefore, these data establish that it is perfectly proper to designate this mitochondrial enzyme 17β-hydroxysteroid dehydrogenase type 10 [4,18,19], which is encoded by the human *HSD17B10* gene, as designated by the Human Gene Nomenclature Committee (HGNC) [53].

Nonetheless, it was reported [6] that "the combination of Aβ binding properties and generalized alcohol dehydrogenase activity, in addition to HADH activity, lead us to propose the new name Aβ binding alcohol dehydrogenase or ABAD to better describe the unusual properties of the enzyme previously referred to as ERAB or HADH II". This statement conflicts with the facts about 17β-HSD10. Although 17β-HSD10 is known to have an affinity for other peptides, including Aβ [30], it exhibits only a negligible alcohol dehydrogenase activity (Table 1). The aim of this review is to dispel the mischaracterization of the generalized alcohol dehydrogenase activity of ABAD/ERAB. Only then can the research on this important brain enzyme 17β-HSD10 move forward.

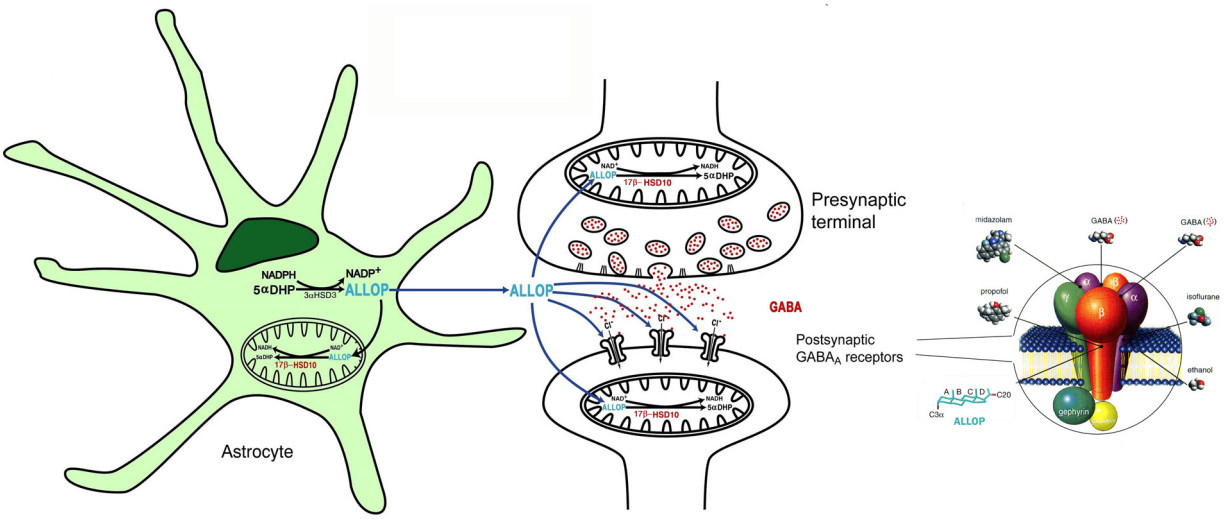
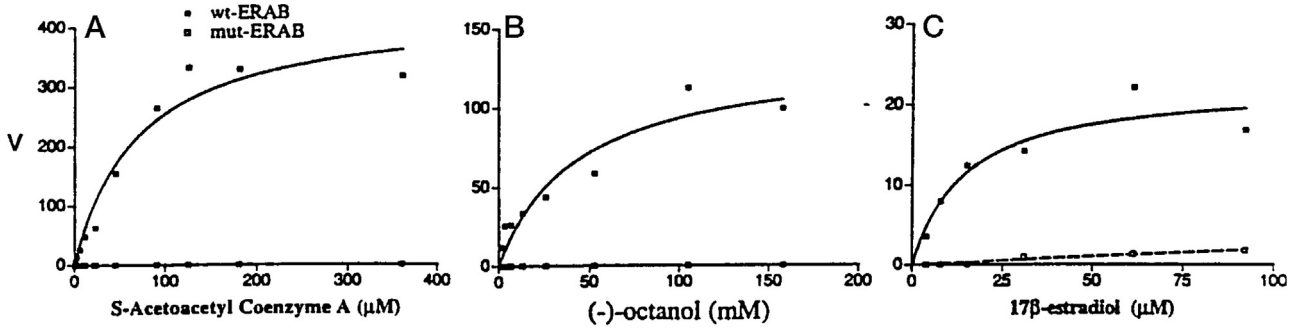
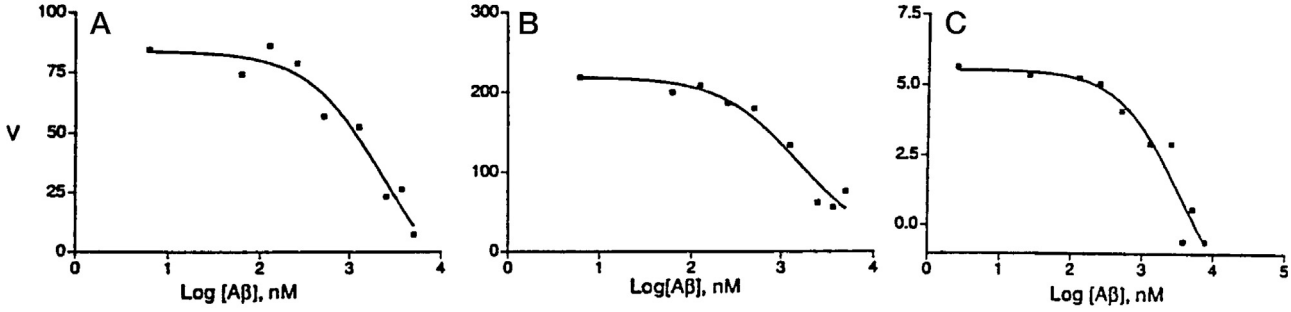


