Expression of 17β-hydroxysteroid dehydrogenase types 1, 2, 3 and 4 in the human temporal lobe

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Abstract

Sex steroid hormones exert important biological effects on the brain. Moreover, an extensive sex steroid metabolism occurs in the brain. In sex steroid metabolism 17β-hydroxysteroid dehydrogenases (17β-HSDs) play essential roles in catalyzing the final steps in androgen and estrogen biosynthesis. Recently four types of human 17β-HSDs and a pseudogene of the type 1 isoform were identified. To date, 17β-HSD 1 has not been extensively studied in the human brain. Therefore, we investigated the mRNA expression of the four isozymes of 17β-HSD as well as the pseudogene of the type 1 isoform in the human temporal lobe to determine the predominant isoforms and, moreover, to elucidate the existence of possible sex and age differences. We studied biopsy materials from the temporal lobe of 34 women, 32 men and 10 children. Quantification of different mRNAs was achieved by competitive reverse transcription-PCR. 17β-HSD 1, 17β-HSD 3 and 17β-HSD 4 were expressed in the human temporal lobe of children and adults, whereas 17β-HSD 2 and the pseudogene of 17β-HSD 1 were not expressed. In adults, 17β-HSD 3 and 17β-HSD 4 mRNA concentrations were significantly higher in the subcortical white matter (17β-HSD 3: 14 591 ± 3457 arbitrary units (aU), mean ± s.e.m.: 17β-HSD 4: 1201 ± 212 aU) than in the cortex (17β-HSD 3: 5428 ± 1057 aU, P<0.0002; 17β-HSD 4: 675 ± 74 aU, P<0.004). 17β-HSD 1 concentrations did not differ significantly between the white matter (3860 ± 1628 aU) and the cortex (2525 ± 398 aU) of adults. In conclusion, the present study demonstrates the expression of 17β-HSD 1, 3 and 4 mRNAs in the human temporal lobe. Together with CYP19α and 5α-reductase, known to be expressed in the human brain, the expression of 17β-HSD 1, 3 and 4 mRNAs indicates the major importance of local steroid biosynthesis in the brain.


Introduction

The brain is an important target organ of sex steroid hormones and an extensive sex steroid metabolism including aromatization and 5α-reduction occurs in several regions of the brain (Martini & Melcangi 1991, Lephart 1993, Naftolin 1994, Stoffel-Wagner et al. 1998a,b). Specific receptors for sex steroids have been identified in several regions of the brain, through which sex hormones could effect a genomic response (Sholl et al. 1989, Simerly et al. 1990).

In sex steroid metabolism 17β-hydroxysteroid dehydrogenases (17β-HSDs) catalyze the final steps in androgen and estrogen biosynthesis, thus playing a crucial role in the biosynthesis and inactivation of sex steroid hormones. Recently four types of human 17β-HSDs were identified and their structure elucidated. Type 1 17β-HSD, which is expressed in placenta, granulosa cells and a number of other tissues, primarily catalyzes the interconversion of the weak estrogen estrone (E1) and the strong estrogen estradiol (E2) with a predominance of the reductive pathway (Peltoketo et al. 1988, Luu–The et al. 1989, Dumont et al. 1992). An in-tandem pseudogene of 17β-HSD 1 has also been identified (Luu–The et al. 1990). Type 2 17β-HSD is expressed in liver, placenta, endometrium and small intestine and converts E2 to E1, testosterone to androstenedione as well as the inactive progestin, 20α-dihydroprogesterone, to the active progestin, progesterone (Wu et al. 1993). Type 3 17β-HSD is an enzyme responsible for the conversion of the weak androgen androstenedione to the potent androgen testosterone, but it also catalyzes the conversion of dehydroepiandrosterone (DHEA) to androstenediol (Adiol) and E1 to E2 (Geissler et al. 1994). To date, it has been shown to be expressed only in the testes. Type 4 17β-HSD is expressed in many tissues including liver, testis, ovary, prostate and heart, but not in placenta, and it preferentially inactivates E2 to E1 and also converts Adiol to DHEA (Adamski et al. 1995).
In the 1970s the presence of 17β-HSD activity in human fetal and adult brain (Jenkins & Hall 1977) and rat brain (Rommerts & van der Molen 1971, Pérez et al. 1975) had been demonstrated in a few tissue specimens, but systematic studies on 17β-HSD activity or expression of the mRNAs of 17β-HSD isoforms in brain tissue are lacking.

