Mechanisms of Cardioprotective Effect of Estradiol

A. Zh. Mambetova and A. I. Matyushin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 1, pp. 67-69, January, 2000
Original article submitted November 2, 1998

Estradiol suppresses contractile activity of isolated atrium, inhibits LPO, and stabilizes lysosomal membranes in rat heart. A small content (3.1-16.5 fmol/mg protein) of estradiol-binding sites is detected in the cytoplasm of ventricular cells from dogs and rats of both sexes.

Key Words: *estradiol; heart; experimental myocardial infarction*

Considerable attention is now focused on clinical application of estrogens and gestagens for preventing and treating cardiovascular diseases. There is evidence that female sex hormones have a protective effect towards the appearance and development of cardiovascular diseases. It explains low prevalence of these diseases in women at the reproductive age in comparison with men and increased morbidity during the postmenopausal period [4]. Prophylactic administration of estrogens during the postmenopausal period decreases cardio- and cerebrovascular mortality by 30-50% and 50%, respectively.

The mechanism of protective effect of estrogens against vascular diseases is not clear.

Our aim was to study the effect of estradiol on some functional and metabolic processes in the heart.

MATERIALS AND METHODS

Experiments were carried out on outbred albino rats of both sexes weighing 200-300 g and mature outbred dogs weighing 8-12 kg. 17β-Estradiol (Merck) was injected intraperitoneally in a dose of 10 mg/kg (in 20% ethanol) one hour before coronary occlusion. Myocardial infarction (MI) was provoked by ligating the descendant branch of the left coronary artery immediately below the auricula [8].

The effect of 17β-estradiol on the area of MI was assessed after 4-h coronary occlusion.

The effect of 17β-estradiol on myocardial contractile activity was studied on isolated fragments of the auricula from outbred albino male rats. Norepinephrine content in the heart was determined by high-performance liquid chromatography with electrochemical detection. Changes in chemiluminescence parameters, which accompany LPO, were assessed [3]. The intensity of spontaneous LPO was evaluated by accumulation of thiobarbituric acid (TBA)-reactive metabolites [11]. The content of free low-molecular-weight thiols and total content of nonprotein sulfur-containing substances were estimated as previously described [12], and the level of reduced glutathione was determined [14].

Free and total activity of lysosomal enzymes β-galactosidase and β-glucosidase was determined [13], acid phosphatase activity was estimated as described elsewhere [6].

The content estradiol receptors in the heart of rats and dogs was determined by the radioligand method. Protein was estimated by the method of Lowry. The results were statistically analyzed using Student's t test at $p<0.05$.

RESULTS

Cardioprotective effect of estradiol was demonstrated in experiments on intact animals and rats with experimental MI.

Estradiol restricted the damage area in the myocardium and, in particular, decreased the ischemic area by 31% in comparison with the control, which is surely of clinical importance [1].

Estradiol modulated some functional and metabolic indices characterizing viability of myocardial cells, in particular, contractile activity of the heart. The decrease in this parameter reduced oxygen demand of the heart and improved survival of ischemic cells.

Department of Molecular Pharmacology and Radiobiology, Medico-Biological Faculty, Russian State Medical University, Moscow

0007-4888/00/0001-57$25.00

TABLE 1. Effect of 17β-Estradiol on Single Contractile Response of Rat Auricula (% of Control, *M±m*)

Concentration, mol/liter	Exposure period, min	Contraction amplitude	Contraction rate	Relaxation rate
10^{-5}	5	100.1±3.1	100.0±0.21	100.0±1.9
	15	96.2±2.2	97.0±1.2	99.9±2.5
	30	88.5±3.0*	90.7±3.1*	92.4±3.2*
	45	83.2±1.2*	85.0±1.3*	88.9±2.5*
	60	81.1±2.2*	83.1±1.3*	82.5±2.5*
10^{-4}	5	71.5±3.4*	82.5±3.2*	93.5±4.5
	15	59.7±1.4*	69.7±3.3*	73.3±3.8*
	30	56.5±3.4*	59.3±1.3*	59.5±3.4*
	45	54.1±1.9*	57.5±3.4*	58.5±1.9*
	60	47.2±2.7*	48.3±1.6*	51.3±4.2*

Note. Parameters of single contractile response before addition of hormone into Krebs solution was taken as 100%. *$p<0.05$ compared to 100%.

We revealed an inhibitory effect of 17β-estradiol on contraction of isolated auricula (Table 1).

The steroid inhibited the processes accompanying the damage to myocardial cells.

LPO plays a role in the pathogenesis of diseases related to hypoxia and ischemia.

Low-molecular-weight thiols specifically, glutathione, protect tissues against LPO due to the existence of the glutathione redox-system and direct interaction with peroxides and free radicals [2].

Rat myocardium contains different fractions of nonprotein thiols. Mixed disulfides with proteins constitute about 60% low-molecular-weight thiols. Free thiols are available predominantly in reduced form and only 15-20% of them were in oxidized form.

The presence of large thiol pool can explain low intensity of LPO in the myocardium. The latency of slow chemoluminescence flash in the myocardium 2.3-fold surpassed that for the liver, while the content of TBA-reactive metabolites in the myocardium after 60-min incubation was 3.5-fold lower than in the liver (1.1 and 0.3 mmol/mg, respectively).

Judging from the concentration of TBA-reactive metabolites, estradiol in concentrations of 10^{-9}-10^{-7} M did not inhibited LPO in the myocardium. In a concentration of 10^{-6} M estradiol significantly inhibited LPO in heart homogenate (Table 2), while in concentrations of 10^{-8} and 10^{-6} M it did not affect the content of reduced glutathione in rat myocardium (38.2± 4.6 and 36.8±2.9 nmol/mg protein, respectively, *vs.* 36.5±5.3 nmol/mg protein in the control).

