Intrinsic alcohol dehydrogenase and hydroxysteroid dehydrogenase activities of human mitochondrial short-chain L-3-hydroxyacyl-CoA dehydrogenase

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The alcohol dehydrogenase (ADH) activity of human short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD) has been characterized kinetically. The k_{cat} of the purified enzyme was estimated to be 2.2 min^{-1}, with apparent K_m values of 280 mM and 22μM for 2-propanol and NAD^+, respectively. The k_{cat} of the ADH activity was three orders of magnitude less than the L-3-hydroxyacyl-CoA dehydrogenase activity but was comparable with that of the enzyme's hydroxysteroid dehydrogenase (HSD) activity for oxidizing 17β-oestradiol [He, Merz, Mehta, Schulz and Yang (1999) J. Biol. Chem. **274**, 15014–15019]. However, the k_{cat} values of intrinsic ADH and HSD activities of human SCHAD were found to be two orders of magnitude less than those reported for endoplasmic-reticulum-associated amyloid β-peptide-binding protein (ERAB) [Yan, Shi, Zhu, Fu, Zhu, Zhu, Gibson, Stern, Collison, Al-Mohanna et al. (1999) J. Biol. Chem. **274**, 2145–2156]. Since human SCHAD and ERAB apparently possess identical amino acid sequences, their catalytic properties should be identical. The recombinant SCHAD has been confirmed to be the right gene product and not a mutant variant. Steady-state kinetic measurements and quantitative analyses reveal that assay conditions such as pH and concentrations of coenzyme and substrate do not account for the kinetic differences reported for ERAB and SCHAD. Rather problematic experimental procedures appear to be responsible for the unrealistically high catalytic rate constants of ERAB. Eliminating the confusion surrounding the catalytic properties of this important multifunctional enzyme paves the way for exploring its role(s) in the pathogenesis of Alzheimer's disease.

Key words: Alzheimer's disease, brain, endoplasmic-reticulum-associated amyloid β-peptide-binding protein, oestrogen, steady-state kinetics.

INTRODUCTION

Human brain short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD) is a homotetramer with a molecular mass of 108 kDa [1]. The primary structure of its subunit is identical to that of an amyloid β-peptide-binding protein reportedly associated with the endoplasmic reticulum (endoplasmic-reticulum-associated amyloid β-peptide-binding protein, ERAB) [1,2]. SCHAD is capable of catalysing the third reaction of the fatty acid β-oxidation spiral [1], and in that respect is similar to the classic L-3-hydroxyacyl-CoA dehydrogenase (L-3-HAD) [3,4], although the latter is a two-domain dehydrogenase whereas human SCHAD is a single-domain dehydrogenase [1,5]. We have demonstrated that the human *SCHAD* gene product is a multifunctional enzyme, which is transported into mitochondria and functions in two different pathways of lipid metabolism [6]. This mitochondrial fatty acid β-oxidation enzyme plays a significant role in the metabolism of steroid hormones [6].

Human mitochondrial SCHAD also displays alcohol dehydrogenase (ADH) activity. In contrast to other ADHs, ethanol is an extremely poor substrate for human SCHAD [6]. Although the specific activity of human SCHAD for oxidizing 2-propanol was found to be one order of magnitude greater than that for oxidizing 1-propanol, the reduction of acetone to 2-propanol by this enzyme was not detected [6]. The catalytic properties of the intrinsic ADH activity of human SCHAD need to be studied further, because Yan et al. reported recently that ERAB displays 'generalized ADH' activities [7], catalysing the oxidation of 17β-oestradiol and alcohols such as ethanol with much faster rates than reported for human brain SCHAD [6]. The striking differences between reported enzymic activities of ERAB [7] and human SCHAD [1,6] prompted us to investigate the catalytic properties of human SCHAD by completing the kinetic characterization of the intrinsic ADH activity of this multifunctional enzyme.