The cloning of the cDNAs of the different 17β-HSD isozymes has enabled this first investigation of the isozyme expression of 17β-HSDs in the human brain. It was carried out on a large number of temporal lobe tissue specimens from children and adults to determine the predominant isoforms and to elucidate the existence of possible sex and age differences.

Subjects and Methods

Subjects

Biopsy materials removed at neurosurgery from 34 women (32·5 ± 1·3 years; mean ± s.e.m.), 32 men (34·8 ± 1·6 years) and 10 children (6 boys and 4 girls; 8 ± 1·4 years) with temporal lobe epilepsy undergoing partial temporal lobe resection were utilized. All patients received carbamazepine (CBZ) as monotherapy or as co-medication with one additional antiepileptic drug.

Tissues

Temporal lobe biopsy materials were separated into cortex and subcortical white matter by inspection and transferred into liquid nitrogen immediately after removal and stored at −80 °C for further use. Cortex tissue specimens were available from 19 women, 16 men and 9 children, white matter tissue specimens from 6 women, 7 men and 1 child and both cerebral cortex and white matter tissue specimens from 9 women, 9 men and 1 child.

Liver tissue (n=3) was obtained from biopsies to exclude liver diseases in a transplantation program, and testis tissue (n=4) from patients with prostate carcinoma undergoing orchiectomy. Adrenal tissue (n=4) originated from tumoral kidneys. Placental tissue was obtained from six normal deliveries. Tissues were transferred to liquid nitrogen immediately after removal and stored at −80 °C for further use.

The study was approved by the local ethics committee and informed consent was obtained from all tissue donors or their parents.

mRNA quantification

The mRNAs of 17β-HSD 1, 2, 3 and 4 as well as the pseudogene of 17β-HSD 1 were quantified with only a few modifications according to a nested competitive reverse transcription (RT)-PCR protocol previously described (Watzka et al. 1997, Waha et al. 1998).

Total RNA was extracted from 25 to 50 mg tissue using the Trizol reagent (Gibco-BRL, Paisley, Strathclyde, UK). Traces of DNA were removed by treatment with RNase-free DNase I (Boehringer-Mannheim, Mannheim, Germany) followed by a second RNA extraction. RNA was taken up in RNase-free water and quantified by its spectrophotometric absorption at 260 nm.

Competitive RNA standards were prepared by two-step mutagenesis of 17β-HSD 3 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Watzka et al. 1997) or single-step mutagenesis of 17β-HSD 1 and its pseudogene, 17β-HSD 2 and 4 (Waha et al. 1998) resulting in the loss of 39, 23, 14 and 27 bp for 17β-HSD 1, 2, 3, 4 and the pseudogene of 17β-HSD 1 respectively. Successful mutagenesis was confirmed by sequencing on a semiautomated sequencer (373A, Applied Biosystems, Foster City, CA, USA). 17β-HSD 3 and GAPDH cDNAs were cloned with the pCR-script cloning kit (Stratagene, La Jolla, CA, USA). Standard RNAs for all investigated genes were produced by in vitro transcription using an RNA in vitro transcription kit (Stratagene) with T7 polymerase as previously described (Watzka et al. 1997, Waha et al. 1998).

