Recent studies have demonstrated the existence of two estrogen receptor subtypes α (ERα) and β (ERβ) with significant differences of expression among organs. Since important pathologies of human eye could be linked to hormonal status, we looked for the expression of ERβ in ocular posterior segment.

Immunohistochemical localization of ERβ and ERα protein and detection of ER mRNA by reverse transcription-polymerase chain reaction (RT-PCR) were performed in macular and extramacular regions of the retina and in the choroid on male and female donors eyes. ERβ protein was localized in the ganglion cell layer and in the choroid. At the transcriptional level, mRNA for ERβ and for ERα were both present. Local differences in the expression level were however observed suggesting the possibility of variation in the ratio of ERα vs ERβ.

The coexistence of two estrogen receptor subtypes in the human ocular posterior segment raises acute questions about their potential physiological role, but offers a perspective for preferential targeting of a specific receptor subtype.

A- Abstract

B- MATERIALS AND METHODS

Tissue collection
Human male and female donor eyes (8 males and 5 females) with a limited post-mortem enucleation time (1h-19h) were collected from the Cornea Bank, (University of Liège, Belgium). Mean age was 65 yrs (range 44-82). After removal of the anterior segment structures, 5 mm diameter punches were made in the macular region and in the peripheral retina. Neural retina was then separated from the retinal pigmented epithelium and from the choroid (RPE-choroid complex) and tissues were stored at -80°C. Alternatively, posterior segments were fixed in 4% formalin, dehydrated and embedded in paraffin.

Immunocytochemical localization of ERα
Frozen sections of 5mm were fixed in 5 min in acetone at room temperature, air-dried, and covered with the 3% normal goat serum. Then, rabbit anti-human estrogen α or β receptor (Santa Cruz Biotechnology, CA) diluted 1/250 were applied for 1 hr, sections were rinsed in Tris HCl pH 7.4 saline, and covered with one drop of EnvisionTM (Dako, ready-to-use goat anti-rabbit peroxidase-conjugated antibody) for 30 min. After rinsing in Tris/HCl, one drop of AEC+ (Dako, 3-amino-9-ethylcarbazole) was added. Sections were washed in H2O, counterstained 1 min in haematoxylin and mounted in Aquamount. Deparaffinized sections were similarly treated excepting for a preliminary microwave (350 W, four times for 5 min in 10 mM sodium citrate buffer, pH 6.0) step to unmask antigenic sites. Negative controls were obtained by omitting the primary antibody, while for positive controls, uterine tissue known to express estrogen β was used (data not shown).

Detection of ERα and ERβ by RT-PCR
Total RNA from 5 mm punches were extracted using RNeasyMini Kit (QiAGEN) as described by the manufacturer. 28S rRNA, ERα and ERβ mRNA were measured in 10μg aliquots of total RNA using the GeneAmp Thermocycler and three pairs of primers (Gibco BRL-Life Technologies) (Sense: 5'-GTTTCCCCCCACTCAACAGCGT-3' and Reverse : 5'-ACTTCCCTTGTCATTGGTACGGC-3' for ERα mRNA; Sense: 5'-TTCCAGGAAATGCTCATTACACT-3' and Reverse : 5'-CTTCTGAACTGSGAGCAAGT-3' for ERα mRNA and; Sense: 5'-GTTACCCCACTAATAGGGAACGTGA-3' and Reverse: 5'-GGATTCTGACTTAGAGGCGTTCAGT-3' for 28S rRNA). Reverse transcription was performed at 70°C for 15 min. followed by 2 min. incubation at 95°C for denaturation and, amplify for 15 sec. at 94°C, 20 sec at 58°C and 15 sec. at 72°C (35 cycles for RNA-DNA heteroduplexes. Amplification started by 15 sec. at 94°C, 20 sec at 58°C and 15 sec. at 72°C (35 cycles for 28S rRNA). Reverse transcription was performed at 70°C for 15 min. followed by 2 min. incubation at 95°C for denaturation of RNA-DNA heteroduplexes. Amplification started by 15 sec. at 94°C, 20 sec at 58°C and 15 sec. at 72°C (35 cycles for 28S rRNA). Reverse transcription was performed at 70°C for 15 min. followed by 2 min. incubation at 95°C for denaturation of RNA-DNA heteroduplexes. Amplification started by 15 sec. at 94°C, 20 sec at 58°C and 15 sec. at 72°C (35 cycles for ERα and ERβ and 19 cycles for 28S) and terminated by 2 min. at 72°C. RT-PCR products were resolved on 10% acrylamide gels and analysed using a Fluor-S MultiImager (BioRad) after staining with Gelstar dye (FMC BioProducts).

C- RESULTS

1) ER immunolocalization on posterior segment sections

• Immunolocalization of ERα is widespread in the neural retina with more intensity in the ganglion cell layer whereas ERβ is found in the ganglion cell layer of the retina and in the choroid.
• The staining was similar in tissues of both sexes.

2) ER mRNA expression in the posterior segment

• ERα and ERβ mRNA expression was detected in ocular tissues from different age and gender patients, regardless of the original location of the sample (inside or outside the macula).
• The expression of ERβ mRNA was relatively constant between different donors, while there was more variation with ERα.

Both receptor subtypes mRNA were detected in the RPE-choroid complex, but ERα was inequally distributed between the retina and the RPE-choroid.

D- CONCLUSIONS

• We demonstrate the presence of ERβ in human male and female ocular posterior segment by immunohistochemistry and RT-PCR analysis. At the transcriptional level, while the expression of ERβ was relatively constant, much more variability between different specimens was observed for ERα.
• Our results contribute to explain, at least partly, the beneficial effects observed in retinal pathology with genistein treatment. Genistein is a naturally occurring phytoestrogen with a 20-fold affinity difference for ERβ vs ERα.
• Our observations suggest that under certain circumstances, ERβ/ERα expression ratio could vary in the eye. This provides a mechanism by which estrogen could exert different effects on the same cell type.