Human brain short chain 1-3-hydroxyacyl-CoA dehydrogenase (SCHAD) was found to catalyze the oxidation of 17β-estradiol and dihydroandrosterone as well as alcohols. Mitochondria have been demonstrated to be the proper location of this NAD⁺-dependent dehydrogenase in cells, although its primary structure is identical to an amyloid β-peptide binding protein reportedly associated with the endoplasmic reticulum (ERAB). This fatty acid β-oxidation enzyme was identified as a novel 17β-hydroxysteroid dehydrogenase responsible for the inactivation of sex steroid hormones. The catalytic rate constant of the purified enzyme was estimated to be 0.66 min⁻¹ with apparent $K_m$ values of 43 and 50 μM for 17β-estradiol and NAD⁺, respectively. The catalytic efficiency of this enzyme for the oxidation of 17β-estradiol was comparable with that of peroxisomal 17β-hydroxysteroid dehydrogenase type 4. As a result, the human SCHAD gene product, a single-domain multifunctional enzyme, appears to function in two different pathways of lipid metabolism. Because the catalytic functions of human brain short chain 1-3-hydroxyacyl-CoA dehydrogenase could weaken the protective effects of estrogen and generate aldehydes in neurons, it is proposed that a high concentration of this enzyme in brain is a potential risk factor for Alzheimer’s disease.

The human short chain 1-3-hydroxyacyl-CoA dehydrogenase (SCHAD) gene, mapped at chromosome Xp11.2, encodes a single-domain dehydrogenase composed of four identical subunits (1). The primary structure of this 1-3-hydroxyacyl-CoA dehydrogenase is identical to an amyloid β-peptide-binding protein called endoplasmic reticulum-associated-binding protein (ERAB) (1–3). Human short chain 1-3-hydroxyacyl-CoA dehydrogenase has a molecular mass of 108 kDa, and its structure is quite distinct from other 1-3-hydroxyacyl-CoA dehydrogenases that bear the signature pattern of the 1-3-hydroxyacyl-CoA dehydrogenase family (4, 5). Although this enzyme is not homologous to the classic 1-3-hydroxyacyl-CoA dehydrogenase (1-3-HAD), which is encoded by the human HADHC gene located at chromosome 4q22–26 (6), functional convergence during evolution has conferred on them the capability of catalyzing the same reaction: 1-3-hydroxyacyl-CoA + NAD⁺ ⇌ 3-ketoacyl-CoA + NADH + H⁺ (1).

Transiently expressed ERAB was reportedly localized at the endoplasmic reticulum of the cultured cells (2). However, this finding does not agree with the presumed function of human SCHAD/ERAB in fatty acid β-oxidation (1). Therefore, the intracellular distribution of this dehydrogenase needed to be re-examined. Determination of the role(s) that human SCHAD plays in normal cells is of paramount importance for elucidating its role(s) in the pathogenesis of Alzheimer’s disease. Because some members of the short chain dehydrogenase family display activities toward several structurally distinct substrates (7), we set out to determine whether human SCHAD harbors more than one enzymatic activity. Human SCHAD is clearly homologous to the amino-terminal domain of the peroxisomal multifunctional protein-2 (MFP-2), which exhibits d-3-hydroxyacyl-CoA dehydrogenase (d-3-HAD) activity of opposite stereospecificity (8). Interestingly, this domain also displays some 17β-hydroxysteroid dehydrogenase (17β-HSD) activity (9). MFP-2 was found to be identical to the previously described 17β-hydroxysteroid dehydrogenase type 4 (8, 9), although the maximal velocity ($V_{max}$) of the 17β-HSD was only about 1/8000 of that of d-3-HAD (9). These clues prompted us to further characterize the catalytic properties of human brain SCHAD.

In this report, we provide evidence that human brain SCHAD is a new 17β-hydroxysteroid dehydrogenase. Moreover, it also harbors alcohol dehydrogenase activity. This single domain multifunctional enzyme not only plays a part in the mitochondrial fatty acid β-oxidation, but also functions in steroid hormone metabolism.