To estimate the amount of standard RNA required for quantification of individual RNA samples, three to six RNA samples of the respective tissue groups were pooled. To aliquots of these mixtures containing 250 ng RNA each, defined amounts of standard RNAs were added. Serial dilutions ranged from 500 pg to 5 ag (attograms) for GAPDH and 100 pg to 1 ag for 17β-HSD 1 and its pseudogene, 17β-HSD 2, 3 and 4. Each mixture containing the respective amount of RNA standard and patient RNA was reverse transcribed followed by PCR amplification. The optimal titration point was defined as the concentration of standard RNA where PCR products yielded signals of comparable intensity for standard and native RNA (Fig. 1). A stock solution was prepared containing standard RNAs for 17β-HSD 1 and its pseudogene, 17β-HSD 2, 3, 4 and GAPDH at the optimal titration point. The concentration of this stock solution was selected in a way that 1 µl stock was sufficient for the RT of 250 ng total RNA. RT was performed at 42 °C for 60 min using 100 U Superscript II (Superscript preamplification System, Gibco-BRL). Resulting cDNA was diluted 20-fold with water and for GAPDH, 17β-HSD 2, 3 and 4 PCR was performed in a final volume of 20 µl containing 2 µl diluted cDNA, 10 mM Tris–HCl pH 8·3, 40 mM KCl, 1·5 mM MgCl2, 200 µM of each dNTP, 0·5 U Taq polymerase (Boehringer–Mannheim) and 4 pmol of each primer (Table 1). Primers were obtained from Genosys, Cambridge, UK or Applied Biosystems. Primers used for GAPDH have previously been published (Watzka et al. 1997). For 17β-HSD 1 and its pseudogene,
recombinant Pfu polymerase (Stratagene) instead of Taq polymerase was used. One primer of the primer pairs used for GAPDH PCR or nested PCR (17β-HSD 2, 3 and 4) was labeled with fluorescent dye. For 17β-HSD 1 and its pseudogene fluorescently labeled primers did not work due to the demanding primer sequences, which could not be avoided because of the highly homologous sequences. In these cases we used unlabeled primers, agarose gel electrophoresis (4% agarose: 19:1 acrylamide:bisacrylamide, 1 : TBE) and analyzed. Peak areas were calculated with the Genescan program (Applied Biosystems, Version 1·2.1). The ratio of native PCR product to standard GAPDH PCR products was used for the differential determination of gene expression. Initial differences in the amounts of total RNA which were subjected to RT were corrected by calculating the ratios of native GAPDH PCR products to standard GAPDH PCR products.

### Statistical analysis

Results were calculated as means ± s.e.m. The statistical difference between groups was calculated using the **Statistical analysis**

Results were calculated as means ± s.e.m. The statistical difference between groups was calculated using the
Mann–Whitney U test. P values <0·05 were considered to reflect statistical significance.

Results

Determination of titration points for 17β-HSD mRNAs in reference tissues and temporal lobe tissue

In order to validate our mRNA quantification protocol we determined the expression of 17β-HSD mRNAs in placental tissue (17β-HSD 1 and 2), testis tissue (17β-HSD 3), liver tissue (17β-HSD 4) and adrenal tissue (pseudogene of 17β-HSD 1). Employment of competitive RT-PCR requires knowledge of the amount of standard RNA which yields a signal of approximately equal density when co-amplified with total RNA. The optimal titration point was 10 pg standard RNA for 17β-HSD 1 on 250 ng total RNA (Fig. 1). The equivalent titration points were 1 pg standard RNA for 17β-HSD 2, 500 fg standard RNA for 17β-HSD 3, 10 pg for 17β-HSD 4 and 1 pg for the pseudogene of 17β-HSD 1. In all tissues the optimal titration point for GAPDH was 250 pg standard RNA based on 250 ng total RNA (Fig. 1).

In the same manner the titration points of 17β-HSD mRNAs in human temporal lobe tissue were determined. The optimal titration point was 1 fg standard RNA for 17β-HSD 1 and 250 pg for GAPDH based on 250 ng total RNA, 10 fg standard RNA for 17β-HSD 3 and 10 pg standard RNA for 17β-HSD 4 (Fig. 1). For 17β-HSD 2 and the pseudogene of 17β-HSD 1 even an RNA standard amount of 1 ag did not yield a detectable fluorescence signal of native RNA (Fig. 1). Conclusively, their mRNAs are not expressed in the human temporal lobe, only illegitimate transcription was detectable when the standard RNA was omitted in the RT step.