Fig. 4. Roles of brain 17β-HSD10 in the metabolism of allopregnanolone (ALLOP). ALLOP is a positive steroid modulator of $GABA_A$ receptors that potentiates GABA to increase the opening of Cl^- channels. ALLOP is generated from 5α-dihydroprogesterone (DHP) by cytosolic 3α-HSD3 catalysis, whereas ALLOP is oxidized to 5α-DHP that has no effect on $GABA_A$ receptors by the catalysis of mitochondrial 17β-HSD10 with the concomitant reduction of NAD^+. —• indicates the binding sites of individual modulators on the $GABA_A$ receptor. (Adapted from Fig. 4 of [48] and the cover page of Neuropharmacology).



Legend: **Characterization of ERAB/HADH II enzymatic activity: reduction of *S*-acetoacetyl-CoA (*A*) and oxidation of octanol (*B*) and 17β-estradiol (*C*).** Experiments utilized either wild-type ERAB/HADH II (*filled squares*) or mutant ERAB(Y168G/K172G) (*open squares*). The same concentration of wild-type and mutant ERAB was used in each case. *A,* ERAB/HADH II (0.33 μg/ml) was incubated with the indicated concentration of *S*-acetoacetyl-CoA and NADH (0.1 mM). *B,* ERAB/HADH II (20 μg/ml) was incubated with the indicated concentration of (−)-octanol and NAD^+ (7.5 mM). *C,* ERAB/HADH II (30 μg/ml) was incubated with the indicated concentration of 17β-estradiol and NAD^+ (0.4 mM). The velocity (*V*) of the reaction (units/mg of protein) is plotted *versus* added substrate concentration. Details of the experimental methods are described in the text. The *broken line* represents the theoretical curve according to the K_m and V_{max} values (see Table I) calculated by the computer program. Experimental procedures are described in the text.



Legend: **Effect of Aβ(1–40) on ERAB/HADH II enzymatic activity.** *A,* reduction of *S*-acetoacetyl-CoA. ERAB/HADH II (0.67 μg/ml) was incubated with *S*-acetoacetyl-CoA (0.18 mM), NADH (0.1 mM), and the indicated concentrations of Aβ. *B,* oxidation of octanol. ERAB/HADH II (10 μg/ml) was incubated with (−)-octanol (210 mM), NAD^+ (7.5 mM), and the indicated concentrations of Aβ. *C,* oxidation of 17β-estradiol. ERAB/HADH II (25 μg/ml) was incubated with 17β-estradiol (61 μM), NAD^+ (0.4 mM), and the indicated concentrations of Aβ. Velocity (*V*) of the reaction (units/mg of protein) is plotted *versus* log [Aβ] (nM). Data were fit to a one-site model for competitive inhibition.

Fig. 5. Kinetic analysis of the endoplasmic reticulum-associated Aβ-binding protein/Aβ-binding alcohol dehydrogenase (17β-HSD10) activity and its inhibition by Aβ. The "mutERAB" represents a mutant 17β-HSD10 carrying Y168G/K172G mutations. (The upper part and bottom part were adapted from Fig. 2 and Fig. 5 of [6], respectively).