In this article, we report the kinetic features of the intrinsic ADH activity of human SCHAD, and demonstrate that the dramatic discrepencies between catalytic efficiencies of SCHAD and ERAB are due to experimental procedures employed for determining the kinetic constants of ERAB.

EXPERIMENTAL PROCEDURES

Overexpression and purification of human L-3-HAD

The cDNA insert in an expression plasmid pSBET-HBHAD containing the entire coding region of human brain SCHAD [1] was re-sequenced using the dideoxy method [8]. Isopropyl β-D-thiogalactoside induction of *Escherichia coli* BL21(DE3)pLysS/pSBET-HBHAD transformants, and the purification of human SCHAD from the transformants containing the overexpressed enzyme, were performed as described previously [1].

Determination of the C-terminal sequence of human SCHAD

Digestion mixtures contained 10 mM Mes (pH 5.5), 50 mM NaCl, 1 mM EDTA, 0.1 mM dithiothreitol, 27 μg of purified

Abbreviations used: L-3-HAD, L-3-hydroxyacyl-CoA dehydrogenase; SCHAD, short-chain L-3-HAD; ADH, alcohol dehydrogenase; ERAB, endoplasmic-reticulum-associated amyloid β-peptide-binding protein; HSD, hydroxysteroid dehydrogenase.

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human SCHAD, 0.81 μg of carboxypeptidase-Y (Sigma) and 0.08 μg of pepstatin in a total volume of 135 μl. After incubation at 47 °C for 40 min, the digestion reaction was stopped by adding PMSF to a final concentration of 0.8 mM as described previously [9]. Parallel experiments omitting either carboxypeptidase-Y or human SCHAD were run as controls. Released amino acids were determined using a Beckman 6300 amino acid analyser.

Protein and enzyme assays

Protein concentrations were determined by the method of Bradford with BSA (Sigma) as the standard [10]. The ADH activity of human SCHAD was measured at 25 °C with various concentrations of 2-propanol as substrate in 0.1 M potassium phosphate (pH 8.0) containing 0.1 mg/ml fatty acid-free BSA at several fixed concentrations of NAD^+ [11,12]. Kinetic parameters of the enzyme were estimated by analysis of the kinetic data with the computer program Leonora [13]. In addition, the activities of L-3-HAD, ADH and hydroxysteroid dehydrogenase (HSD) were assayed with acetoacetyl-CoA, 2-propanol and 17β-oestradiol, respectively, as substrate under the same conditions as described previously [7], except that the absorbance change at 340 nm was measured as a function of time using a continuously recording spectrophotometer (Hitachi U-3010) rather than by observing the absorbance every 5 min for a total of 2 h. The molar extinction coefficient used for calculating rates was 6220 $M^{-1} \cdot cm^{-1}$. A unit of activity is defined as the amount of enzyme that catalyses the conversion of 1 μmol of substrate to product per min. Molecular modelling studies of SCHAD suggest that each subunit contains a single domain. The N-terminal half of the domain is capable of binding to coenzyme, and the C-terminal half of the domain contains a catalytic triad [14] (tyrosine-168, lysine-172 and serine-155) capable of converting substrate into product (D. Lin and S.-Y. Yang, unpublished work). Since a subunit consists of 261 amino acid residues and its molecular mass is 27 kDa [1], 1 mg of human brain SCHAD was estimated to be 37.04 nmol of enzyme for the purpose of calculating the catalytic rate constant (k_{cat}), defined as the number of catalytic cycles the enzyme can undergo per unit time.