It can be suggested that estradiol as an endogenous antioxidant inhibits LPO due to interaction with free $RO_2^{\bullet}$-radicals initiating oxidation chains [5].

Apart from free radical oxidation, lysosomal enzymes play an important role in the development of hypoxia-induced damage to myocardial cells.

Stabilization of lysosomal membranes in the myocardium was observed 1 h after injection of estradiol diphosphates (10 mg/kg) as a decrease in free β-glucosidase and β-galactosidase activities from 74.5 to 46.0 and from 89.7 to 57.0%, respectively (percent of total enzyme activity).

The direct effect of sex steroids on myocardial lysosomes was assessed *in vitro*.

Incubation of myocardial lysosome suspension with estradiol (10^{-8} M) did not affect free β-glucosidase and β-galactosidase activities. In a concentration

TABLE 2. Effect of Steroid Hormones (10^{-6} M) on the Content of TBA-Reactive Substances (nmol/mg protein) in Rat Heart (*M±m*)

Hormone	Incubation period, min		
	60	120	180
Control	0.278±0.012	0.468±0.044	0.546±0.068
Estradiol	0.145±0.010*	0.283±0.011*	0.338±0.017*
Testosterone	0.299±0.017	0.466±0.031	0.516±0.048
Hydrocortisone	0.281±0.009	0.494±0.028	0.562±0.051

Note. *$p<0.05$ compared to the control.

TABLE 3. Content of Estradiol-Binding Sites in Rat and Canine Hearts (fmol/mg protein, *M*±*m*)

Sex	Rats	Dogs	
		left ventricle	right ventricle
Females	3.1±0.5	15.7±2.4	10.6±1.3
Males	7.3±1.9	11.4±3.0	16.5±4.9

of 10^{-6} M this hormone decreased free β-glucosidase and β-galactosidase activities by 32 and 41%, respectively.

Therefore, estradiol in high concentrations can stabilize lysosomal membranes from rat myocardium.

Catecholamines are the most important bioactive substances regulating functional and metabolic processes in the heart. There is evidence on a pathogenic relation between hyperactivation of the sympathoadrenal system and the development of acute coronary insufficiency and myocardial infarction [9].

In experimental myocardial infarction, the content of norepinephrine in the infarction area is significantly below the control (1.68±0.18 *vs.* 2.22±0.18 nmol/g tissue, $p<0.05$). Injection of 10 mg/kg estradiol 1 h before provoking infarction normalized the content of norepinephrine in the myocardium (2.36±0.40 nmol/g tissue, $p<0.05$ compared to untreated infarction).

The regulatory action of estradiol on cardiac function and metabolism can be mediated by its interaction with specific receptor in myocardial cells similarly to reaction of steroid hormones with classical target organs (liver, uterus, *etc.*).

Radioligand assay showed that the content of estradiol-binding sites in the cytoplasm of cardiomyocyte in rats and dogs of both sexes was significantly lower than in the uterus, where their concentration is as high as 150-200 fmol/mg protein (Table 3). It can be hypothesized that the effects of high doses of estradiol are mediated via its interaction with binding sites on cardiomyocyte membranes, which explains diversity variety and rapid development of these effects in the heart.

Therefore, estradiol protects the heart under conditions of experimental infarction by affecting a number of functional and metabolic processes: contractile activity, LPO, state of lysosomal membranes, and catecholamine level. These data and previously reported antiarrhythmic [7] and coronarodilating [10] effects of estradiol attest to its cardioprotective properties.

Since estradiol possesses cardioprotective activity, it can be used the in treatment of cardiac disturbances.

REFERENCES

1. J. S. Alpert, G. S. Frencis, *Handbook of Coronary Care*, Boston (1993).
2. M. V. Bilenko, *Ischemic and Reperfusion Damage to Organs* [in Russian], Moscow (1989).
3. Yu. A. Vladimirov and A. I. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972).
4. N. A. Gratsianskii, *Klin. Farmakol. Ter.*, **3**, 30-39 (1994).
5. V. M. Gukasov and V. K. Fedorov, in: *The Role of Changes in Membrane Structure in Cell Pathology*, Ed. Yu. A. Vladimirov [in Russian], Moscow (1997), pp. 8-52.
6. I. N. Ivanov, B. F. Korovkin, and I. M. Markelov, *Introduction into Clinical Enzymology* [in Russian], Leningrad (1974).
7. V. I. Kobrin, M. Manoakh, M. Belokopytov, *et al.*, *Byull. Eksp. Biol. Med.*, **121**, No. 4, 370-373 (1996).
8. L. N. Sernov and V. V. Gatsura, *Ibid.*, **107**, No. 5, 534-535 (1989).
9. P. Daly and M. Sole, *Circulation*, **82**, No. 2, Suppl., 135-143 (1990).
10. C. Jiang, Ph. M. Sarrel, Ph. A. Poole-Wilson, and P. Collins, *Am. J. Physiol.*, **263**, No. 1, Pt. 2, H271-H275 (1992).
11. A. Kitabchi, D. Challoner, and R. Williams, *Proc. Soc. Exp. Biol.*, **127**, 647-649 (1968).
12. H. Modig, *Biochem. Pharmacol.*, **17**, 177-186 (1968).
13. V. Patel and A. Tappel, *Biochem. Biophys. Acta*, **191**, 86-94 (1969).
14. J. Sedlak and R. Lindsay, *Anal. Biochem.*, **25**, 192-205 (1968).