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Dossier: Cardiovascular pathologies

Time dependence of estrogen receptor expression in human hearts

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ABSTRACT

Objectives and aims. – Transcriptional effects of estrogens are primarily mediated by the two nuclear estrogen receptors (ER), ERα and ERβ. Both receptors are present in the vasculature and in the human heart and have been shown to act antiatherogenic and to be protective against the development of cardiac hypertrophy. The aim was to quantify ER mRNA expression in left ventricular specimens from patients with coronary heart disease (CHD, $n = 15$) and dilated cardiomyopathy (CMP, $n = 38$) and compare their levels with those from healthy heart donors ($n = 9$). Additionally, a possible variation of ERmRNA expression in human hearts in respect to time of day was studied.
Methods and results. – mRNA expression of both ER receptors was detected by real-time PCR in all of the human specimens. There was no difference in the relative quantity of the receptors between CHD and CMP patients. However, control specimens showed significant lower levels of either receptor in the healthy myocardium ($p < .001$ each). Analyzing the time dependency of receptor expression with a cosinor analysis showed a significant 8-hour period rhythm for ERβ in CMP- but no rhythm in CHD patients. Due to the low patient number, rhythmic analysis was not possible in controls.
Conclusions. – The increased ERα and ERβ mRNA expression in left ventricular specimens from CHD and CMP patients might reflect a compensatory mechanism to counteract the decline in ventricular function. Furthermore, we provided evidence for a time dependent variation of ERβ receptor expression in the human heart.

1. Introduction

Estrogens play a major role in reproduction and sexual development and also participate in various physiological processes in different tissues including the cardiovascular system [1]. Estrogens, particularly 17β-estradiol (E2) and to a lesser extend estrone (E1), bind and activate estrogen receptors (ER) α and β in order to modulate growth and differentiation of target cells [2]. ERα and ERβ are nuclear receptors. After hormone binding they undergo conformational changes and bind to estrogen-responsive elements in target gene promoters mediating the transcriptional activation of estrogen responsive genes. Thereby, ERα and ERβ activate different signalling pathways causing a balance between growth and differentiation [3]. While activation of ERα, the predominant receptor in the female reproductive organs, was found to stimulate cell growth, ERβ preferentially expressed in the intestinal, cardiovascular, respiratory and urinary system, often counteracts this process [4].

Both ERs were shown to be expressed in rat cardiac myocytes and fibroblasts [5] and in the human heart [6,7]. Studies using ER-knock out mouse models revealed that both ERα and ERβ can mediate protective effects of estrogens on the cardiovascular-system [8,9] like augmenting growth or repair and NO release in vascular endothelial cells. Estrogens also regulate a number of functions of vascular smooth muscle cells, including contractility, proliferation, matrix formation, and composition [10]. Furthermore, estrogens can exert antiatherogenic effects in rodents [11], and genetic variations of ERα are associated with an increased risk of myocardial infarction in humans [12].

In addition to vessels, estrogens also exert protective effects in cardiac cells like reducing cardiac injury upon ischemia [13]. In addition, ERs have also been suggested to be protective in the development of cardiac hypertrophy. The underlying mechanisms might be a protection from higher intracellular calcium levels [14] and an inhibition of calcineurin via ERβ activation [15]. In humans, higher ventricular pressure loads can result in increased

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expressions of ERα and β as it was shown in biopsy samples from patients with aortic valve stenosis [7]. Furthermore, ERα was demonstrated to be upregulated in human heart failure [16].

In summary, estrogens, via coupling to ERα and β receptors, exert clear cardioprotective effects, and pathological adaptations of the myocardium to pathological processes result in an upregulation of ERs. Therefore, we intended to study the myocardial expression of ERα and β in hearts from patients with coronary heart disease (CHD) and dilated cardiomyopathy (CMP), respectively, and compared the levels with those in hearts from healthy controls. A further aim of this study was to analyze, whether these two ERs are expressed in rhythmic patterns during 24 h.

2. Material and methods

2.1. Study samples

We obtained specimens of left papillary muscle tissue from 64 hearts of 52 males and 12 females that had been extracted during orthopic heart transplantation. Samples were acquired from 16 patients with CHD, 39 subjects with CMP, and nine healthy donors. Prior to further analyses, we excluded one patient of the CHD and one of the CMP-group as their mRNA values were more than two standard deviations from the mean. Considering the remaining patients ($n = 62$), CHD patients featured at least one anamnestic myocardial infarction and evidenced existing occlusion of one or more coronary arteries. Three had been stable at home and eight were stable in the hospital. Three of those had received inotropic drug support and two assist device prior to heart explantation. CMP patients had no history of any clinically relevant occlusions of the coronary arteries. Thirteen of these patients were stable in the hospital, whereas two of those received drug support, and three were on an assist device before transplantation. The control group consisted of nine healthy donors without history of any cardiovascular disease. Four of them died from subarachnoidal bleeding, four suffered from lethal craniocerebral injury, and one committed suicide by a self-inflicted gunshot to the head. The control hearts had been intended for transplantation, but were not implanted, mostly due to an increased risk of infection during removal. In these hearts, there was no macroscopic indication of any myocardial disease or mechanical trauma (e.g., endocardial bleeding). Although we do not have special information on a per person basis, subjects were presumed to be adhering to a normal daytime activity/nighttime sleep routine. A summary of anthropometric characteristics of the subjects is shown in Table 1.