3.1.3. Invalid competitive inhibition constants (K_i) of Aβ

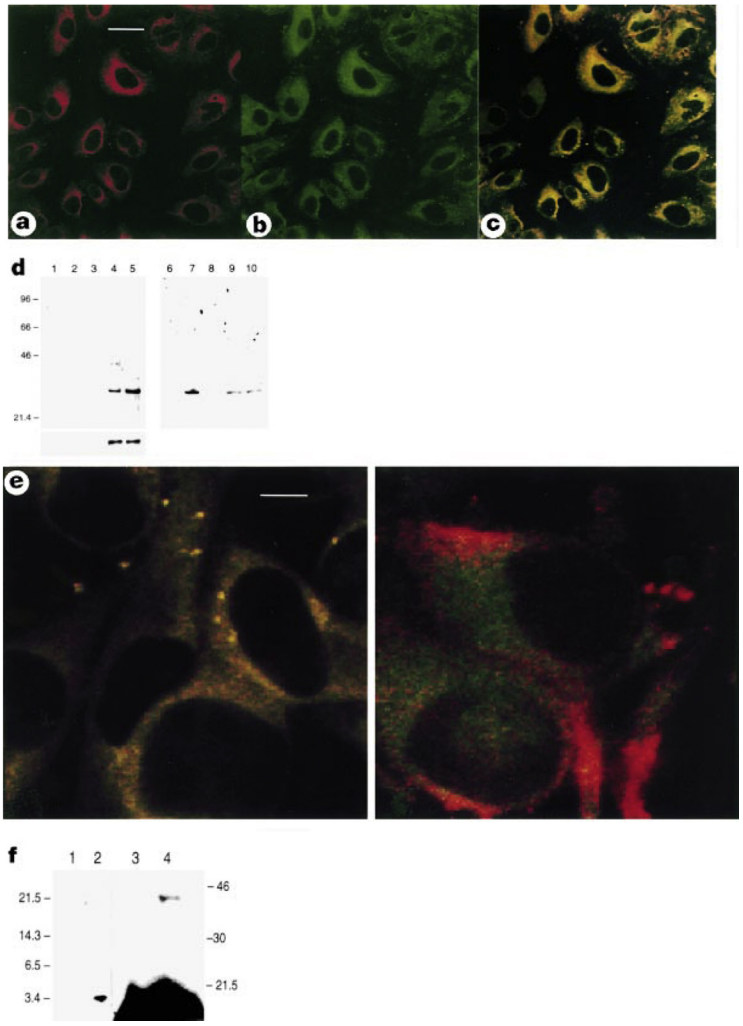
Further confusion concerning 17β-HSD10 was engendered by reports of kinetic studies of ABAD/ERAB. 17β-HSD10 is inhibited by the binding of estrogen receptor alpha [21] or Aβ peptides that include residues 12–24 [22]. The inhibition of 17β-HSD10 activities by Aβ reportedly follows a one-site model for competitive inhibition [6], for which a well-known enzyme kinetics book [54] had been cited as the specific reference. However, only a single, fixed substrate concentration was used to study the competitive inhibition of ABAD/ERAB, which is inadequate to establish the competitive inhibition. More surprisingly, competitive inhibition constants (K_i) of Aβ were reportedly obtained by fitting data to a one-site model for competitive inhibition (Fig. 5 bottom part A, B, and C). In fact, no such "one-site model for competitive inhibition" is described in the cited reference [54]. Thus, it seems unlikely that any of the K_i values of Aβ reported for ABAD/ERAB [6,55,56] would be valid. The one-site competitive inhibition model is another misconception that has emerged from ABAD/ERAB studies [6,27,28]. The validity of ABAD/ERAB reports in AD research [55–63] needs to be reexamined.

3.2. Does 17β-HSD10 have triple intracellular localization?

ERAB, a misnomer of 17β-HSD10, was reportedly localized in ER, and would migrate to the plasma membrane after cells are incubated with Aβ peptides [23] (Fig. 6). However, the reported migration of 17β-HSD10 from ER to plasma membrane [23,24] is not reproducible except those shown in Fig. 6 of [6], because 17β-HSD10 is, indeed, localized in mitochondria but not ER according to honest immunocytochemical studies (Fig. 7) [4,15].

