PERGAMON

Journal of Steroid Biochemistry and Molecular Biology 70 (1999) 237–241

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Expression of CYP19 (aromatase) mRNA in different areas of the human brain

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Received 5 October 1998; accepted 19 March 1999

Abstract

The conversion of androgens to estrogens by CYP19 (cytochrome $P450_{AROM}$, aromatase) is an important step in the mechanism of androgen action in the brain. CYP19 expression has been demonstrated in the brain of various animal species and in the human temporal lobe. Studies on postnatal CYP19 expression in various other areas of the human brain are rare and carried out in a limited number of post mortem obtained tissue. Therefore, we investigated CYP19 mRNA expression in fresh human frontal and hippocampal tissues and compared them to the expression in temporal neocortex tissues.

We studied biopsy materials removed at neurosurgery from 45 women and 54 men with epilepsy. Quantification of CYP19 mRNA was achieved by nested competitive reverse transcription-PCR. CYP19 mRNA concentrations were significantly higher in temporal (2.29 ± 0.40 arbitrary units, AU, mean $\pm$ SEM; $n = 57$) than in frontal neocortex specimens (0.92 ± 0.17 AU; $n = 18$; $P < 0.04$). In hippocampal tissue specimens CYP19 expression (1.41 ± 0.18 AU; $n = 24$) was lower than in temporal neocortex specimens, but the difference did not reach statistical significance. Sex differences were not observed in any of the brain regions under investigation. In conclusion, CYP19 mRNA is expressed in the human temporal and frontal neocortex as well as in the hippocampus. Regardless of sex, CYP19 expression was significantly higher in the temporal than in the frontal neocortex.

1. Introduction

Aromatase cytochrome P450 (EC 1.14.14.1), the product of the CYP19 gene, catalyses the conversion of androgens to estrogens in specific brain areas [1]. Brain CYP19 activity is not distributed uniformly throughout the central nervous system, but is readily detectable in neurons of discrete hypothalamic and limbic structures as well as in the hippocampus, different neocortical regions and the mid-brain [2–4]. During development local estrogen formation in discrete brain regions is thought to play a central role in the mechanisms by which sex steroids perform their central neuroendocrine function. For example, they influence differentiation of the brain, regulation of gonadotropic control and behaviour [3]. The presence of CYP19 activity in the cerebral cortex has been attributed to general growth-stimulating properties of estrogens rather than to their reproductive function per se [5]. However, one of the best studied and most critical influences of local biosynthesis on neural development is that of synaptic and dendritic organisation during perinatal development and of synaptic remodelling during postnatal development [6]. CYP19 expression has been demonstrated in the brain of various animal species and in the human temporal lobe of children and adults [7]. Studies on postnatal CYP19 expression or activity in various other areas of the human brain are rare and carried out in a limited number of tissue specimens obtained post mortem [8,9]. Therefore, we investigated CYP19 mRNA expression in human fron-

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0960-0760/99/$ - see front matter
PII: S0960-0760(99)00114-4

tal and hippocampal tissue and compared it with its expression in temporal neocortex tissue.

2. Subjects and methods

2.1. Subjects

We studied biopsy materials removed at neurosurgery from 45 women (31.9 ± 1.6 years; mean $\pm$ SEM) and 54 men (35.2 ± 1.5 years) suffering from epilepsy. The study was approved by the local ethics committee and informed consent was obtained from the patients.

2.2. Tissues

Brain biopsy material was divided macroscopically into cortex and subcortical white matter, and cortex tissue specimens were transferred into liquid nitrogen immediately after removal and stored at $-80°C$ until further use. Temporal neocortex tissue specimens (neocortex from 2/3 resections of the temporal lobe pole) were available from 29 women and 28 men, frontal lobe tissue specimens from 5 women and 13 men and hippocampal tissue specimens (allocortex from selective amygdalahippocampectomies) were available from 11 women and 13 men.