EXPERIMENTAL PROCEDURES

Plasmid Constructs and Transient Transfections—The cDNA insert (BamHI-EcoRI) containing the whole coding region of human brain SCHAD was removed from the recombinant plasmid pGEM-T-HBHAD (1) by digestion with proper restriction enzymes and subcloned into the BamHI-EcoRI site of the vector pDNA3.1 (Invitrogen) to yield a mammalian expression plasmid designated pDNA3.1/SCHAD. The cDNA of human brain SCHAD was also amplified from pGEM-T-HBHAD by polymerase chain reaction using a pair of primers: 5’-AAGCTTCCGC-CACCATGGCAGCAGCGTGTCGGA-3’ and 5’-CTCGAGAGGCTG-CATACGGAATTGCCC-3’. The resulting polymerase chain reaction product was cloned into the pcDNA3.1/CT-GFP-TOPO vector (Invitrogen) according to the procedures of the manufacturer to generate a SCHAD-GFP (green fluorescent protein) fusion protein expression plasmid designated pDNA3.1/SCHAD-CT-GFP. COS-7 and PC-12 cells were grown according to the instructions of the supplier (ATCC). All culture media were purchased from Life Technologies, Inc. Subconfluent cells

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† The abbreviations used are: SCHAD, short chain 1-3-hydroxyacyl-CoA dehydrogenase; ERAB, endoplasmic reticulum-associated-binding protein; HSD, hydroxysteroid dehydrogenase; AD, Alzheimer’s disease; Aβ, amyloid β-peptide; GFP, green fluorescent protein; IPTG, isopropyl-1-thio-b-D-galactopyranoside.

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were plated in 35-mm plates. The next day, cells were washed with fresh medium and transfected with 1 μg of pcDNA3.1/CT-GFP (Invitrogen), pcDNA3.1/SCHAD-CT-GFP, pcDNA3.1/SCHAD, and pcDNA3.1, respectively, using LipofectAMINE PLUS reagent (Life Technologies, Inc.) in accordance with the protocol of the manufacturer. From 24 to 48 h post-transfection, the cells were examined for GFP expression or were used for immunocytochemical staining.

**Antibody Preparation and Immunocytochemistry**—Anti-SCHAD antibodies were generated by immunizing a rabbit with the peptide RLDDAIRMQP coupled to keyhole limpet hemocyanin with glutaraldehyde. Cells transfected with pcDNA3.1/SCHAD or pcDNA3.1 and growing on coverslips were incubated with primary anti-SCHAD antibody. The bound primary antibody was then detected with fluorescein isothiocyanate-labeled goat anti-rabbit IgG (Sigma) according to a published protocol (10). Cells were imaged using a laser scanning confocal microscope as described below.

**Mitochondria Staining and Confocal Microscopy**—Living cells were incubated in culture medium containing 1 μM MitoTrackerRed CMXRos (Molecular Probes) for 15 min at 37 °C. The live cells were then either mounted in fresh culture medium and examined directly or were immunostained as described above. Fluorescence-labeled cells were examined using a Nikon Eclipse E800 microscope coupled to a Nikon PCM 2000 dual laser scanning confocal microscopy system. Images were analyzed with a C-Imaging-SIMPLE32TM image analysis system (Compix Inc.).

**Overexpression and Purification of Human Brain Long-3-Hydroxyacyl-CoA Dehydrogenase—** IPTG induction of Escherichia coli BL21 (DE3)pLysS/pSBET-HBH transformants, and the purification of human brain SCHAD from the transformants containing the overexpressed enzyme, were performed as described previously (1).

**Protein Analysis and Enzyme Assays**—Protein concentrations were determined by the method of Bradford (11). Proteins were separated on a 4—20% gradient gel at pH 8.3, as described previously (12). The activity of L-3-hydroxyacyl-CoA dehydrogenase was measured with acetocetin-CoA as substrate according to the published method (13). Alcohol dehydrogenase and hydroxysteroid dehydrogenase activities were assayed at 25 °C by spectrophotometrically measuring the absorbance change at 340 nm as a function of time (14, 15). The molar extinction coefficient used for calculating rates is 6220 M−1 cm−1 for NADH. The molar extinction coefficient used for calculating rates is 2.0 × 10^3 M−1 cm−1 for the dehydrogenation of 1-propanol. However, secondary alcohols were found to be better substrates. For example, 2-propanol was oxidized approximately 9-fold faster than was 1-propanol in a standard assay. Nevertheless, the rate of the backward reaction, i.e. the reduction of acetone to produce 2-propanol, was not detected. These observations suggest that human brain SCHAD, in contrast to the classic L-3-hydroxyacyl-CoA dehydrogenase, is a multifunctional enzyme.