Patients received ACE-blockers and beta-blockers as required for their individual needs. On the other hand, donors were administered 2–10 μg/kg BM /min Dobutamin or Dopamin and Vasopressin substitution (1-Desamino-8-D-Arginin-Vasopressina-cetate) between the fatal incident and heart explantation. The time spans between the incidences and the acquisitions of samples was < 24 h. Drug administration did not follow any special chronotherapeutic pattern.

Left ventricular specimens were obtained and shock frozen on dry ice within 5 min after heart explantation. They were kept at −70 °C until further laboratory analyses. The times of explantation were monitored and linked to the minute with the ER mRNAs. The study adheres to the ethical and methodological standards expected for the conduct of medical biological rhythm research [17] and Declarations of Helsinki (updated in 2000).

2.2. Real time TaqMan® PCR

Gene expression levels for mRNAs of ERα and ERβ were analyzed. Specimens were first ground in liquid nitrogen and thereafter homogenized in TRIzol® (Invitrogen®, Carlsbad, CA, USA). Afterwards, a spectrophotometrical quantification (260 nm absorbance) was performed. Following the manufacturer's instructions, reverse transcription to cDNA was done with 2 μg total RNA using random hexamer primers and the "Superscript II RT system®" (Invitrogen®). A prefabricated "TaqMan® Gene Expression Assays" (Applied Biosystems®, ABI, Foster City, CA) for the mentioned ERs was applied.

In a preliminary experiment we determined 18S (M10098) as an appropriate endogenous control gene with highest expression stability in human heart tissue from a panel of 12 housekeeping genes by applying a "geNormTM housekeeping gene selection kit" (PrimerDesign® Ltd, Southhampton, UK).

Relative quantification of the ER mRNAs by real-time PCR was done in an amplification mixture volume of 20 μl containing 10 μl "2 × TaqMan® Universal PCR Master Mix" (ABI, #4324018), 1 μl of the appropriate Gene Expression Assay Mix for the specific gene, and 10 ng template cDNA diluted in 9 μl nuclease-free water. Thermal cycling consisted of 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 1 minute at 60 °C. An ABI PRISM 7700 Sequence Detection System® was used to measure fluorescence generation by the 5'→3' exonuclease activity of the DNA polymerase due to TaqMan® probe cleavage. All samples were amplified in duplicates and repeated twice. Relative mRNA expression levels of the specific genes were calculated by the ΔΔCt (threshold cycle) method [18] in relation to our calibrator which we generated by pooling sample cDNAs. All calculations were done according to the manufacturer's instructions. The expressions of the specific target genes are given as fold change of the target genes in relation to the calibrator normalized to the endogenous control.

2.3. Data analysis, statistics

Initially, we analyzed the distribution of age between the three groups by Student *t*-tests for non-paired samples. The only significant difference was between subjects of the control group and CHD patients (controls younger than patients, $p = .04$).

Table 1
Anthropometric data of subjects mean.

	Total	CHD-patients	CMP-patients	Controls
n (males)	62 (51)	15 (14)	38 (30)	9 (6)
Age (years)	55.7 (±11.2; 16–70)	58.7 (±7.2; 44–67)	56.6 (±11.1; 16–70)	41.3 (±11.9; 22–70)
Body mass (kg)	74.5 (±12.3; 37–105)	74.7 (±14.7; 37–96)	74.0 (±12.6; 48–105)	75.7 (±7.2; 65–88)
Diabetes mellitus II	11	5	6	0
Smoking	24	9	15	–
Creatinine (mg/dL)	1.28 (±.31; .61–2.20)	1.3 (±.37; .83–2.20)	1.26 (±.29; .61–2.13)	–
Blood urea nitrogen (BUN in mg/dL)	26.22 (±14.39; .1–94.0)	22.39 (±10.24; .01–37.6)	27.9 (±15.58; .1–94.0)	–
Cholesterol (mg/dL)	203.3 (±64.5; 109–444)	244.5 (±94.9; 135–444)	190.1 (±46.5; 109–318)	–
Triglycerides (mg/dL)	160.7 (±124.4; 41–680)	220.2 (±197.5; 72–680)	143.6 (±92.7; 41–448)	–

CHD: coronary heart disease, CMP: cardiomyopathy, "–": data not available.