These data show that in the human temporal lobe 17β-HSD 1 mRNA concentrations were 10 000-fold lower than in the placenta, while 17β-HSD 3 mRNA concentrations were 50-fold lower than in testis tissue and 17β-HSD 4 concentrations were of the same magnitude as in liver tissue.

Expression of 17β-HSD mRNAs in temporal lobe tissue

from children and adults

17β-HSD 1, 3 and 4 mRNA concentrations in the cerebral cortex did not differ significantly between men and women (Table 2). 17β-HSD 3 and 4 mRNA concentrations were significantly higher in the subcortical white matter of adults than in cortex tissue (P<0·0002; P<0·004; Table 2) while these differences could not be observed in 17β-HSD 1 mRNA expression. Mean 17β-HSD 1, 3 and 4 mRNA concentrations in the cortex of children were lower than in adults, but as only a few cortex specimens from children were available a statistical analysis of the mRNA expression in cortex tissue of adults and children was not carried out.

Discussion

Neurons and glial cells are capable of synthesizing various steroid hormones such as estrogens via CYP19AROM (Naftolin 1994, Stoffel-Wagner et al. 1998a) or dihydrotestosterone via 5α-reductase (Martini & Melcangi 1991, Stoffel-Wagner et al. 1998b). Over 20 years ago the presence of 17β-HSD activity was demonstrated in a few human (Jenkins & Hall 1977) and rat brain tissue specimens (Rommerts & van der Molen 1971, Pérez et al. 1975). To date, systematic studies on 17β-HSD activity or expression of the mRNAs of 17β-HSD isoforms in brain tissue are lacking.

Four isoforms of 17β-HSD (type 1, 2, 3 and 4) and an in-tandem pseudogene of the type 1 isofor with differential tissue distribution and biochemical differences have been identified in humans (Luu-The et al. 1989, 1990, Dumont et al. 1992, Wu et al. 1993, Geissler et al. 1994, Adamski et al. 1995).

Although brain tissue homogenates are able to interconvert androstenedione to testosterone as well as E1 to E2 (Martel et al. 1992, 1994), the activity and substrate specificity of each isozyme of 17β-HSD are greatly influenced by the in vitro conditions used for the assay, particular cofactor addition and the pH of the incubation. Thus, to determine which isozymes are present within a tissue, investigations on the presence of activity should be followed by an examination of mRNA for the different 17β-HSD isoforms.

The present study is the first to determine the expression of 17β-HSD isoforms in a large number of fresh human temporal lobe tissue specimens. The sensitive competitive RT-PCR approach used permitted us to demonstrate expression of 17β-HSD type 1, 3 and 4 in the human temporal lobe, but the pseudogene of the type 1 isofor and 17β-HSD type 2 were not expressed.

A previous study, using Northern blot analysis (Casey et al. 1994), demonstrated that 17β-HSD type 2 mRNA was not present in the human brain, a finding which is now confirmed by RT-PCR.

Previously, the presence of type 1 17β-HSD primarily catalyzing the interconversion of E1 and E2 with a predominance of the reductive pathway was demonstrated in the rat, frog and human fetal brain using immunocytochemical techniques (Milewich et al. 1990, Pelletier et al. 1995, Mensah-Nyagan et al. 1996). We found that also in the human temporal lobe 17β-HSD type 1 is expressed with mRNA concentrations about 10 000-fold lower than in the placenta.

Our system for quantification of 17β-HSD 1 was primarily designed to distinguish between 17β-HSD 1 and its pseudogene and different splicing variants cannot
be differentiated. Thus, we cannot exclude that different splicing variants occur in temporal lobe or placenta tissue.