**Characterization of the Intrinsic Dehydrogenase Activities of Human Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase**—A variety of steroid substrates were tested for the ability of human brain SCHAD to oxidize or to reduce them. We found that SCHAD could oxidize the most potent estrogen 17β-estradiol to a very weak one, estrone. This dehydrogenase can also oxidize and thereby inactivate androstenedione to androstanediol. In addition, SCHAD can use NADH as coenzyme to reduce the potent androgen, 5α-dihydrotestosterone, to a very weak one, dihydroandrosterone. SCHAD also displayed low activities toward C21 steroid hormones (Table I). Details of this investigation will be described in a subsequent report. Our results reveal that human brain SCHAD is a new type of oxidative 17β-hydroxysteroid dehydrogenase, which possesses some 3α-hydroxysteroid dehydrogenase activity as well. Its primary catalytic properties were determined by steady-state kinetic measurements (16, 18) and are summarized in Table II. To our knowledge, porcine 17β-hydroxysteroid dehydrogenase type 4 was the only known oxidative 17β-HSD that had been purified to near homogeneity (9). It is noteworthy that the Vmax and the Km relaxed significantly that those reported for porcine 17β-HSD type 4 (9). Therefore, the actual catalytic efficiencies of these two different oxidative 17β-HSDs are comparable.

The effects of pH on the 17β-HSD and L-3-HAD activities of SCHAD/antibody complex co-localized with the red fluorescence of mitochondria (see Fig. 1, h and i). When premune rabbit serum was used instead of the anti-SCHAD antibodies, such green fluorescence was not observed (data not shown). Cells transfected with pcDNA3.1 vector emitted little green fluorescence after treatment with anti-SCHAD and FITC-labeled secondary antibody (data not shown). We obtained similar results when using PC-12 instead of COS-7 cells (data not shown).

The human SCHAD gene product was shown to be identical to the αβ-binding-protein, ERAB (1). This finding may cause some confusion about whether human SCHAD participates in mitochondrial fatty acid β-oxidation, particularly in light of a recent report localizing ERAB to the endoplasmic reticulum in transiently transfected cells (2). Our study, employing confocal microscopy and using either the immunocytochemistry or GFP method, resulted in fluorescence images differing from those published previously (2). We show that mitochondria are a major location for human SCHAD in living cells (see Fig. 1). These results strongly support our proposition (1) that this human dehydrogenase could act as a mitochondrial fatty acid β-oxidation enzyme.

**Capability of Human Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase to Oxidize Alcohols**—Ethanol is usually a good substrate for alcohol dehydrogenases (14); however, its rate of oxidation catalyzed by human brain SCHAD is not detectable (data not shown). Because we considered SCHAD to be a new member of the short chain dehydrogenase superfamily (1), we were interested in determining whether this mitochondrial β-oxidation enzyme is capable of converting alcohols to aldehydes or ketones. Our experiments revealed that propanol and longer chain primary alcohols could be slowly oxidized. For example, the specific activity of the purified enzyme was estimated to be 2.0 ± 0.3 nmol·min⁻¹·mg⁻¹ for the dehydrogenation of 1-propanol. However, secondary alcohols were found to be better substrates. For example, 2-propanol was oxidized approximately 9-fold faster than was 1-propanol in a standard assay. Nevertheless, the rate of the backward reaction, i.e. the reduction of acetone to produce 2-propanol, was not detected. These observations suggest that human brain SCHAD, in contrast to the classic L-3-hydroxyacyl-CoA dehydrogenase, is a multifunctional enzyme.

