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Comparative Contents of mRNAs of Sex Steroid Receptors and Enzymes of Their Metabolism in Arterial Walls of Men

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Abstract—The potential role of estrogens in regulation of metabolism in arteries of men was studied. Contents of mRNAs of sex hormone receptors, of some enzymes of their metabolism, and of some potential markers of the hormone effects were determined by real-time polymerase chain reaction in fragments of 18-54-year-old men's large arteries with and without atherosclerotic lesions. Contents of estrogen receptor alpha (ERα) and transferrin receptor mRNAs were significantly different in undamaged fragments of the aorta and of the carotid and coronary arteries. Contents of some mRNAs in the carotid artery and aorta were found to correlate, which suggested a similarly directed regulation of their expressions. The levels of ERα and aromatase mRNAs negatively correlated with the blood plasma concentration of estradiol. Levels of steroid sulfatase and aromatase mRNAs were lower and the level of estrogen sulfotransferase mRNA was higher in blood vessel fragments with atherosclerotic lesions than in undamaged fragments. It is suggested that large arteries should be different in sensitivity to estrogens and that atherosclerotic lesions could lead to local suppression of the effect of estrogen on the cells of arteries.

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Key words: sex hormone receptors, steroid sulfatase, aromatase, estrogen sulfotransferase, arteries, atherogenesis, polymerase chain reaction

According to epidemiological data, the incidence of cardiovascular diseases in women of reproductive age is several times lower than in men of the same age. Upon menopause, the morbidity in women rapidly increases and approximates that for men. Therefore, it was supposed that estrogens should have a protective effect on the cardiovascular system [1]. However, rather extensive clinical studies have revealed no positive effect of replacement therapy with combined estrogen and progestin preparations on the cardiovascular system in postmenopausal women [2, 3]. But, considering the inadequacy of hormonal preparations compared to natural hormones [4] and differences between the injection regimen and natural hormonal rhythm during the ovarian cycle, conclusions concerning the influence of estrogen on the condition of the cardiovascular system should be considered with care. Replacement therapy with selective estrogen receptor modulators (SERM) also failed to definitely answer the question about the protective effect of estrogens [5, 6]. Experimental and clinical studies have shown that the favorable effect of estrogens on the cardiovascular system includes two main components: mod-

Abbreviations: AR) androgen receptor; Arom) aromatase; GAPDH) glyceraldehyde phosphate dehydrogenase; ER) estrogen receptor; E-sel) E-selectin; EST) estrogen sulfotransferase; ICAM) intercellular adhesion molecule; PCR) polymerase chain reaction; StS) steroid sulfatase; TfR) transferrin receptor; VCAM) vascular cell adhesion molecule.

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ulation of lipid metabolism and a direct action on the vascular wall. The relative contribution of these components can vary from 1 : 3 to 2 : 3 [7]. The direct action of estrogens on blood vessels leads to vasodilation due to stimulation of NO synthase activity in the endothelium [8], inhibition of smooth muscle cell proliferation [9], and suppression of inflammation components, such as adhesion molecules (ICAM-1, VCAM-1, and E-selectin) and proinflammatory cytokines [10, 11]. The above-listed effects in totality are supposed to decrease the risk of atherogenesis. The principal cellular elements of arteries, endothelial and smooth muscle cells, are capable of independent reception of the estrogen signal owing to expression of two estrogen receptor isoforms, α and β [12]. Isoform α is in cells as a classic nuclear and shortened membrane variant of the receptor produced as a result of alternative splicing [13]. Moreover, a system modulating the level of estrogenic signal, which includes enzymes of *de novo* biosynthesis (aromatase), activation (steroid sulfatase, 17β-hydroxysteroid dehydrogenase), and inactivation of estrogens (estrogen sulfotransferase) has been found in blood vessels [14]. The vascular wall cells are also sensitive to androgens, which are physiological antagonists of estrogens in some tissues, but their role in the vascular wall is not quite clear [15, 16].

To reveal potential physiological and pathogenetic roles of estrogens and components of their reception and metabolism, in the present work contents of mRNAs of sex hormone receptors and of some enzymes of their metabolism, as well as of some proteins which could be involved in atherogenesis have been determined in undamaged and atherosclerotic regions of large arteries in men, for whom estrogens are not dominant hormones. This study was reasoned by results of clinical testing of an aromatase inhibitor in male volunteers. This testing revealed the suppression of vasodilation caused by inhibition of estrogen biosynthesis [17] that indicated a real involvement of estrogens in regulation of blood vessel conditions in men. The involvement of estrogens in regulation of metabolism in men's blood vessels is also supported by an early development of atherosclerosis in men with mutations in the genes of aromatase and estrogen receptor α. Moreover, injection of estrogen into patients with aromatase deficiency improved the state of the vascular channel and decreased some other risk factors of myocardial infarction [18].

