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PROTECTIVE ROLE OF ESTRADIOL IN EXTREMAL STATES

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Hormones and, in particular, corticosteroids and their synthetic analogs, in large doses, are widely used in extremal states [6, 18]. Information on their efficacy is contradictory in character. The possibility of using other types of steroid hormones, namely estrogens and androgens, in the treatment of extremal states has virtually not been investigated because of the absence of water-soluble forms suitable for intravenous injection.

Taking the above facts into consideration, it was decided to study the action of estradiol on survival and on some functional-metabolic parameters of the cardiovascular system of intact animals and of animals with hemorrhagic shock.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats of both sexes weighing 150-220 g and on dogs weighing 8-15 kg. Acute blood loss in the dogs and rats was induced by unrestricted bleeding from the femoral or carotid artery under pentobarbital anesthesia.

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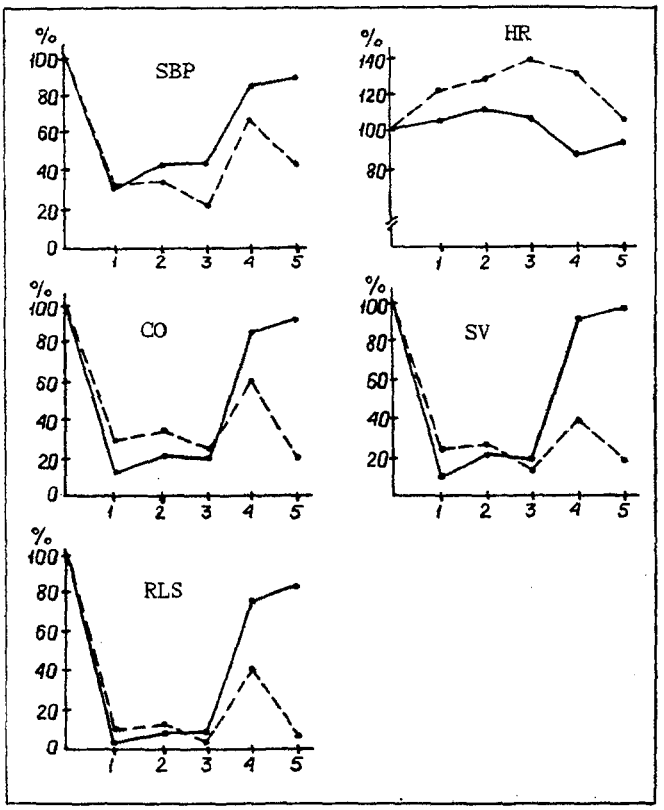

Fig. 1 Parameters of cardiohemodynamics of dogs with hemorrhagic shock. Broken line, control; continuous line – experiment. 1) 60 min of hypotension; 2 and 3) 15 and 60 min respectively after injection of estradiol in a dose of 10 mg/kg; 4 and 5) 15 and 60 min respectively after reinfusion of lost blood.

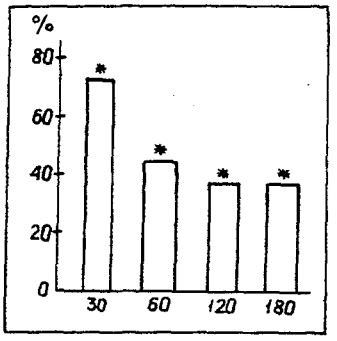

Fig. 2. Effect of estradiol on malonic dialdehyde level in rat heart. Abscissa, incubation time (in min); ordinate, degree of inhibition (in % of control). Estradiol concentration 10^{-6} M. *$p < 0.05$ compared with control.

In experiments on heparinized dogs the blood pressure was lowered to 40 mm Hg and kept at that level for 60 min (Wiggers' model). Against this background, without making good the blood loss, water-soluble estradiol phosphate in the form of the sodium salt was injected into the animals in a dose of 10 mg/kg. The lost blood was returned by transfusion after 60 min.