3.2.1. Confirmation of erroneous data by distorted subcellular fractionation

The localization of 17β-HSD10 in the mitochondria, ER, and plasma membrane has been confirmed by subcellular fractionation data [6,27]. The fractionation experimental procedure employed in ABAD/ERAB studies was reportedly described in a particular reference [64]. Unfortunately, there is only half of a sentence relevant to the fractionation experimental procedure, i.e., "[f]ractionation of cell lysates was performed as described (24), ...". As a matter of fact, the actually employed procedure [6,27] has nothing to do with the subcellular fractionation described in the indirectly cited literature [65], the purpose of which is to isolate nuclei for the purification of a transcription factor [65]. If the conventional method instead of the distorted subcellular fractionation procedure was performed, 17β-HSD10 cannot be detected in the ER fraction (Fig. 8). Data from the honest immunocytochemical studies were perfectly corroborated by data obtained from the conventional subcellular fractionation experiments, because 17β-HSD10 possesses a non-cleavable



Legend. Expression of ERAB in cultured cells: localization to the endoplasmic reticulum and change in distribution following addition of Aβ. **a**–**c**, Confocal microscopy demonstrating immunofluorescence staining for ERAB alone (**a**; red), protein disulphide isomerase (PDI) alone (**b**; green), or these two antigens simultaneously co-localized (**c**; yellow). Scale bar in **a**–**c** is 25 μm. **d**, Transfected cells (5×10^8) were pelleted and fractionated in a series of sucrose steps (38, 30 and 20%) by ultracentrifugation: layered (lanes 1–4) and pelleted fractions (lane 5) were western blotted using either anti-ERAB IgG (top) or anti-GRP78 IgG (bottom). Lanes correspond to cytosol (lanes 1 and 6), plasma membrane (lanes 2 and 7), Golgi apparatus (lanes 3 and 8), and endoplasmic reticulum (lanes 4, 5 and 9, 10). In lanes 6–10, subcellular fractionation of ERAB-transfected human neuroblastoma cells was performed following exposure to Aβ(1–42) (1 μM) for 14 h at 37 °C. **e**, Neuroblastoma cells were incubated in buffer alone or in the presence of Aβ(1–42) (1 μM) for 14 h at 37 °C, and the distribution of ERAB determined by double-immunofluorescence staining for ERAB (red) and PDI (green). The two antigens, which were initially co-localized (yellow, left), then separate, allowing visualization of ERAB deposits near the cell membrane (red) distinct from PDI (green) (right). Scale bars, 6 μm. **f**, Neuroblastoma cells (2×10^7) were incubated with ^{125}I-Aβ (100 nM) for 6 h at 37 °C, and cells dissolved in lysis buffer[10] followed by immunoprecipitation with anti-ERAB IgG (10 μg ml^{-1}; lane 2) or non-immune IgG (10 μg ml^{-1}; lane 1), and Tris-tricine gel electrophoresis. Alternatively, neuroblastoma cells (2×10^7) were incubated with ^{125}I-Aβ (100 nM) for 6 h at 37 °C, disuccinimidyl suberate (0.2 mM; Pierce) was added for 30 min at 25 °C, cells were washed extensively, followed by dissolution of cells in lysis buffer and electrophoresis on non-reducing SDS-PAGE (10%): immunoprecipitation with non-immune IgG (lane 3) and anti-ERAB IgG (lane 4).

Fig. 6. Proposed intracellular localization of the endoplasmic reticulum-associated Aβ-binding protein (17β-HSD10) and its redistribution to the plasma membrane. (Adapted from Fig. 4 of [23]).

mitochondrial targeting signal at its *N*-terminus (residues 1–11) [15,18,19].

3.2.2. Cytochrome C used as the mitochondrial marker

As indicated by Nobel laureate Christian de Duve, cytochrome C is readily released from damaged mitochondria. The use of cytochrome C rather than cytochrome C oxidase as a marker for mitochondria in ABAD/ERAB studies [6,27] resulted in additional artifacts [18] that further promoted ABAD/ERAB's erroneous and astonishing story.

Misconceptions that emerged from ABAD/ERAB studies have severely impacted 17β-HSD10 research. It is hoped that in the future, erroneous triple intracellular localization of 17β-HSD10 in public databases (Supplementary Materials S1) based upon reports that are not supported by any reliable data, will be corrected, and that the underlying reports [6,23,24,27,28] will be withdrawn.

4. Significance of 17β-HSD10 for Alzheimer's disease (AD)

4.1. Elevated levels of 17β-HSD10 in AD brains

The 17β-HSD10 sequence and the coding nucleotide sequence of the *HSD17B10* gene are highly conserved among animals and appear to be indispensable for life [66,67]. 17β-HSD10 is present in brain regions at different levels, and is most abundant in the hippocampus [48]. 17β-HSD10 concentrations in the CSF may reflect its quantity in the brain. It was reported [68] that 17β-HSD10 concentration in the CSF declines with age, yet there is 5.7-fold more 17β-HSD10 in AD patients' CSF than in corresponding age-matched controls. Furthermore, abundant 17β-HSD10 is found in the brains of AD patients [48] as well as of an AD mouse model [26] (Fig. 9). It has been reported [11] that overexpression of 17β-HSD10 results in mitochondrial matrix condensation and partial loss of cristae structure.