2.3. mRNA quantification

CYP19 mRNAs were quantified by a nested competitive RT-PCR protocol previously described using GAPDH as house-keeping gene [7,10].

In brief, total RNA was extracted from 30 to 50 mg tissue using the Trizol™ reagent (Gibco BRL, Paisly, UK) according to the manufacturer's instructions. RNA was taken up in RNase free H_2O and quantified by its spectophotometric absorption at 260 nm. Competitive RNA standards for CYP19 and GAPDH were prepared by overlap extension mutagenesis as previously described [10]. The mutant cDNAs were cloned with the pCR-script™ cloning kit (Stratagene, La Jolla, USA). From these plasmids cDNA templates were amplified and used to generate standard RNAs (in vitro transcription kit, Stratagene). cDNA templates were removed by treatment with RNase free DNase I (1 U/μg template; Boehringer–Mannheim, Germany). Standard RNA was extracted with the RNeasy™ total RNA kit (Qiagen, Hilden, Germany) and its concentration was measured spectrophotometrically.

To estimate the amount of standard RNA required for quantification of individual RNA samples we pooled 10 brain RNA samples. 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 for GAPDH and 100 pg to 1 ag for CYP19. Each mixture containing

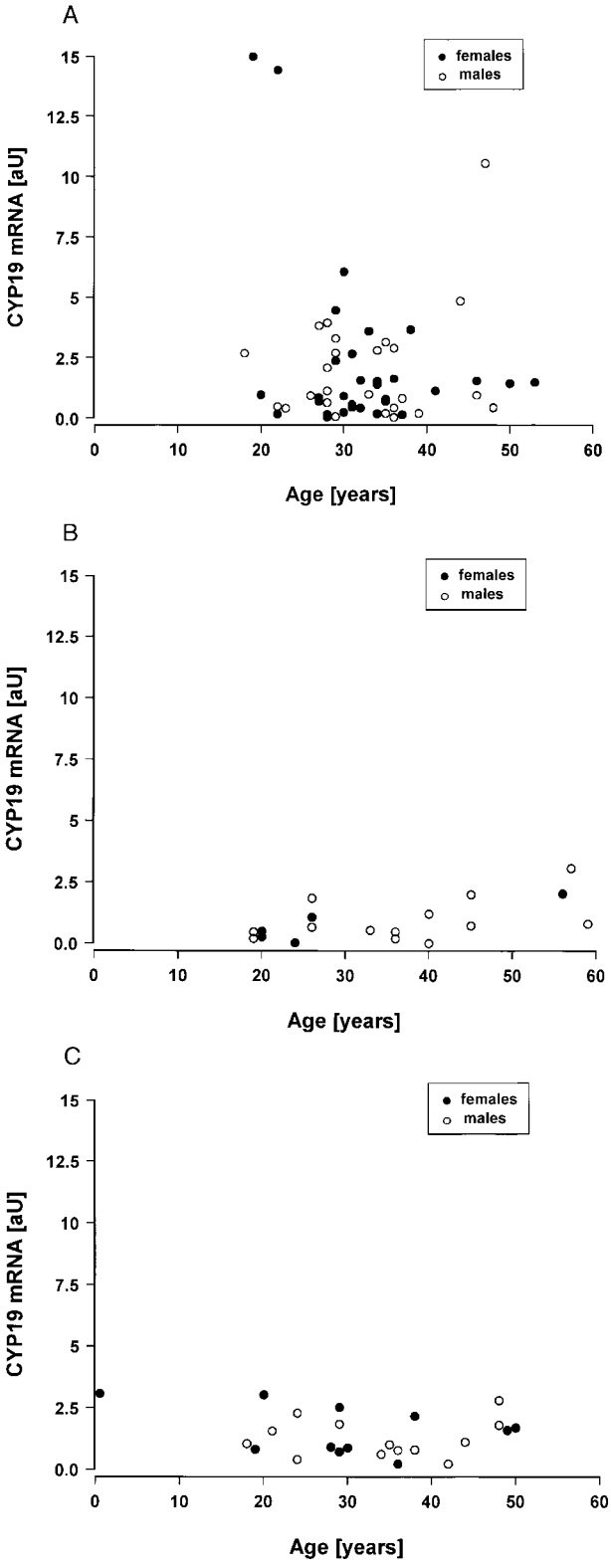

Fig. 1. Expression of CYP19 mRNA in human temporal lobe tissue (A), frontal lobe tissue (B) and hippocampal tissue (C). AU, arbitrary unit