RESULTS

Catalytic efficiencies of human SCHAD and ERAB

2-Propanol was chosen as a substrate for the steady-state kinetic characterization of SCHAD [11,13] because it is the best of the known substrates to assay the intrinsic ADH activity of the enzyme. The catalytic rate constant of the purified enzyme was estimated to be 2.2 min^{-1}, with apparent K_m values of 280 mM and 22 μM for 2-propanol and NAD^+, respectively (Table 1). The catalytic efficiency for both substrates in the ordered Bi Bi reaction would be better expressed by the term $k_{cat}/(K_{ia} \cdot K_{mb})$, which describes the termolecular reaction of enzyme with both substrates and is proportional to the free energy of activation for the overall reaction [15]. However, K_{ia} values have not previously been reported for ERAB [7]. Therefore, in order to directly compare the kinetic behaviour of human SCHAD with that of ERAB, the term k_{cat}/K_{mb} must be employed to reflect catalytic efficiency. Nevertheless, this parameter may be employed for the second substrate in the ordered Bi Bi reaction to describe the rate of reaction of the substrate with the enzyme–coenzyme complex to produce the product and the enzyme–coenzyme complex [15]. As shown in Table 1, the catalytic efficiencies of human brain SCHAD for the oxidation of 2-propanol and 17β-oestradiol were 1.3×10^{-1} and 2.6×10^2 $M^{-1} \cdot s^{-1}$, respectively; the latter is comparable with that of peroxisomal 17β-hydroxysteroid dehydrogenase (17β-HSD) type 4 [16]. These values are two to three orders of magnitude lower than those reported for ERAB. Also, ERAB reportedly catalyses the reduction of acetoacetyl-CoA with a catalytic efficiency of 2.8×10^6 $M^{-1} \cdot s^{-1}$, about seven times greater than human brain SCHAD (see Table 1). The catalytic rate constants of the 17β-HSD activity and the ADH activity inherent to human SCHAD were found to be about 909- and 444-fold, respectively, less than those reported for ERAB; the k_{cat} of human SCHAD for the reduction of acetoacetyl-CoA was also five times lower than that of ERAB (see Table 1). These significant discrepancies underscored the need to determine whether mutant variations in human SCHAD or differences in assay conditions for recombinant human ERAB and SCHAD are responsible for the divergent reports.

Evidence that human SCHAD is not a mutant protein

In order to exclude the possibility that one or more mutation(s) exist in recombinant human SCHAD, we again sequenced the entire coding region of the cDNA insert in our expression plasmid by the dideoxy method [8]. Furthermore, the C-terminal sequence of human SCHAD was determined with carboxypeptidase-Y according to a published procedure [9]. SCHAD's N-terminal sequence was determined by automated Edman degradation [17]. The N- and C-terminal sequences, with 10 and 6 residues respectively, of purified recombinant human SCHAD were found to be exactly the same as predicted by the conceptual translation from the human *SCHAD* gene sequence, except for missing the first methionine residue [1] (results not shown). It is noteworthy that both SCHAD isolated from bovine liver [18] and recombinant human ERAB [7] lack the initial methionine. Together, these observations indicate that the significant discrepancies in catalytic properties reported for human ERAB and SCHAD are not due to mutations in recombinant human SCHAD. The evidence indicates that the recombinant human SCHAD employed to determine the catalytic functions and kinetic parameters of SCHAD is the authentic product of the human *SCHAD* gene, identical to the form present in human mitochondria [6].

Impact of assay conditions on specific activities of human SCHAD

The reported catalytic rate constant of ERAB for oxidation of 17β-oestradiol is greater than those of all known human 17β-HSDs [6]. To determine whether variant assay conditions are responsible for these discrepant results, human SCHAD was assayed under conditions identical to those employed in assays of human ERAB [7] to generate a set of directly comparable kinetic parameters. However, human SCHAD assays were performed using a continuously recording spectrophotometer to determine initial velocities rather than by observing absorbance changes at 5-min intervals over 120 min, as employed in ERAB assays [7]. Because ERAB's kinetic parameters were determined at a single fixed coenzyme concentration, the rate equation employed to generate the previously reported ERAB data was the Michaelis–Menten equation [7]. The L-3-HAD, 17β-HSD and ADH activities exhibited by human SCHAD were empirically determined employing substrate concentrations equal to the apparent K_m values reported for ERAB (see Table 1). The empirically determined reaction rates should have been equal to half the apparent maximal velocities ($0.5 \cdot V_{max}$). However, the velocities (v) obtained in this manner were found to be much lower than half the V_{max} reported for ERAB (Table 2). In fact, this study established that the actual specific activity of the ADH of human SCHAD is quite low, i.e. it is at least two orders of magnitude lower than the value previously reported for ERAB (see Tables