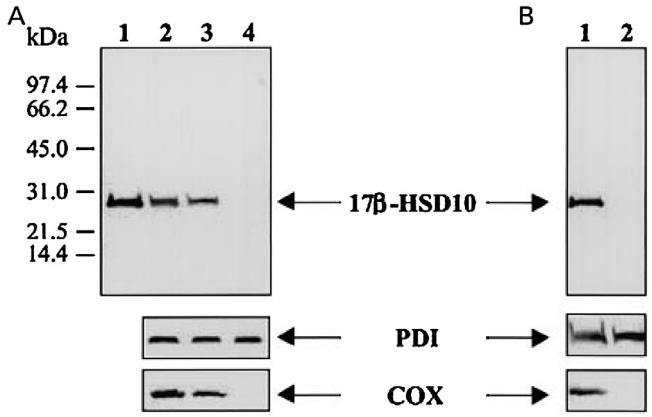


Fig. 8. 17β-HSD10 detectable only in mitochondria after the performance of a conventional subcellular fractionation. (A) Purified human 17β-HSD10 (0.2 μg) (lane 1), cell homogenate (lane 2), pellet (lane 3) and supernatant (lane 4); (B) the ER fraction obtained from the altered subcellular fractionation [6,27] (lane 1) and that obtained by use of the conventional procedure [105] (lane 2). PDI, protein disulfide isomerase was used as the ER marker; COX, cytochrome C oxidase as the mitochondria marker. (Adapted from Fig. 5 of [18]).

4.2. Oligomeric Aβ bound to 17β-HSD10

The D loop of 17β-HSD10 has a short β hairpin structure and extends the subunit interface in the homotetramer by contacting the helix αE2 of an adjacent monomer (Fig. 1). A unique insertion I region of the D loop can bind Aβ [3]. The dissociation constant of

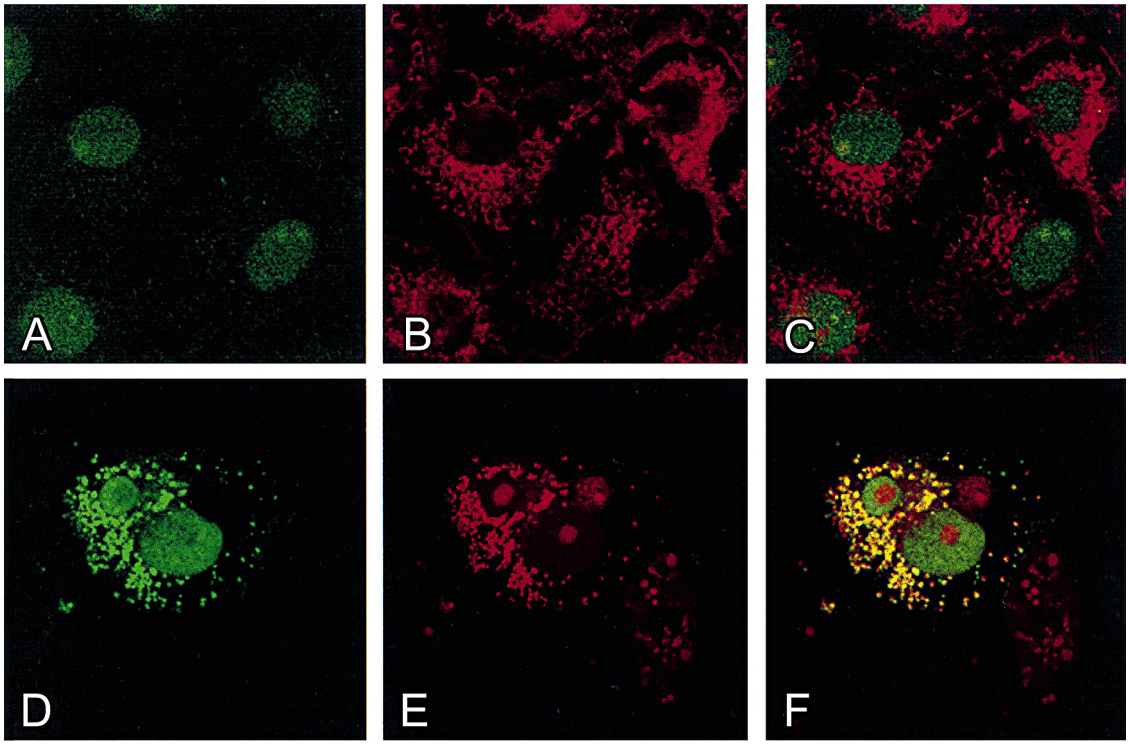


Fig. 7. Intracellular localization of 17β-HSD10 expressed in COS-7 cells. All cells either transfected with a 17β-HSD10 expression vector (D–F) or a control vector (A–C) were immunostained with the primary anti-17β-HSD10/SCHAD antibody and then with the fluorescence-tagged secondary antibody, and 17β-HSD10 is shown in green (D, F). Mitochondria stained with MitoTracker™ Red CMXRos are shown in red (B, C, E, F). Laser scanning confocal microscopic merged images of (A) and (B), and (D) and (E) were shown in (C) and (F), respectively. The results shown are representative of three independent experiments. (Adapted from Fig. 5 of [15]).