The individual levels of 17β-HSD 1 mRNA vary considerably. This is due to a few individuals presenting very high expression levels. We have no good explanation

Figure 1 Titration of RNA standards for 17β-HSD 1 and its pseudogene, 17β-HSD 2, 3 and 4. PCR was performed from total RNA of temporal lobe tissue (left lane) and reference tissues (right lane): placental tissue (17β-HSD 1 and 2), testis tissue (17β-HSD 3), liver tissue (17β-HSD 4) and adrenal tissue (pseudogene of 17β-HSD 1). Each lane corresponds to cDNA reversely co-transcribed from 250 ng total RNA with decreasing standard RNA concentrations of the respective 17β-HSD isozymes. The amounts of standard RNAs were from left to right: 100 pg, 10 pg, 1 pg, 100 fg, 10 fg, 1 fg, 100 ag, 10 ag and 1 ag.
for the runaways; regional differences within the temporal lobe itself might account for them.

17β-HSD type 3 concentrations were 50-fold lower in the temporal lobe than in testis tissue. To date, the presence of 17β-HSD type 3 mRNA in brain tissue has not been reported. Since in the brain of rats and rhesus monkeys (Rommerts & van der Molen 1971, Pérez et al. 1975, Martel et al. 1994), as well as in human temporal lobe specimens (Steckelbroeck et al. 1997), androstenedione can be converted to testosterone, these interconversions may be associated with the type 3 isoform. However, in a study using Northern blot analysis the expression of 17β-HSD 3 was not detected in the human brain, but only in the testes (Geissler et al. 1994). This discrepancy probably results from either the 50-fold lower expression of this isoform in the temporal lobe compared with testis tissue reaching the borders of sensitivity of Northern blot analysis, or from differences in the brain region under investigation. These, however, were not further identified by Geissler and his coworkers.

Type 4 17β-HSD was also expressed in the human temporal lobe with mRNA concentrations of the same magnitude as in liver tissue. Northern blot analysis of 17β-HSD type 4 in various human tissues, including brain tissue, revealed a widespread expression of the enzyme (Adamski et al. 1995). The formation of E1 from E2 has been demonstrated in various rat tissues including the brain (Martel et al. 1992). This interconversion may be associated with 17β-HSD type 4, as 17β-HSD type 2 mRNA was not expressed in the brain.

The expression of 17β-HSD 1, 3 and 4 in the temporal lobe did not differ between sexes. 17β-HSD 4 concentrations were lower in the cortex of children than in that of adults. 17β-HSD 4 activity favors the oxidation of the most potent estrogen E2 to the weak estrogen E1 so as to inactivate estrogens. Recently, we demonstrated in the same tissue specimens that CYP19<sub>AROM</sub> expression is significantly lower in the cortex of children than in that of adults (Stöffel-Wagner et al. 1998a). Thus, in children local E2 formation from testosterone via CYP19<sub>AROM</sub> might be lower than in adults and this might reduce the need for an enzyme that inactivates E2.

In the subcortical white matter of adults, 17β-HSD 3 and 4 mRNA concentrations were significantly higher than in cortex tissue, whereas for 17β-HSD 1 mRNA expression this difference did not reach significance. Recently we demonstrated in a few human temporal lobe specimens that the conversion of androstenedione to testosterone was significantly higher in the subcortical white matter than in cortex tissue of both men and women (Steckelbroeck et al. 1997). These data support the finding of higher 17β-HSD 3 mRNA levels in white matter than in cortex tissue. In the rat and frog brain, 17β-HSD 1-immunoreactive material appeared to be present only in glial and ependymal cells (Pelletier et al. 1995, Mensah-Nyagan et al. 1996). 17β-HSD 3 and 4 immunoreactivity have not been studied to date. Our data confirm the predominant 17β-HSD expression in non-neural cells and suggest that glial cells could play an important role in the biosynthesis and inactivation of sex steroid hormones. On