MATERIALS AND METHODS

In this work, fragments of arteries obtained by autopsy 4-12 h after death caused by accident were used. The fragments were separated into specimens of 50-200 mg, either without noticeable lesions or containing a fatty stripe or a lipofibrous plaque. The specimens were stored in liquid nitrogen until extraction of RNA. Altogether 51 specimens isolated from 17 men of 18-54 years old (35.5 ± 10.9, 37 years as a median) were studied. Blood serum obtained from some of the donors was stored at −50°C before sex hormone concentrations were determined.

Isolation of RNA and synthesis of cDNA. RNA was isolated by homogenization of frozen pieces in TRIzol Reagent (Invitrogen, USA), 0.8 ml per 100 mg tissue; then the RNA was transferred to aqueous phase by addition of 1/5 volume of chloroform and precipitated with isopropanol according to the protocol. The precipitates washed with 75% ethanol were diluted in water, and the quantity of isolated RNA was measured with a spectrophotometer. Synthesis of cDNA was performed on 2-6 μg of total RNA using a Promega ImProm-II™ Reverse Transcription System kit (USA) as recommended by the producer. Hexameric primers (0.5 μg) were annealed for a random primer per 1 μg RNA, and a mixture was added which contained dNTP in the final concentration of 0.5 mM each, a ribonuclease inhibitor RNasin (1 U/μl), and reverse transcriptase ImProm-II™ in buffer with 4.8 mM $MgCl_2$.

Real-time PCR. The synthesized cDNA was used as a template for real-time PCR on a Rotor-Gene 3000 amplifier (Corbett Research, Australia) with a kit of reagents including the intercalating dye SYBR Green I (Sintol, Russia) as recommended. The primers were chosen using the Beacon Designer 6.00 program (www.PremierBiosoft.com). The primers were chosen on consideration of the intron–exon structure of the corresponding gene. The primer pairs were chosen without amplification of the genomic DNA during the real-time PCR. This was controlled using 4 μg isolated RNA as a template. Table 1 presents the primers used.

The reaction mixture containing all necessary components including the enzyme Taq-DNA polymerase and an intercalating reagent was supplemented with primers in the final concentration of 160 nM and with the synthesized cDNA in three or four dilutions with each pair of primers. The PCR conditions were as follows: denaturation at 95°C for 5 min, then 45-50 cycles in the following regimen: denaturation at 95°C for 10 sec, annealing at 58°C for 17 sec, elongation at 72°C for 20 sec. The fluorescence intensity of SYBR Green bound with double-stranded DNA was determined at the end of the elongation stage of each cycle at 72°C. Upon the last cycle, the melting curve of the PCR products was recorded.

Analysis of PCR results. Amplified products were analyzed by electrophoresis in 2% agarose gel. Initially, PCR products obtained with each pair of primers were isolated from agarose using a QIAEX II kit for extraction from gel (QIAGEN, Germany) according to the producer's recommendation and sequenced. DNA was sequenced with an ABI PRISM® BigDye™ Terminator v.3.1 kit of reagents with subsequent analysis of the reaction products using an ABI PRISM 3100-Avant automated DNA sequencer. In all cases, the nucleotide sequence

Table 1. Primers used for real-time PCR

cDNA	Primer nucleotide sequence	Amplification product size, bp
GAPDH	for 5'-GAGCCCGCAGCCTCCCGCT-3' rev 5'-CGCCCAATACGACCAAATC-3'	145
StS	for 5'-ACTGCAACGCCTACTTAAATG-3' rev 5'-AGGGTCTGGGTGTGTCTGTC-3'	289
Arom	for 5'-GTGAAAAAGGGGACAAACAT-3' rev 5'-TGGAATCGTCTCAGAAGTGT-3'	215
EST	for 5'-TGGTGGGAAAAGGGAAAGAGTC-3' rev 5'-CTGGCAGTGTTGTGTAATTTGTGG-3'	200
AR	for 5'-CCTGGCTTCCGCAACTTACAC-3' rev 5'-GGACTTGTGCATGCGGTACTCA-3'	168
ERα	for 5'-CTGGCTACATCATCTCGGTTC-3' rev 5'-AGGTGGATCAAAGTGTCTGTG-3'	182
ERβ	for 5'-GCTCCTGGCAACTACTTCAAG-3' rev 5'-ATCACCCAAACCAAAGCATCG-3'	186
TfR	for 5'-GTTGAATTGAACCTGGACTATGAG-3' rev 5'-GTCTGGAAGTAGCACGGAAG-3'	154
E-sel	for 5'-CCAGCCCAGGTTGAATGC-3' rev 5'-GGACCCATAACGGAAACTGC-3'	147
VCAM-1	for 5'-AGCGGAGACAGGAGACACAG-3' rev 5'-AATGGCAGGTATTATTAAGGAGGATG-3'	226
ICAM-1	for 5'-CAAGAAGATAGCCAACCAATGTGC-3' rev 5'-ACCGTGGTCGTGACCTCAG-3'	266