**Subcellular Location of Human Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase**—The GFP and GFP-tagged human brain short chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD-GFP) were expressed in COS-7 cells transiently transfected with pcDNA3.1/CT-GFP and pcDNA3.1/SCHAD-CT-GFP, respectively. Because GFP and its fusion protein fluoresce under UV light, GFP is a convenient molecular reporter to monitor patterns of protein localization in living cells (17). As shown in Fig. 1a, GFP was distributed uniformly throughout the cytoplasm and nucleus. In contrast, the resulting SCHAD-GFP fusion protein displayed a granulated pattern of localization (see Fig. 1d). Once mitochondria were specifically stained by a mitochondria-selective dye with red fluorescence (Fig. 1, b and c), the punctate organelles where the GFP-tagged SCHAD localized were unequivocally identified as mitochondria (see Fig. 1f). Because GFP itself cannot enter mitochondria (18e); these observations suggest that mitochondria are the proper location for this dehydrogenase.

The native SCHAD expressed in COS-7 cells transiently transfected with pcDNA3.1/SCHAD was located by immunocytochemistry (see Fig. 1g). The green fluorescence of the SCHAD/antibody complex co-localized with the red fluorescence of mitochondria (see Fig. 1, h and i). When premune rabbit serum was used instead of the anti-SCHAD antibodies, such green fluorescence was not observed (data not shown). Cells transfected with pcDNA3.1 vector emitted little green fluorescence after treatment with anti-SCHAD and FITC-labeled secondary antibody (data not shown). We obtained similar results when using PC-12 instead of COS-7 cells (data not shown). The human SCHAD gene product was shown to be identical to the αβ-binding-protein, ERAB (1). This finding may cause some confusion about whether human SCHAD participates in mitochondrial fatty acid β-oxidation, particularly in light of a recent report localizing ERAB to the endoplasmic reticulum in transiently transfected cells (2). Our study, employing confocal microscopy and using either the immunocytochemistry or GFP method, resulted in fluorescence images differing from those published previously (2). We show that mitochondria are a major location for human SCHAD in living cells (see Fig. 1). These results strongly support our proposition (1) that this human dehydrogenase could act as a mitochondrial fatty acid β-oxidation enzyme.
human brain short chain L-3-hydroxyacyl-CoA dehydrogenase were determined. The reduction of acetoacetyl-CoA to L-3-hydroxybutyryl-CoA exhibited a pH optimum of 6.5–7.0 (Fig. 2), which is quite distinct from that reported for the bovine liver enzyme (19). In the latter case, reaction rates continuously increase with decreasing pH (19). For the oxidation of 17β-estradiol to estrone, human brain SCHAD showed a pH optimum of 9.5–10.0 (Fig. 2), which is similar to that reported for human 17β-HSD type 2 (20). SCHAD is different from other 17β-HSDs because of its inability to catalyze the reduction of either estrone or androstenedione by NADH or NADPH at an appreciable rate (Table I). However, its pH-activity profile for the reduction of acetoacetyl-CoA appears to be similar to the patterns determined for the reduction of estrone or androstenedione catalyzed by human 17β-HSD type 2 (20).

**Human Short Chain L-3-Hydroxyacyl-CoA Dehydrogenase Is a Novel 17β-Hydroxysteroid Dehydrogenase**—Thus far, five different types of human 17β-HSD have been identified (20–24). However, only type 1, which catalyzes the reduction of estrone to 17β-estradiol, has been purified to near homogeneity (25). Recently, rat 17β-HSD type 6 (26) and mouse 17β-HSD type 7 (27) and Ke 6 (28) were identified and reported to be new isoenzymes. However, their corresponding enzymes from human tissues have not yet been identified. The sequence identity between human 17β-HSD isoenzymes is less than 22% except that human brain SCHAD and the amino-terminal domain of peroxisomal 17β-HSD type 4 show 29% identity. Nevertheless, all of them have eight conserved residues that represent “fingerprints” typical of the short chain dehydrogenase family (29) (see Fig. 3). By comparing the structural and functional features of SCHAD with the known 17β-HSDs (see Table III), we conclude that human SCHAD represents a new type of 17β-HSD.