Table 1 Comparison of kinetic parameters of human SCHAD and ERAB

ND, not determined.

	SCHAD					ERAB‡				
Substrate	k_{cat} (s^{-1})	K_{ma} (μM)	K_{mb} (μM)	K_{ia} (μM)	k_{cat}/K_{mb} ($M^{-1} \cdot s^{-1}$)	k_{cat} (s^{-1})	K_{ma} (μM)	K_{mb} (μM)	K_{ia} (μM)	k_{cat}/K_{mb} ($M^{-1} \cdot s^{-1}$)
Acetoacetyl-CoA	37 ± 1.6*	–	89 ± 5.4*	–	4.2×10^5	190	–	68 ± 20	–	2.8×10^6
NADH	–	20 ± 2.8*	–	10 ± 0.9	–	–	ND	–	ND	–
17β-Oestradiol	$11 \times 10^{-3} \pm 0.2 \times 10^{-3}$†	–	43 ± 2.1†	–	2.6×10^2	10	–	14 ± 6	–	7.4×10^5
NAD^+	–	50 ± 6.4†	–	188 ± 13	–	–	ND	–	ND	–
2-Propanol	$36 \times 10^{-3} \pm 2.3 \times 10^{-3}$	–	$2.8 \times 10^5 \pm 3.3 \times 10^4$	–	1.3×10^{-1}	16	–	$1.5 \times 10^5 \pm 1.7 \times 10^4$	–	1.1×10^2
NAD^+	–	22 ± 6.2	–	74 ± 11	–	–	ND	–	ND	–

* Data are from Table 2 of [1].
† Data are from Table 2 of [6].
‡ Data are from Table 1 of [7].

Table 2 Specific activity of human SCHAD determined under ERAB assay conditions*

The only exception is that in the SCHAD assays initial velocities were measured instead of monitoring absorbance changes every 5 min for 2 h. KPi, potassium phosphate; NaPPi, sodium pyrophosphate; NaPi, sodium phosphate.

Assay buffer (pH)	Coenzyme (μmol/ml)	Substrate (μmol/ml)	Max. absorbance change* (at 340 nm)	Enzyme (mg/ml)	Enzyme (μmol/ml)	V† (determined) (μmol/min/mg)	$0.5 \cdot V_{max}$ (reported) (μmol/min/mg)	Absorbance change/min (at 340 nm)‡
97 mM KPi (7.3)	NADH	Acetoacetyl-CoA§						
	0.1	68×10^{-3}	0.423§	33.3×10^{-5}	1.23×10^{-5}	21.8 ± 1.6	215§	0.445§
20 mM NaPPi (8.9)	NAD^+	17β-Oestradiol§						
	0.4	14×10^{-3}	0.087§	30×10^{-3}	1.11×10^{-3}	$2.1 \times 10^{-2} \pm 0.5 \times 10^{-3}$	11.5§	2.15§
22 mM NaPPi/0.3 mM NaPi (8.8)	NAD^+¶	2-Propanol						
	7.5	150	46.7¶	20×10^{-3}	7.4×10^{-3}	$4.2 \times 10^{-2} \pm 2.5 \times 10^{-3}$	18¶	2.24¶

* Multiplying the concentration of substrate or coenzyme, whichever is limiting, by 6220 $M^{-1} \cdot cm^{-1}$.