Table 2. Results of two independent measurements of mRNA in a lipofibrous plaque from the coronary artery specimen of a 46-year-old man

Measurement	mRNA, % of GAPDH mRNA									
	StS	Arom	EST	AR	ERα	ERβ	TfR	E-sel	ICAM	VCAM
1	12.4	0	1.7	53.1	7.0	0.2	272	53.5	32.6	287
2	8.8	0	0	47.2	6.2	0	262	42.0	52.3	215
Deviation from mean, %	17	0	100	6	6	100	2	12	23	14

corresponded to the expected DNA fragment. Only those PCR results were taken in consideration when the melting temperatures and electrophoretic mobilities of the amplification products corresponded to the expected ones. The levels of mRNAs under study were calculated in percent of the housekeeping gene mRNA content in the specimen analyzed (glyceraldehyde phosphate dehydrogenase, GAPDH).

The reproducibility of results was assessed by comparing the mRNA contents in the same specimen measured with the interval of 2 months (Table 2). For seven parameters whose values were different from zero for both measurements, the average relative deviation from the mean was 11%.

Determination of hormones. Serum concentrations of testosterone and estradiol were determined by enzyme

immunoassay using, respectively, EIA1559 and EIA2693 kits (DRG, USA), as recommended by the producer.

Statistical analysis. The results were analyzed with the Statistica 6.0 program. Independent samples were compared using the Mann–Whitney U-test. Dependent samples, which included parameters measured in different blood vessels of the same donors, were compared using the Wilcoxon test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

In the male donors studied, no correlations were found between their age and the parameters under determination; therefore, the analysis was performed without subdivision into age-related groups. Blood concentrations of sex hormones and contents of some mRNA species in undamaged regions of three types of arteries are shown in Table 3. The table does not show the content of the estrogen receptor β (ERβ) mRNA because we failed in its reliable measurement in most of the specimens. In cases with reliable measurements, the content of this mRNA was 0.04-0.8% of GAPDH mRNA. Our data on the low level of ERβ mRNA seem to contradict the data of work [19] where the levels of ERα and ERβ mRNAs were approximately equal in human blood vessels, while in our work the ratio ERα/ERβ mRNAs was no less than 10. However, first, in work [19] only smooth muscle part of the vascular wall was used and not the whole wall as in the present study. Second, the tissue was cultured for 3 days before the isolation of RNA (our preliminary data have shown that the content of ERα mRNA in the cultured cells of the aorta intima is about an order of magnitude lower than in the whole fragment of the blood vessel and, by contrast, the ERβ mRNA content is increased).

Table 3. Sex hormone levels in blood serum and contents of some mRNA species in the undamaged wall of men's large arteries

	Estradiol, pg/ml	Testosterone, ng/ml	mRNA, % of GAPDH mRNA											
			androgen receptor			estrogen receptor α			steroid sulfatase			aromatase		
Blood vessel			aorta	carot.	coron.	aorta	carot.	coron.	aorta	carot.	coron.	aorta	carot.	coron.
Mean	41	2.9	86	83	46	2.3	5.2	5.4	19	13	17	1.2	1.0	2.3
Standard deviation	24	1.4	83	47	18	2.2	3.8	4.0	11	9	10	1.3	0.9	2.1
Median	34	3.1	57	72	45	1.7	4.8	5.7	18	9.6	19	0.9	0.8	2.0
Quartiles	19-62	1.6-4.0	38-84	53-106	39-55	0.6-3.3	0.9-8.5	1.4-9.0	14-30	8.4-17	13-23	0.3-1.2	0.3-1.8	0.7-4.0
Number of measurements	10	13	12	15	5	11	15	5	12	14	5	5	8	4