Table 1
CYP19 mRNA concentrations in temporal, frontal and hippocampal tissue of male and female subjects[a]

Tissue	Female subjects	Male subjects
Temporal lobe	2.64 ± 0.69 AU ($n = 29$)	1.93 ± 0.41 AU ($n = 28$)
Frontal lobe	0.74 ± 0.37 AU ($n = 5$)	0.93 ± 0.24 AU ($n = 13$)
Hippocampus	1.45 ± 0.28 AU ($n = 11$)	1.26 ± 0.21 AU ($n = 13$)

[a] There were no significant differences between female and male subjects in the brain regions under investigation. AU, arbitrary unit.

the respective amount of RNA standard and patient RNA was reversely transcribed followed by PCR amplification. After reverse transcription (RT) and PCR the optimal titration points were evaluated.

RT was performed as previously described [10]. The resulting cDNA was diluted 50-fold with water and PCR was performed in a final volume of 10 μl containing 1 μl diluted cDNA, 10 mM Tris–HCl pH 8.3, 40 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.25 U Taq polymerase, 2 pmol of each primer (Genosys, Cambridge, UK or Applied Biosystems, Weiterstadt, Germany). The primer sequences and PCR cycling conditions were published previously [10]. One primer of each primer pair was labeled with fluorescent dyes. PCR products labeled with fluorescent dyes were separated on 6% denaturing acrylamide gels (50% w/w urea, 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 PCR product was used for the differential determination of gene expression. Initial differences in the amounts of total RNA subjected to RT were corrected by calculating the ratios of native GAPDH PCR products to standard GAPDH PCR products.

2.4. Statistical analyses

Results were calculated as means ±SEM. The statistical difference between groups was calculated using the Mann–Whitney *U*-test. *P* values < 0.05 were considered to reflect statistical significance.

3. Results

CYP19 expression did not show any detectable sex or age related differences in any of the brain regions investigated. (Fig. 1, Table 1).

CYP19 mRNA concentrations were significantly higher in temporal lobe (2.29 ± 0.40 arbitrary units, (AU), mean ± SEM; $n = 57$) than in frontal lobe tissue specimens (0.92 ± 0.17 AU; $n = 18$; $P < 0.04$; Fig. 1). In hippocampal tissue specimens CYP19 expression (1.41 ± 0.18 AU; $n = 24$) was lower than in temporal lobe specimens, but the difference did not reach statistical significance (Fig. 1). CYP19 expression also did not differ significantly between frontal lobe and hippocampal tissue.

4. Discussion

The brain is an important target organ of sex steroid hormones and in several regions of the brain an extensive sex steroid metabolism including aromatisation, 5α-reduction and 17β-reduction as well as 17β-oxidation occurs; the two major active products, estrogens and 5α-androstanes are responsible for many behavioral and physiological effects of the parent steroid [7,11–13].

Brain CYP19 activity is not distributed uniformly throughout the brain. CYP19 has been demonstrated not only in the diencephalon [14] where its effects on brain development would explain its location, but also in the cortex of developing rats and monkeys [2,15,16] and human fetuses [3]. The majority of physiological, biochemical and behavioral studies were carried out in rodents and other vertebrate species. However, little is known of its characteristics in the human brain. This is probably due to the difficulty in obtaining fresh human brain tissue coupled with the pesumably low expression levels of CYP19.

Recently, we demonstrated CYP19 mRNA expression in a large number of human temporal neocortex tissue specimens from children and adults [7]. In the present paper we extended our studies to the investigation of CYP19 mRNA expression in fresh human frontal and hippocampal tissues and compared it to that in temporal neocortex tissues.