TABLE 1. Parameters of Myocardial Energy Metabolism (in μmoles/g) of Dogs with Hemorrhagic Shock in Response to Injection of Estradiol in a Dose of 10 mg/kg

Parameters	Intact animals	60 min of hypotension	Recovery period (60 min)	
			control	estradiol
ATP	7,1±0,5	3,4±0,2*	4,6±0,6*	5,6±0,3*
ADP	1,9±0,2	1,5±0,2	1,0±0,2*	1,5±0,5
AMP	1,4±0,1	1,9±0,2*	1,4±0,2	2,0±0,4
Total adenine nucleotides	10,5±0,6	6,8±0,4*	6,9±0,2*	9,1±0,6**
CP	8,2±0,7	3,2±0,2*	4,9±0,2*	6,9±0,9*

Legend: *$p < 0.05$ compared with intact animals; **$p < 0.05$ compared with control.

TABLE 2. Effect of Estradiol on Dehydrogenation of NADH

Parameters	Concentration, M	Number of experiments	Io	I
Control	—	11	103,7±3,1	—
Estradiol	10^{-5}	11	—	105,2±3,9
Control	—	9	123±1,3	—
Estradiol	10^{-4}	9	—	112,0±1,3*

Legend: Io, I) Intensity of luminescence of NADH without and with preparation respectively; *$p < 0.05$ compared with control.

Parameters of the cardiodynamics and hemodynamics studied, namely systolic blood pressure (SBP), heart rate (HR), cardiac output (CO), and others were studied in the initial state of the dogs, and 15 and 60 min after blood loss and transfusion (recovery period) [5].

In the experiments on rats acute blood loss was caused by one-stage exsanguination from the carotid artery in a volume of 2.5% of body weight. Water-soluble estradiol was injected in a dose of 10 mg/kg 10 min before and 10 min after blood loss. Survival of the animals was noted for 1 and 24 h. Acute hypobaric hypoxia was created in a pressure chamber by raising the animals to an "altitude" of 11,000 m. Resistance to hypoxia was determined from the reserve life span.

Lipid peroxidation (LPO) was studied as accumulation of malonic dialdehyde by the thiobarbiturate method [12]. Free and total β-galactosidase and β-glucosidase activity was determined by the method in [14], acid phosphatase activity by the method in [3], and protein by Lowry's method. Adenine nucleotides (ATP, ADP, AMP) were determined microchromographically [4] and creatine phosphate (CP) was determined by the method in [10]. To assess the dehydrating (electron-acceptor) activity of estradiol, the test of dehydrogenation of NADH by a luminescence method was used [5]. The results were subjected to statistical analysis by the usual methods. Differences between the values compared were taken to be statistically significant at the $p < 0.05$ level.

EXPERIMENTAL RESULTS

The experiments demonstrated that the presence of estrogens in the body, whether determined by the sex of the animals or their exogenous administration, may have a positive effect on the resistance of the animals to extremal factors, including hypoxic states of different genesis.

Determination of resistance of the rats to oxygen deficiency on a model of acute hypobaric hypoxia showed that female rats are more resistant to this extremal factor than miles. Whereas the reserve life span (RLS) of the male rats during exposure to hypoxia was 7.8 ± 0.7 min, in females it was higher, namely 14.2 ± 1.8 min.

In experiments to study the effect of water-soluble estradiol on survival of male rats following acute blood loss, the hormone in a dose of 10 mg/kg was found to reduce the mortality of the animals when administered before or after the beginning of bleeding. Whereas in the control 73% of rats survived 1 h after blood loss and 53% after 24 h, injection of estradiol before and after blood loss increased the survival rates to 90 and 95% 1 h and 70 and 75% 24 h after blood loss respectively.

There is information in the literature also on the greater resistance of female animals and women to the action of various unfavorable factors. For instance, females tolerate better than men various types of hypoxia [11, 15, 17], shock [1, 9], virulent infection [13], and a fall of body temperature [19]; the prevalence of myocardial infarction among women is lower than among men [16], and so on.

It can be tentatively suggested that the greater resistance of female animals and women is somehow linked with the favorable effect of estrogens, although the exact mechanism of their protective action is unclear.