† The concentration of substrate equals the corresponding K_m value for ERAB so that the initial velocity determined in this study (v) should represent half of the maximal velocity (V_{max}) of the enzyme for the indicated substrate. The kinetic constants (K_m and V_{max}) of ERAB are identical to those in Table 1.

‡ Absorbance change per min equals enzyme concentration $\times (0.5 \cdot V_{max}) \times 6220\ M^{-1} \cdot cm^{-1}$.

§ If the previously reported high catalytic rate constants of ERAB are accurate, almost all the substrate would have been consumed well before the investigators started to observe an absorbance change at 5 min due to the high concentration of enzyme used in the assay, as described in the Experimental procedures section of [7] and Figure 2 of [7].

¶ When the coenzyme is depleted, the reaction stops regardless of the amount of substrate remaining in the assay system. If the reported kinetic constants of ERAB are correct, the reaction would have been completed within 30 min. This would conflict with the published protocol [7] that '[t]he reaction was run for 2 h at 25 °C, and the absorbance at 340 nm was monitored every 5 min'.

Table 3 Reported ERAB activities and ERAB assay methods

The assay described in [7] was reportedly run for a total of 2 h at 25 °C under steady-state conditions, and the change of absorbance at 340 nm was determined every 5 min. The final concentrations of substrate, coenzyme and enzyme in the assay mixtures and the reported v_r are taken from Experimental Procedures and Results sections of [7].

Coenzyme (μmol/ml)	Substrate (μmol/ml)	Maximal absorbance change* (at 340 nm)	[Enzyme] (μg/ml)	[Enzyme] (μmol/ml)	v_r (reported) (μmol/min/mg)	Absorbance change/min† (at 340 nm)
NADH	Acetoacetyl-CoA‡					
0.1	1.5×10^{-3}	0.009‡	0.333	1.23×10^{-5}	≈ 9‡	0.019‡
0.1§	360×10^{-3}	0.622§	0.333	1.23×10^{-5}	≈ 300‡	0.621‡
NAD^+	17β-Oestradiol¶					
0.4	3.8×10^{-3}	0.024¶	30	1.11×10^{-3}	≈ 5¶	0.933¶
0.4	92×10^{-3}	0.572¶	30	1.11×10^{-3}	≈ 20¶	3.73¶
NAD^+	(−)-2-octanol					
7.5	2	12.4	20	7.4×10^{-4}	≈ 6	0.746
7.5§	105#	46.7§	20	7.4×10^{-4}	≈ 115‡	14.3‡

* Multiplying the concentration of substrate or coenzyme, whichever is limiting, by 6220 $M^{-1} \cdot cm^{-1}$.

† The value is equal to enzyme concentration $\times$ $v_r \times 6220$ $M^{-1} \cdot cm^{-1}$.

‡ If the kinetic parameters of ERAB in Table 1 are taken as accurate, all these reactions would have proceeded to near completion long before an experimenter could observe the first absorbance change at 5 min

§ When the coenzyme is depleted, the reaction stops regardless of the amount of substrate remaining in the assay system; coenzyme is limiting.

¶ The substrate would be virtually depleted within a matter of seconds, provided that the recently reported specific activities of ERAB (v_r) are accurate. It does not appear to be possible to measure reaction rates under steady-state conditions without the use of a stopped-flow system.

The fact that the solubility of (−)2-octanol is very low ($\approx$ 6 mM) has not been considered in ERAB assays.

1 and 2). Moreover, the intrinsic ADH and HSD activities of human SCHAD were found to be two to three orders of magnitude lower than its L-3-HAD activity, even for assay conditions employed in ERAB assays [7] (see Table 2). The results of this study reveal that assay conditions such as pH and the concentrations of coenzyme and substrate do not account for the kinetic differences reported for ERAB and SCHAD. Furthermore, the differences between the reported catalytic rate constants of SCHAD and ERAB shown in Table 1 cannot be attributed to analysis of the bireactant kinetics of ERAB by using the equation for a unireactant mechanism, as was done in studies of ERAB.