	mRNA, % of GAPDH mRNA														
	estrogen sulfotransferase			transferrin receptor			E-selectin			ICAM-1			VCAM-1		
Blood vessel	aorta	carot.	coron.	aorta	carot.	coron.	aorta	carot.	coron.	aorta	carot.	coron.	aorta	carot.	coron.
Mean	3.6	0.64	1.7	78	60	80	14	7.6	32	1.0	4.2	5	137	96	290
Standard deviation	5.1	0.74	0.5	44	41	74	10	10	–	–	5.8	–	123	136	–
Median	0.4	0.3	2.0	60	45	48	15	3.6	32	1.0	0.8	5	92	37	290
Quartiles	0.2-6.9	0.2-1.0	–	53-120	26-91	39-122	7.6-21	0.6-13	–	–	–	–	49-224	18-107	–
Number of measurements	7	6	3	11	15	4	4	8	2	1	3	1	7	11	2

Note: Here and further "carot." stands for carotid artery and "coron." for coronary artery.

Third, in work [19] a fixed number of amplification cycles (35) were used, which allowed an outcome of one or both products beyond the exponential stage of the amplification.

We have quantitatively determined ICAM-1 mRNA in only nine of 34 specimens (26%) of the undamaged and damaged vascular fragments; therefore, we do not consider further this mRNA type.

Contents of mRNAs in different blood vessels were not significantly different. But it should be noted that the samples for measurement in different blood vessels were overlapped only partially. To more correctly compare the mRNA contents in different blood vessels, the mRNA ratios were analyzed in the blood vessels obtained from the same donors. The results are presented in Table 4. Data for the coronary artery and aorta ratios are not shown because the samples were too small. The ERα mRNA level in the aorta was lower than in the carotid artery. In turn, the levels of ERα and transferrin receptor (TfR) mRNAs in the carotid artery were lower than in the coronary artery.

The differences found indicated that the mRNA expression could be regulated differently in different blood vessels. To reveal potential common regulatory factors, multiple correlations were analyzed between the mRNA contents in arteries of the same donors and of testosterone and estradiol contents in their blood plasma. Coronary arteries were not considered in this analysis because of small size of the sample. Correlations with coefficients higher than 0.6 between the studied parameters are presented in Table 5. Positive correlations found between contents of mRNAs of AR, ERα, TfR, and vascular cell adhesion molecules (VCAM-1) in the aorta and carotid artery indicate that regulatory factors common for both types of blood vessels are likely to influence the corresponding mRNAs in the same direction.

The sex steroid concentration in the bloodstream is likely to be such a common regulatory factor because we have found correlations between the estradiol concentration and ERα mRNA contents in the aorta and carotid artery. Note that this correlation is negative, but in some other organs and tissues, including vascular endothelium [20], estrogens induce expression of their own receptor. However, ERα gene expression is controlled by multiple promoters [21] and estrogens can inhibit activities of some of them in some cells, including smooth muscle cells in the human aorta [22, 23].

A tendency for negative correlation was also found between the estradiol concentration and the AR mRNA contents in the two types of blood vessels. The literature data on direction of the estrogen-caused regulation of AR gene expression in human tissues are contradictory. In the

Table 4. Ratio of mRNAs in undamaged regions of the aorta and carotid and coronary arteries of the same donors

	Androgen receptor		Estrogen receptor α		Steroid sulfatase		Aromatase	
	carot./aorta	coron./carot.	carot./aorta	coron./carot.	carot./aorta	coron./carot.	carot./aorta	coron./carot.
Mean	1.44	1.27	3.86*	2.42*	0.85	1.30	0.27	69
Standard deviation	0.85	0.63	2.40	2.12	0.61	0.84	–	113
Median	1.19	1.27	3.00	1.46	0.79	1.16	0.27	6.8
Quartiles	0.65-1.97	0.75-1.78	2.10-6.50	1.30-3.60	0.44-1.15	0.60-2.00	–	–
Number of measurements	11	4	11	4	10	4	2	3

	Estrogen sulfotransferase		Transferrin receptor		E-selectin		VCAM	
	carot./aorta	coron./carot.	carot./aorta	coron./carot.	carot./aorta	coron./carot.	carot./aorta	coron./carot.
Mean	0.50	14.5	0.84	1.92*	8.6	1.98	0.98	1.66
Standard deviation	–	–	0.49	0.63	14.5	–	0.64	–
Median	0.50	14.5	0.75	1.85	0.43	1.98	1.16	1.66
Quartiles	–	–	0.52-1.08	1.44-2.41	–	–	0.36-1.17	–
Number of measurements	2	2	10	4	3	2	5	1

* Values significantly different from 1 (at $p < 0.05$).