the Aβ-17β-HSD10 complex is about 88 nM, and oligomeric rather than monomeric Aβ would inhibit 17β-HSD10 [56]. It was claimed that the D-loop is disordered and submerged to a large solvent channel as shown in Fig. 2D of ref. 28. The D-loop could perhaps binds with several Aβ peptides. Nevertheless, no electron density for Aβ in the 17β-HSD10-Aβ crystal was observed [28] (Supplementary Materials S2). The precise oligomeric state of Aβ that binds to 17β-HSD10 has not been established. Since the inhibition of 17β-HSD10 requires micromolar concentration of Aβ [22], aggregation of Aβ is necessary to distort the NAD^+ binding site [69].

4.3. Limited role of 17β-HSD10 in the production of energy

17β-HSD10 was originally identified as short chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) [2]. It is clear that L-3-hydroxyacyl-CoA dehydrogenase (HAD) but not 17β-HSD10/SCHAD plays an essential role in the mitochondrial fatty acid β-oxidation [7,70–72]. Unfortunately, for the rationalization of reports in literature claiming 17β-HSD10 to be a genuine alcohol dehydrogenase and therefore designated as amyloid beta-peptide-binding alcohol dehydrogenase (ABAD) [6,27,28,56,57], it was asserted [56] that the catalytic mechanism of L-3-hydroxyacyl-CoA dehydrogenase [73] is the reaction mechanism of ABAD catalyzing the oxidation of alcohols to ketones (Scheme 1. Reduction and oxidation of alcohols and ketones by ABAD of [56]). In fact, L-3-hydroxyacyl-CoA dehydrogenase (HAD) and 17β-HSD10/SCHAD are two distinct enzymes belonging to two different dehydrogenase families, respectively [7,70,71,74].

The conflation of one with the other serves to confuse the function of 17β-HSD10 as an alcohol dehydrogenase that oxidizes alcohols and ketone bodies to supply energy. Although this proposition sounds plausible because of the observation that glucose metabolism is reduced in AD brains [75], 17β-HSD10 plays only a limited role in the production of energy, indeed. It is well known that the oxidation of ketone bodies is catalyzed by human D-3-hydroxybutyrate dehydrogenase [76,77] rather than by 17β-HSD10, for which the catalytic efficiency for D-3-hydroxybutyrate is negligible (Table 1). Thus, the proposition that an amyloid beta-peptide-binding alcohol dehydrogenase is a component of the cellular response to nutritional stress [27] sends a misleading message.

4.4. Impairment of mitochondria due to abnormality of 17β-HSD10

It has been reported [6] that cells expressing both βAPP(V717G) and 17β-HSD10 (ABAD/ERAB) generate toxic aldehydes, e.g., 4-hydroxy-2-nonenal (4-HNE) whereas those expressing both βAPP (V717G) and mutant 17β-HSD10(Y168G/K172G) do not. However, it was later reported [78] that 17β-HSD10 can actually detoxify 4-HNE.

17β-HSD10 is a putative intracellular mediator for Aβ neurotoxicity because it is one of dozen proteins that can bind to Aβ [30,62,79]. However, no coherent mechanistic explanation has yet been postulated for this toxicity [80]. Mitochondrial dysfunction and oxidative stress seem to play a key role in the pathogenesis of AD [81]. Oxidative damage occurs early in the brains of AD patients, and also precedes Aβ deposition in an AD mouse model [82]. The binding of Aβ to 17β-HSD10 in mitochondria might increase lipid peroxidation and reactive oxygen species (ROS) release. ROS, in turn, would activate β- and γ-secretase and facilitate the amyloidogenic cleavage of the amyloid-β precursor molecule. Aβ would further induce free radicals and so affect glycolysis, the tricarboxylic acid (TCA) cycle, and the respiratory chain – especially complex IV activity –