<table>
<thead>
<tr>
<th>Tissue</th>
<th>17β HSD 1</th>
<th>17β HSD 3</th>
<th>17β HSD 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults, cortex</td>
<td>2525 ± 398</td>
<td>5428 ± 1057</td>
<td>675 ± 74</td>
</tr>
<tr>
<td></td>
<td>(n=51)</td>
<td>(n=53)</td>
<td>(n=53)</td>
</tr>
<tr>
<td>Adults, swm</td>
<td>3860 ± 1628</td>
<td>14 591 ± 3457*</td>
<td>1201 ± 212**</td>
</tr>
<tr>
<td></td>
<td>(n=28)</td>
<td>(n=31)</td>
<td>(n=31)</td>
</tr>
<tr>
<td>Children, cortex</td>
<td>1237 ± 403</td>
<td>3395 ± 1506</td>
<td>359 ± 86</td>
</tr>
<tr>
<td></td>
<td>(n=7)</td>
<td>(n=9)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Women, cortex</td>
<td>2350 ± 459</td>
<td>7041 ± 1909</td>
<td>584 ± 74</td>
</tr>
<tr>
<td></td>
<td>(n=28)</td>
<td>(n=28)</td>
<td>(n=28)</td>
</tr>
<tr>
<td>Women, swm</td>
<td>5933 ± 3431</td>
<td>11 534 ± 2459***</td>
<td>1184 ± 204****</td>
</tr>
<tr>
<td></td>
<td>(n=13)</td>
<td>(n=15)</td>
<td>(n=15)</td>
</tr>
<tr>
<td>Men, cortex</td>
<td>2739 ± 691</td>
<td>3620 ± 53</td>
<td>777 ± 133</td>
</tr>
<tr>
<td></td>
<td>(n=23)</td>
<td>(n=25)</td>
<td>(n=25)</td>
</tr>
<tr>
<td>Men, swm</td>
<td>2064 ± 543</td>
<td>17 457 ± 6315*****</td>
<td>1218 ± 371****</td>
</tr>
<tr>
<td></td>
<td>(n=15)</td>
<td>(n=16)</td>
<td>(n=16)</td>
</tr>
</tbody>
</table>

swm: subcortical white matter.

*P<0.0002, swm vs cortex tissue, **P<0.004, swm vs cortex tissue, ***P<0.01, swm vs cortex tissue,

****P<0.05, swm vs cortex tissue, *****P<0.002, swm vs cortex tissue.

Table 2 Expression of 17β HSD 1, 17β HSD 3 and 17β HSD 4 mRNAs in the human temporal lobe. Data are expressed as means ± S.E.M. in arbitrary units.
the other hand, glial cells are involved in the formation of myelin, suggesting a possible correlation between sex steroids, these enzymatic activities and the formation of myelin, or its functions.

The importance of 17β-HSDs in the maintenance of physiological levels of E2 or testosterone is supported by its ubiquitous distribution in the human (Martel et al. 1992, 1994). The expression and regulation of the counteacting enzymes CYP19AROM and 17β-HSD 1 on the one hand and 17β-HSD 4 on the other hand in the human temporal lobe might well determine the estrogenic microenvironment and may be important in terms of intracrine and paracrine effects in the brain. The brain might possess the ability to adjust the rate of sex steroid formation according to its individual needs.

Temporal lobe specimens used for our study were from patients who were treated with either CBZ as mono-therapy or as co-medication with another antiepileptic drug. It has been reported that CBZ interacts competitively with enzymes of the cytochrome P450 superfamily such as CYP17 in testicular tissue (Kühn-Velten et al. 1990); effects on the mRNA expression of these enzymes or on the expression as well as the activity of 17β-HSDs have not yet been reported. If there is an unknown effect of CBZ on the mRNA expression of 17β-HSDs in the brain, it would be the same for all subjects under investigation.

In conclusion, the present study demonstrates for the first time the expression of 17β-HSD 1, 3 and 4 mRNAs in a large number of temporal lobe specimens from children and adults, but 17β-HSD 2 mRNA and the pseudogene of 17β-HSD 1 were not expressed. Together with CYP19AROM and 5α-reductase, which are known to be expressed in the human brain, the expression of 17β-HSD 1, 3 and 4 mRNAs indicates the major importance of local steroid biosynthesis in the brain.

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