Table 5. Correlations between the studied parameters

Parameter 1	Parameter 2							
	AR		ERα		Arom		EST	
	aorta	carot.	aorta	carot.	aorta	carot.	aorta	carot.
Estradiol	$r = -0.60$ $p = 0.15$	$r = -0.64$ $p = 0.06$	$r = \mathbf{-0.95}$ $p = 0.004$	$r = \mathbf{-0.81}$ $p = 0.008$	$r = \mathbf{-0.99}$ $p = 0.048$			
Testosterone	$r = 0.62$ $p = 0.07$					$r = 0.67$ $p = 0.09$		
AR (aorta)		$r = \mathbf{0.68}$ $p = 0.02$			$r = -0.66$ $p = 0.22$			
AR (carot.)				$r = \mathbf{0.66}$ $p = 0.007$				
ERα (aorta)				$r = \mathbf{0.71}$ $p = 0.02$			$r = 0.70$ $p = 0.12$	
StS (aorta)							$r = \mathbf{0.90}$ $p = 0.006$	
Arom (carot.)								$r = \mathbf{0.92}$ $p = 0.03$

Parameter 1	Parameter 2					
	TfR		E-sel		VCAM-1	
	aorta	carot.	aorta	carot.	aorta	carot.
Estradiol				$r = -0.62$ $p = 0.18$		
Testosterone				$r = \mathbf{-0.76}$ $p = 0.046$		
AR (aorta)					$r = \mathbf{0.84}$ $p = 0.017$	
StS (aorta)					$r = 0.64$ $p = 0.12$	
TfR (aorta)						
TfR (carot.)		$r = \mathbf{0.74}$ $p = 0.009$				$r = \mathbf{0.88}$ $p = 0.0004$
VCAM-1 (aorta)						$r = \mathbf{0.90}$ $p = 0.014$

Note: Correlations with $p < 0.05$ are shown in bold type.

promoter region of the hamster's AR gene [24] both an estrogen-response element that provides for inhibition of the promoter activity by estrogens and an androgen-response element that provides for stimulation by androgens in the Harder's gland were detected. The promoter region of the human AR gene is very much like the corresponding sequence of the hamster's AR gene.

We have found a negative correlation between estrogen level and aromatase mRNA in the aorta. Expression of the aromatase gene is controlled by numerous promoters [25], and sex hormones can influence their activities either in the same [26] or opposite direction [27].

The tendency for positive correlation revealed by us between the testosterone blood concentration and the AR

mRNA content in the aorta is consistent with the literature data on androgen-stimulated AR expression in smooth muscle cells of blood vessels [28].

A tendency for positive correlation was also observed between the testosterone blood concentration and the aromatase mRNA level in the carotid artery, which supported data on the stimulation of aromatase activity by androgens in different cells [26, 27].

Testosterone concentration negatively correlated with the E-selectin mRNA level in the carotid artery. For blood estradiol, a tendency for negative correlation with the E-selectin mRNA level was also revealed; thus, it was suggested that in this case the androgen and estrogen acted in the same direction.

The role of the positive correlation found between AR and ERα mRNAs in the carotid artery is unclear. This correlation might indicate parallel regulation of the cell sensitivities to androgens and estrogens. The significant positive correlations between the StS and EST mRNA levels in the aorta and between the aromatase and EST mRNA levels in the carotid artery suggest the existence of factors regulating the expression of enzymes with opposite physiological functions in the same direction. This suggestion is confirmed by data on the influence of atherosclerotic lesions on contents of different mRNAs in blood vessels.

The positive correlation found by us between the levels of AR and VCAM-1 mRNAs in the aorta seems to indicate the involvement of androgens in the regulation of VCAM-1 expression. This explanation is consistent with clinical observations that the concentration of soluble VCAM-1 (sVCAM-1) depends on the testosterone level in women with polycystic ovary [29] and experimental data on the dihydrotestosterone-induced increase in the VCAM-1 promoter activity in human endothelial cells [30]. However, there are also data on a quite opposite effect [31].

We found positive correlation between the levels of transferrin receptor and VCAM-1 mRNAs in the carotid artery. The correlation between these parameters might be due to induction of VCAM-1 expression by reactive oxygen species [32], and the transferrin receptor promotes an increase in their concentration through delivery of iron into cells [33]. Iron accumulation in atherosclerotic patches is also shown to be markedly higher than in the undamaged intima [34].