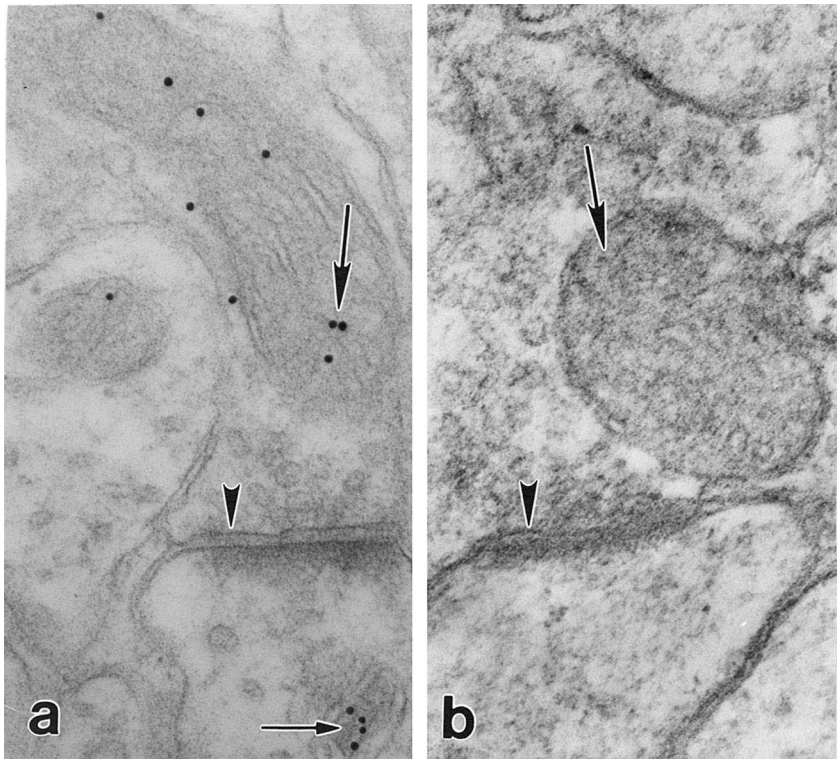


Fig. 9. Elevated levels of mitochondrial 17β-HSD10 in brains of AD model mice. Electron micrographs of an axodendritic synapse in the hippocampus of Hsiao's βAPP transgenic mouse (a) and of an age-matched control (b). Immunogold particles represent the location of 17β-HSD10. The large and small arrows indicate mitochondria of the axon terminal and dendrite, respectively. An arrowhead indicates the presynaptic terminal membrane (magnification, ×75,000). The results are representative of three independent experiments. (Adapted from Fig. 4 of [26]).

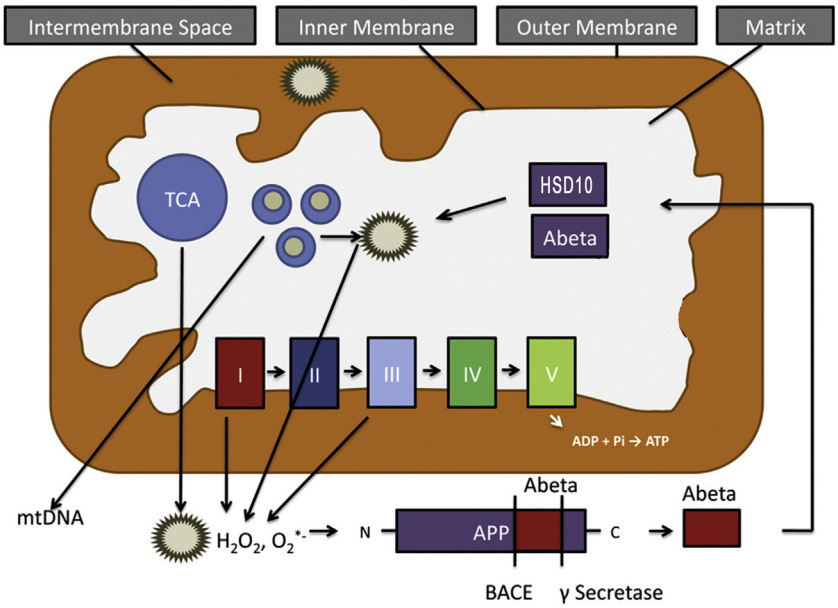


Fig. 10. Binding of Aβ to 17β-HSD10 in mitochondria increases the release of reactive oxygen species (☼, reactive oxygen species). (Adapted from Fig. 3 of [79]).

leading to reduced glucose metabolism and reduced ATP production (Fig. 10) [79]. Opening of the mitochondrial permeability transition pore (mPTP), the disturbance of Ca^{++} homeostasis, the release of cytochrome C, and the activation of caspases would eventually lead to DNA fragmentation and cell death. It was reported [83] that ALLOP would directly inhibit mPTP and thus protect neurons.