A homolog of the human SCHAD gene was found on *Drosophila* chromosome X. A conceptual translation from the open reading frame of the *Drosophila scutellum* gene revealed a 68%
L-3-hydroxyacyl-CoA dehydrogenase was thought to function in fatty acid β-oxidation because such a role agreed with the presence of aberrant mitochondria and cytoplasmic lipid inclusions in scully mutants described previously (30). However, other important scully mutant phenotypes, such as hypogonitalism and germinal cell aplasia, cannot be explained without the knowledge that human brain SCHAD is a multifunctional enzyme with intrinsic 17β-hydroxysteroid dehydrogenase activity. Combining these observations, it seems likely that the invertebrate counterpart of human SCHAD, the scully gene product, retains enough hydroxysteroid dehydrogenase activity to play a significant part in sex steroid hormone metabolism as well as in fatty acid oxidation.

**DISCUSSION**

The results of our present study provide new evidence for a close relationship between fatty acid metabolism and steroid metabolism. The recent observation that the sterol regulatory element-binding protein (SREBP) stimulates the biosynthesis of cholesterol and fatty acids—particularly unsaturated fatty acids—established interdependent regulatory control at the transcriptional level for these two lipid pathways (31). Our study, which shows that the human SCHAD gene product, a mitochondrial β-oxidation enzyme, functions as an oxidative 17β-hydroxysteroid dehydrogenase (17β-HSD), establishes interdependence of metabolites in these two pathways upon a single-domain multifunctional enzyme. Together, these observations suggest that both the biosynthesis and the catabolism of intermediates in these two lipid pathways are interrelated at both the level of gene expression and enzyme function. These important observations could have wide ranging implications.

17β-Hydroxysteroid dehydrogenase is essential for sex steroid metabolism. The reductive 17β-HSDs catalyze the final step in the synthesis of potent androgens and estrogens, whereas the oxidative 17β-HSDs inactivate these androgens and estrogens. The various 17β-HSD isoenzymes display different tissue and cellular distributions and substrate specificities (Table III). Expression of reductive 17β-HSD isoenzymes across tissue types is directly related to the formation of the respective sex steroid in each tissue type. For example, 17β-HSD types 3 and 5 catalyze the formation of testosterone in the testis and peripheral tissues, respectively (22, 24). In contrast, type 1 17β-HSD converts estrone to the potent estrogen 17β-estradiol in both gonadal and nongonadal tissues, including brain (32). Reportedly, 30–50% of total androgens in males and 75% of total estrogens in females prior to menopause are synthesized in peripheral tissues (33). Oxidative 17β-HSD, on the other hand, play a significant role in maintaining the steady-state level of each sex steroid within individual cells. 17β-HSD type 2, expressed in placenta, endometrium, and liver, inactivates androgens as well as estrogens (20). 17β-HSD type 4, expressed in organs that are rich in peroxisomes, catalyzes the oxidation of 17β-estradiol (8, 9). We found that human SCHAD, expressed in a variety of tissues including brain, catalyzes the conversion of 17β-estradiol and dihydrotestosterone to inactive steroids (Table I). This is the first mitochondrial enzyme found to function in the catabolism of steroid hormones although several reactions essential for the conversion of cholesterol into sex steroids and bile acids have been previously shown to occur in mitochondria. Moreover, the oxidation of androsterone catalyzed by human SCHAD would yield androstenedione, a potent inhibitor of the aromatase. Therefore, overexpression of this dehydrogenase may interfere with the generation of 17β-estradiol from androgens. After menopause, all estrogens in women are synthesized in peripheral tissues (33). In this situation, human SCHAD and other oxidative 17β-HSDs would have an exceedingly profound impact on the control of intra-

**Table I**

| Substrate                  | Coenzyme | Specific activity a
|----------------------------|----------|-------------------|
| 17β-Estradiol              | NAD⁺     | 15.6 ± 0.8        |
| Estriol                    | NADH     | ND ^b             |
| Testosterone               | NAD⁺     | 1.1 ± 0.2         |
| Androstanediol             | NADH     | ND ^b             |
| Dihydroandrosterone        | NAD⁺     | 130 ± 1.8         |
| Androsterone               | NAD⁺     | 12.1 ± 0.9        |
| Androsterone               | NADH     | ND ^b             |
| 5α-Dihydrotestosterone     | NAD⁺     | 1.0 ± 0.1         |
| 5α-Dihydrotestosterone     | NADH     | 8.7 ± 2.0         |
| Epiandrosterone            | NAD⁺     | ND ^b             |
| Epiandrosterone            | NADH     | ND ^b             |
| 5-Androstenedioli          | NAD⁺     | 0.7 ± 0.3         |
| 5α-Androstanediol          | NAD⁺     | ND ^b             |
| 5α-Androstanediol          | NADH     | 0.9 ± 0.3         |
| 20α-Dihydroprogesterone    | NAD⁺     | 3.0 ± 0.6         |
| Progesterone               | ND ^b    | 3.6 ± 0.2         |
| Dihydrocortisone           | NAD⁺     | ND ^b             |
| Cortisone                  | NADH     | 6.5 ± 0.8         |
| Cortisol                   | NAD⁺     | 0.9 ± 0.1         |