Considering the important role of the cardiovascular system in the response of the body to extremal states, we studied the effect of estradiol on functional and metabolic parameters of the cardiodynamics and hemodynamics, lipid peroxidation, and activity of lysosomal enzymes in the heart tissue, and also the possibility of dehydrogenating activity of estrogens.

Injection of estradiol in a dose of 10 mg/kg into dogs with hemorrhagic shock normalized the parameters of the cardio- and hemodynamics, and the effect was particularly marked in the recovery period. Whereas in the control dogs a fall of SBP, CO, SV, and RLS was observed at the 15th and 60th minutes of the recovery period compared with the initial values in intact dogs, as Fig. 1 shows, in the experimental series the values of these parameters were close to normal.

In dogs with hemorrhagic shock disparity between energy utilization and production was observed in the heart. The ATP concentration 1 h after the beginning of hypotension was reduced by 52%, CP by 61%. The total high-energy phosphates fell by 35%. Injection of estradiol 1 h after the beginning of hypotension led to better preservation of ATP and CP in the recovery period than in the control animals (Table 1).

The study of the action of estradiol on metabolism in the heart showed that the steroid has an inhibitory effect on processes causing damage to myocardial cells.

One such process is LPO, the role of which in the pathogenesis of diseases associated with hypoxia, ischemia, and emotional and painful stress is well documented [2, 7]. Studies of the effect of estradiol on LPO have shown that it inhibits LPO in rat heart tissue (Fig. 2).

Lysosomal enzymes play an important role in the development of pathological processes in the heart. An increase in activity of lysosomal enzymes in the blood and a change in stability of the lysosomal membranes of the heart have been observed in hypoxia, ischemia, and several other pathological states [8].

The results of our experiments are evidence of sex-related differences in lysosomal function in the rat heart, manifested as a higher total activity of lysosomal enzymes in male than in female rats. In male rats, for instance, total β-galactosidase activity in heart homogenate averaged 41.6 ncat/g protein, β-glucosidase activity 35.6 ncat/g protein and acid phosphatase activity was an order of magnitude higher than activity of the glucosidases, namely 644.5 ncat/g protein. In females (in the stage of diestrus) β-galactosidase activity was 46.8% lower, β-glucosidase activity 18.5% lower and acid phosphatase activity 42.8% lower. Maximal acid phosphatase activity and the strongest binding of β-galactosidase were observed in the proestrus stage, when the highest level of sex hormones is found in the blood.

Experimental investigation of the direct action of estradiol on the properties of myocardial lysosomes in vitro showed that in a concentration of 10^{-5} M the hormone increased total β-glucosidase activity by 20%, without changing the strength of binding of the enzyme with the lysosomal membrane, whereas in a concentration of 10^{-3} M it significantly reduced (by 32.4%) free activity of the enzyme, while the total level of activity was virtually stable. A change in acid phosphatase activity took place in response to the action of estradiol in concentrations of 10^{-5} and 10^{-3} M, and this was reflected in a decrease in free activity of the enzyme by 18 and 32% respectively, although total activity remained unchanged.

These results indicating a stabilizing action of estradiol on the lysosomal membranes of the heart, in conjunction with the lower level of lysosomal enzyme activity in females than in males, prove the importance of estradiol as a factor regulating involvement of the lysosomes in the development of pathological processes in myocardial cells.

The results of the next series of experiments demonstrated that estradiol has dehydrogenating (electron-accepting) activity, as is shown by a decrease in luminescence of NADH in its presence (Table 2). Because of the presence of this dehydrogenating activity, estradiol can diminish the after-effects of disturbance of energy metabolism in the cells under conditions of acidosis due to oxygen deficiency, when dehydrogenation of NADH with the aid of dehydrogenases is inhibited.

The results of these experiments indicate an increase in resistance of experimental animals to the action of hemorrhagic shock under the influence of estradiol, one cause being the fact that this steroid can induce a combination of functional and metabolic effects stabilizing or improving the state of the cardioviscular system (strengthening the contractile function of the myocardium, preserving the ATP and CP pool, and inhibiting LPO and activity of lysosomal enzymes).

It can be postulated on the basis of these results that besides glucocorticoids, estrogens and, in particular, estradiol, may be used by the body to form a defensive reaction.

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