Inconsistency in ERAB activities reported previously

Because human SCHAD contained no mutations, and assay conditions could not have accounted for the discrepant kinetic behaviour reported for human SCHAD and ERAB, we sought to determine whether particular experimental approaches to the study of ERAB were responsible for the reported differences. ERAB studies indicated that ERAB reactions were run for 2 h and absorbance changes were observed at 5-min intervals [7]. On close scrutiny of the method employed to assay ERAB we determined that, assuming that the kinetic constants reported for ERAB [7] are true, our calculations indicate that substrates such as 17β-oestradiol and acetoacetyl-CoA would have been virtually completely consumed well before the first 5-min interval following initiation of the reaction. Even the dehydrogenation of 2-propanol would have ceased in less than 30 min due to depletion of the coenzyme (7.5 mM NAD^+), although measurements were taken for 2 h [7] (see Table 2). Assuming that the reported kinetic constants for ERAB were observed, the use of large amounts of ERAB in ERAB assays [7] would be incompatible with measurements of rates at 5-min intervals over a 2-h period (see Table 3). For example, it was reported that the catalytic efficiency of ERAB towards 17β-oestradiol was 7.4×10^5 $M^{-1} \cdot s^{-1}$, a value that is only about 4-fold lower than the value observed for the reduction of *S*-acetoacetyl-CoA [7]; however, 90 times more ERAB was reportedly employed for assaying 17β-oestradiol oxidation than for assaying the reduction of acetoacetyl-CoA (see Tables 2 and 3). Moreover, under conditions where 3.8–92 μM 17β-oestradiol was present and a high concentration of ERAB, 30 μg/ml (or 1.11 μM), was employed [7], the 3.8 μM substrate would have been depleted within a few seconds, and even 92 μM substrate would have been consumed in about 20 s, taking the reported kinetic constants of ERAB as true. Given this situation it would not seem possible to measure reaction rates without the use of a stopped-flow system [19]; however, ERAB is reportedly assayed by measuring absorbance changes over a 120-min period [7]. Since the total absorbance change at 340 nm (assuming complete consumption of substrate) ranged from 0.024 (for 3.8 μM substrate) to 0.572 (for 92 μM substrate), and if the reported kinetic constants of ERAB are taken as accurate, the specific activity of ERAB should not be greater than 1.1×10^{-3} and 2.6×10^{-2} units/mg for 3.8 and 92 μM 17β-oestradiol, respectively. These calculated values are 4500- and 770-fold, respectively, lower than the reported specific activities of ERAB, as depicted on the curve of v versus [S] for ERAB for 17β-oestradiol [7]. Similar differences between reported and expected ERAB activities are also apparent with respect to the reduction of acetoacetyl-CoA and the oxidation of (−)-2-octanol (see Table 3). Because these calculations do not depend on the identity of ERAB, the calculations are applicable regardless of whether ERAB is a mutant of the human *SCHAD* gene product or not. Altogether, it is concluded that the reported kinetic data for ERAB are unreliable, possibly due to unorthodox methods employed in ERAB assays.

DISCUSSION

A number of aliphatic alcohols were used as substrates in ERAB studies, e.g. the K_m for ethanol was reported to be as high as 1.2 M [7]. When human SCHAD was assayed in 7 % ethanol and

1 mM NAD^+, a very small increase in the absorbance at 340 nm was observed (results not shown). However, this oxidation rate was too small to determine the kinetic parameters of SCHAD's ADH activity spectrophotometrically. It was also observed that the rate of (±)2-butanol oxidation catalysed by human SCHAD was lower than the rate of 2-propanol oxidation under identical experimental conditions (results not shown). The catalytic rate constants of ERAB determined with longer-chain alcohols, except for (±)2-octanol, were not higher than rate constants obtained with 2-propanol. The k_{cat} of ERAB for oxidizing (±)2-octanol was about 7-fold higher than that for the oxidation of 2-propanol [7]. Since the solubility of (±)2-octanol is extremely low (< 0.1 % according to The Merck Index, p. 1161 [20]), and since it has not been reported how a 160-mM solution was prepared [7], 2-propanol is apparently the most appropriate substrate that can be used for the kinetic characterization of the ADH activity of human SCHAD.