^a When NAD⁺ and NADH were replaced by NADPH, respectively, no enzyme activity was detected.
^b The data from triplicate assays are given as a mean ± S.D.
^c ND, not detected, implies activity of <0.0005 unit/mg.

**Table II**

| Substrate                  | kcat | Km  |
|----------------------------|------|-----|
|                           | s⁻¹  | μM  |
| 17β-Estradiol              | 11 ± 0.2 | 43 ± 2.1 |
| NAD⁺                      | 93 ± 2.8 | 34 ± 2.4 |
| Androsterone               | 11 ± 1.3 | 45 ± 9.3 |
| NAD⁺                      | 242 ± 56 |       |

The kinetic data are based on measurements of human brain short chain L-3-hydroxyacyl-CoA dehydrogenase at pH 8 with steroids as variable substrates at several fixed concentrations of NAD⁺.

**Fig. 2.** Effects of pH on the activities of human short chain L-3-hydroxyacyl-CoA dehydrogenase. Solid circles and triangles represent 17β-hydroxysteroid dehydrogenase activity measured with 100 μM 17β-estradiol as substrate and L-3-hydroxyacyl-CoA dehydrogenase activity assayed with 20 μM acetocetyl-CoA as substrate, respectively. Initial rates were measured at 25°C in the presence of 1 mM NAD⁺ (●) or 100 μM NADH (▲) in potassium phosphate buffer adjusted to the appropriate pH.
Recent clinical studies have suggested that estrogen replacement therapy can delay or prevent Alzheimer's disease (AD) (34). A number of putative mechanisms whereby estrogens could interfere with the progression of AD have been proposed, including organizational and activational effects on the central nervous system (35), increase of dendritic spines and synapse formation (36), and reduction of neuronal generation of amyloid β-peptide (37), among others. Brain extracts of AD patients contain significantly more human brain SCHAD/ERAB than those of age-matched controls (2). A high concentration of human brain SCHAD would likely cause an estrogen-deficient state in neurons. The identification of human brain SCHAD as a new 17β-HSD leads us to propose that high concentrations of human brain SCHAD are a potential risk factor for AD.

Estrogens reportedly attenuate excitotoxicity, oxidative injury, and amyloid β-peptide (Aβ) toxicity in hippocampal neurons (38). Overexpression of human brain SCHAD in cultured cells would deplete 17β-estradiol, thus suggesting for the high sensitivity of such cells to Aβ-induced stress and apoptosis described previously (2). Moreover, it was reported that aldehyde reductase confers protection upon PC-12 cells to glyoxal cytotoxicity (39), so the alcohol dehydrogenase activity of human brain SCHAD may play the opposite role of enhancing the toxicity of aldehydes. Because the human brain SCHAD/Aβ complex displays considerable enzymatic activities, the binding of Aβ to human brain SCHAD might exacerbate the adverse effects of this multifunctional enzyme if it would cause a relocation of SCHAD. On the basis of these observations, we believe that tuning down SCHAD expression to restore the protective effects of estrogen may ameliorate the neuronal dysfunction associated with AD. Additionally, because the human SCHAD gene product is responsible for estrogen inactivation, it would be important to assess if its expression in malignant cells of estrogen-responsive tissues such as breast cancer and endometrium cancer differs from that in normal controls. In our effort to expand insight into this important multifunctional enzyme, we are currently studying the regulatory mechanism of human SCHAD gene expression.
L-3-Hydroxyacyl-CoA Dehydrogenase

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