Analysis of ratios of mRNAs of the studied proteins in vascular regions with and without atherosclerotic

Table 6. Ratio of mRNAs in regions of blood vessels with and without atherosclerotic lesions

Blood vessel	Parameter	mRNA, damaged/undamaged region							
		AR	ERα	StS	Arom	EST	TfR	E-sel	VCAM
Aorta	mean	1.27	3.12	1.02	0.51	18.8	6.54*	–	0.81
	standard deviation	0.54	3.23	0.22	–	24.8	5.58	–	–
	median	0.92	1.67	0.94	0.51	9.5	3.21	–	0.81
	quartiles	0.91-1.66	1.41-3.16	0.94-1.20	–	3.6-24.8	2.8-10.8	–	–
	number of measurements	5	5	5	1	4	5	–	1
Carot.	mean	0.88	0.89	0.61	0.67*	76.2	1.28	4.15	3.48
	standard deviation	0.22	0.37	0.48	0.27	69.0	0.71	–	–
	median	0.98	1.04	0.47	0.6	76.2	0.93	4.15	3.48
	quartiles	0.86-1.00	0.62-1.04	0.32-0.67	–	–	0.79-2.02	–	–
	number of measurements	5	5	5	3	2	5	2	2
Coron.	mean	0.64*	1.06	0.47*	0.23	1.74	0.75	0.8	0.50
	standard deviation	0.19	0.22	0.22	–	0.90	0.44	–	–
	median	0.58	1.16	0.50	0.23	1.68	0.56	0.8	0.50
	quartiles	0.52-0.76	0.94-1.17	0.32-0.62	–	–	0.50-0.99	–	–
	number of measurements	4	4	4	2	3	4	1	1
All vessels	mean	0.95*	1.73	0.72*	0.50*	32.2*	3.01	2.47	2.07
	standard deviation	0.43	2.10	0.40	0.28	45.4	4.16	2.78	1.75
	median	0.91	1.16	0.69	0.48	6.36	1.56	1.70	1.76
	quartiles	0.62-1.00	0.73-1.41	0.45-0.94	0.25-0.60	1.68-27.4	0.59-2.80	–	0.66-3.48
	number of measurements	14	14	14	6	9	14	3	4

* Values significantly different from 1 (at $p < 0.05$).

lesions has not revealed significant changes in expression of these mRNAs in the damaged aorta regions (Table 6). Aromatase expression was considerably decreased in the damaged regions of the carotid artery. In the damaged coronary artery, the contents of AR, StS, and aromatase mRNAs were decreased. In total, the expression of StS and aromatase mRNAs were decreased and the content of EST mRNA was increased in all damaged regions of all blood vessels. These findings are in partial agreement with the data of Nakamura et al. [14]; a direct comparison is difficult because the authors used blood vessels with moderate or severe atherosclerotic lesions without subdivision into the damaged and undamaged fragments. The decrease in the AR level found in atherosclerotic lesions of the coronary artery corresponds to data of [35] on the negative correlation between the lesion size and intensity of immunohistochemical staining of AR.

We have found that large human arteries different in the wall structure (the elastic carotid artery, the mainly smooth muscle coronary artery, the mixed type aorta) can be different in the levels of mRNAs of individual proteins. Therefore, it was supposed that effects of sex hormones on these blood vessels should be quantitatively different. Thus, the carotid and coronary arteries would depend more strongly on estrogens due to higher expression of ERα gene than in the aorta. But it is rather difficult to interpret this finding because the relative contribution of each parameter under analysis to regulation of the vascular wall functions is still unclear. Our findings can be associated with the regional sex-related differences in the frequency and/or degree of atherosclerotic lesions of blood vessels [36]. Thus, the more active atherogenesis in men than in women is especially pronounced in the coronary artery (an eightfold stronger calcification in the age group of 61-67 years); the sex-related difference in the severity of atherosclerotic lesions of the coronary artery was less pronounced (a threefold difference); and atherosclerotic lesions of the arteries in extremities were virtually the same in men and women. Similar but not identical data were obtained by another group of researchers who assessed the severity of atherosclerotic lesions by the ratio of atheroma area to the total area of the intima [37]. Thus, it seems that the level of ER (and/or estrogen inactivation under the influence of EST) is more important for the protective action of estrogens than the local production of these hormones.

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