The k_{cat} of the ADH activity of human SCHAD was about three times higher than that of the 17β-HSD activity [6], but was 1028-fold lower than that of the L-3-HAD activity [1]. The catalytic rate constant of the ADH activity relative to those of the 17β-HSD and L-3-HAD activities was 1:0.3:1028 (see Table 1). In contrast, the relative catalytic rate constants of ERAB for the oxidation of 2-propanol, the oxidation of 17β-oestradiol and the reduction of acetoacetyl-CoA were reported to be 1:0.6:12. On the basis of these previously reported observations of ERAB's activities, it has been proposed to rename human SCHAD as a 'generalized ADH' [7]. However, we have demonstrated that these catalytic rate constants are virtually certain to be inaccurate, possibly due to unorthodox methods employed in arriving at ERAB's kinetic constants. Well-established methods for assaying dehydrogenases such as L-3-HAD [21], HSD [14] and ADH [12] are available but have not been employed in the kinetic characterization of ERAB. The present study has not only characterized the ADH functionality of human SCHAD, but also established that the published curves for v versus [S] of ERAB [7] are invalid. Elimination of the confusion about the catalytic functions of human SCHAD and confirming it to be the human *SCHAD* gene product were essential initial steps in the elucidation of the physiological role(s) of this important multifunctional protein and its contribution(s) to the pathogenesis of Alzheimer's disease.

It has been reported recently that an ERAB fusion protein displays no steroid dehydrogenase activity but that it still exhibits marginal L-3-HAD activity [22]. This ERAB fusion protein retained only 1.3 % of the L-3-HAD activity reported for human brain SCHAD [1]. We too have observed that a human SCHAD fusion protein would be devoid of most enzymic activity because of such mutational addition. Obviously, a mutant protein is not suitable for the purpose of characterizing the catalytic properties of the human *SCHAD* gene product, a multifunctional multisubunit enzyme.

The results of our study compel the conclusion that the L-3-HAD activity of the human *SCHAD* gene product is at least several hundred times greater than its other dehydrogenase activities. However, SCHAD's catalytic efficiencies for the redox conversion of sex steroids are nevertheless comparable with those of known HSDs. Steroids are known to be metabolized in the brain, e.g. differentiating neuroblastoma cells [23] and brain astrocytes [24] have been reported to produce 17β-oestradiol. Since it was found recently that 17β-HSD type 2 was not expressed in human temporal lobe [25], human SCHAD may play a highly significant role in steroid metabolism in brain. Owing to the intrinsic HSD and ADH activities of this multifunctional enzyme, a high concentration of SCHAD/ERAB in the brains of Alzheimer's patients [2] may reduce the protective effects of oestrogen and increase the toxicity of intraneuronal aldehydes [6]. This situation could exacerbate neuronal stress due to amyloid β-peptide accumulation and promote or hasten neuronal degeneration.

We thank David Miller for critical reading of the manuscript, and Irwin H. Segel, Charlotte Russell, Tor E. Smeland and Stephen Bobin for helpful discussions. We also thank James Styles and Jeffrey Messing for performing the amino acid analyses. This work was supported in part by United States Public Health Service Grants DK47392, AG04220 (to S.-Y. Y.) and HL30847 (to H. S.) from the National Institutes of Health and by the New York State Office of Mental Retardation and Developmental Disabilities.

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Received 11 August 1999/24 September 1999; accepted 29 October 1999