Our data show that CYP19 mRNA expression levels in all brain areas under investigation did not differ significantly between the two sexes. This observation confirms our previous studies on human temporal neocortex tissue [7]. Recently Sasano et al. [8] examined CYP19 mRNA expression in various parts of post mortem human brain tissue. The number of subjects was limited and apparent differences in the amounts of CYP19 mRNA between male and female brain were not observed. Moreover, in a study on CYP19 enzyme activity in human post mortem temporal and frontal cortex tissue samples no differences between the two sexes were observed [9]. In forebrain tissues of male and female rabbit embryos [4] or in rat brain during embryonic, neonatal and infantile development [17] as well as in cortex and hippocampal tissue of adult rats [18] no sex differences were observed. However, the brains of adult male guinea pigs contain higher CYP19 activity than the brains of females [19].

These discrepant results may be due to species differences, differences concerning the brain regions under investigation or differences between CYP19 activity during fetal and postnatal development.

An important finding of our study was the fact that CYP19 mRNA expression was significantly higher in temporal neocortex specimens than in frontal neocortex specimens ($P < 0.04$) and intermediate in hippocampal tissue. Our observation is in accordance with previously published data demonstrating significantly higher CYP19 enzyme activitiy in human temporal than in frontal brain areas [9]. The authors studied biopsy materials removed at autopsy from normal adult control subjects and from patients with Alzheimer's disease. Temporal CYP19 activity was always higher regardless of sex and/or disease state. In a recent study on CYP19 mRNA expression in various parts of the human brain from four men and two women obtained from autopsy, highest mRNA amounts were found in hypothalamus, thalamus and amygdala [8]. However, for ethical reasons a mapping of CYP19 expression throughout the whole human brain was impossible for us. Sasano et al. [8] studied only five frontal lobe, three hippocampal and one temporal lobe specimen, thus not allowing a statistical comparison between these areas. CYP19 mRNA amounts were relatively variable among the brain regions and individuals (frontal lobe: 0.43 to 8.96 amol/ng RNA, hippocampus: 1.09 to 8.27 amol/ng RNA and temporal lobe: 3.3 amol/ng RNA). The authors suggest that the variability may possibly be due to the effects of terminal and/or post mortem changes on the preservation of mRNA in tissue specimens. In the present study such influences were excluded as all tissue was immediately transferred to liquid nitrogen after removal at neurosurgery. Tissue specimens from different brain areas could not be obtained from the same individuals, as of course temporal lobe tissue derived from patients with temporal lobe epilepsy and frontal lobe from those with frontal epilepsy. Thus, although the number of tissue specimens under investigation is large, interindividual variances in CYP19 expression cannot be completely excluded.

CYP19 expression in the human hippocampus is probably due to estrogen actions in this brain structure involving changes in dendritic branching and synapse formation [20]. However, the higher expression levels of CYP19 in temporal neocortex tissue compared to hippocampal allocortex tissue suggest that local estrogen production might influence higher cognitive processes characteristic for primate species. Even in the rat, in which the cerebral neocortex is not at all as highly developed as in the human brain, immunohistochemical studies on CYP19 distribution in various parts of the brain revealed immunoreactivity not only in the hippocampus, but also in several cortical regions including piriform cortex [18,21], frontal, frontoparietal and temporal cortex [21]. The localization of CYP19 in the cerebral cortex suggests that estrogens locally synthesized by CYP19 might not only be involved in regulating neuroendocrine mechanisms and reproductive functions, but also in modulation of cortical information processing.

Acknowledgements

We thank Drs N. Harada, Fujita Health University, Toyoake, Japan, and E.R. Simpson, University of Texas, Southwest Medical Center, Dallas, USA for the CYP19 cDNAs. This work was supported by the Deutsche Forschungsgemeinschaft (Kl 524/4–1) and by the Herbert-Reeck-Stiftung.

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