Damage to mitochondria would also cause impairment of mitochondrial transport, imbalance of mitochondrial fission/fusion, and abnormal mitochondria dynamics [79,84]. Mitochondrial function, positioning, and shape all impact brain function and cognition [85,86]. Because synaptic mitochondria are more vulnerable than perinuclear mitochondria, synaptic deficiency occurs in the early stage of AD.

4.5. Possible target for treatment of AD

Mitochondrial 17β-HSD10 levels are significantly increased in the brains of individuals with AD and in an AD mouse model [26,48]. It has also been reported that 17β-HSD10 levels are increased in the brains of individuals with multiple sclerosis [68] and are decreased in the brains of a Parkinson's disease (PD) mouse model [87]. 17β-HSD10 is involved in the progression of 17β-HSD10 deficiency and AD because 17β-HSD10 plays an important role in the metabolism of neuroactive steroids, e.g., 17β-estradiol and allopregnanolone [1,4,5,7,8,10–13,15,18,19,30,48,88] (Fig. 4).

The question of whether changes of 17β-HSD10 levels [48,68,71,87,89] in the above-mentioned neurological disorders and ulcerative colitis are due to alterations of *HSD17B10* gene expression remains to be answered. To date, studies on the regulatory mechanism of *HSD17B10* gene expression have been limited [90,91]. The mechanism by which elevated levels of 17β-HSD10 would cause the increase of 'downstream' proteins such as peroxiredoxin-2 [92] and endophilin-1 [93] remains unknown. However, what is known is that the inhibitory effect of Aβ on mtRNase P is not generated via the interaction of Aβ and 17β-HSD10 [94].

It was reported [28] that a decoy peptide synthesized according to the D loop sequence of 17β-HSD10 (residue 92–120) would reduce the binding of Aβ to 17β-HSD10 and thus improve mitochondrial function in an AD mouse model. However, parenteral administration of a synthetic peptide to human may cause immunological responses. Even if such a decoy peptide could pass the blood-brain barrier and eventually enter into mitochondria, it is not known how it might avoid degradation by mitochondrial peptidases such as prolyl endopeptidase [95] and insulin-degrading enzyme [96]. Thus, this decoy peptide does not appear to be a feasible strategy for treatment of AD patients.

The etiology of late-onset AD remains elusive. An imbalance of neuroactive steroid metabolism may have an important part to play in neurodegenerative disorders [4,8,19]. The neuroprotective and neurotrophic effects of 17β-estradiol and allopregnanolone are well documented [43,97–101]. 17β-estradiol and allopregnanolone levels are reduced in the brains of individuals with AD [19,44,98,102]. A proper dosing and treatment regimen will be a key to have success in a clinical trial of the neuroactive steroid supplementation. The inhibition of 17β-HSD10 would increase these neuroprotective steroid levels in the brain [4,88], given that 17β-HSD10 is present in different brain regions and catalyzes the oxidation of 17β-estradiol and allopregnanolone [4,5,10–13,48] (Table 1 and Fig. 4). A suicide inhibitor of 17β-HSD10 has already been tested [88] (Fig. 1). Recently, a reversible, specific steroidal inhibitor of 17β-HSD10 was also developed [103], which may be more suitable to the pharmaceutical industry. Approaches to restore steroid homeostasis in AD brains, including the adjustment of 17β-HSD10 activity, are therapeutic modalities that warrant further exploration.

5. Concluding remarks

17β-HSD10 is the sole 17β-hydroxysteroid dehydrogenase in mitochondria. Earlier studies indicating that 17β-HSD10 is also localized in the plasma membrane, cytoplasm and endoplasmic reticulum have been proved to be incorrect. Furthermore, studies have shown that this enzyme does not possess generalized alcohol dehydrogenase activity. Missense mutations in the *HSD17B10* gene result in 17β-HSD10 deficiency, an infantile neurodegenerative disorder. Since allopregnanolone, a positive steroid modulator of

$GABA_A$ receptors synthesized in cytosol by 3α-HSD3 catalysis, is inactivated by the catalysis of mitochondrial 17β-HSD10, the imbalance of neuroactive steroid metabolism is probably a major cause.

Elevated levels of 17β-HSD10 are present in the brains of individuals with AD and of an AD mouse model, but the implications of these findings have been neglected while investigators focused on the erroneous findings of the ABAD/ERAB studies. The rectification of these misconceptions about 17β-HSD10 should promote more progress in this field. Imbalanced neuroactive steroid metabolism appears to be associated with mitochondrial dysfunction in brain. The restoration of neuroactive steroid homeostasis should be a future research direction, which may create a new approach to the treatment of AD and other neurodegenerative disorders.

Conflict of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsbmb.2014.07.001.

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