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PROTECTIVE EFFECT OF THE ANTIOXIDANT IONOL IN TOTAL CARDIAC ISCHEMIA

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Ischemia and, in particular, subsequent restoration of the blood flow are accompanied by activation of lipid peroxidation (LPO), causing damage to cell membrane structures and leading ultimately to disturbance of function of the organ in the postischemic period [1-3, 5, 6]. In myocardial ischemia, inhibition of LPO by antioxidants enables the zone of necrosis in infarction due to coronary occlusion to be reduced, anoxic and reoxygenation damage to the perfused heart to be prevented, and the degree of contractural changes in the myocardium to be diminished [4, 7-9]. Meanwhile, the effectiveness of antioxidants when used during reperfusion of the myocardium after its total ischemia, i.e., under conditions arising during open heart surgery, and also during transplantation of the heart, still awaits investigation.

The aim of the present investigation was to study the effectiveness of the antioxidant ionol when used to maintain the contractile function and prevent the development of contractural changes in the rat heart after total ischemia for 30-60 min, in experiments conducted at different temperatures. A model of perfusion of the isolated rat heart with saline, and also a model of heterotopic transplantation of a donor's heart into recipient rats were used.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats anesthetized with hexobarbital (70 mg/kg). Ionol (2,6-di-tert-butyl-4-methylphenol) was injected as a single dose of 240 mg/kg 24 h before the operation and heparin was given in a dose of 3 ml/kg intraperitoneally 1 h before the operation. Ionol was not injected in the control experiments. In series I (20 experiments) the heart was subjected to total ischemia for 30 min at 20°C and reperfused with Krebs–Henseleit solution by the method of Langendorf and Neely [14]. A consequence of the experiment was the following: the heart was removed and placed in cold (4°C) Ringer's solution; then, for 15 min, it was perfused with hydroxy-generated (95% O_2 + 5% CO_2) Krebs–Henseleit solution through the aorta by the Langendorf method, after which it was transferred to a model of the working heart according to Neely. In this model the solution was infused through the left atrium under a pressure of 20 mm Hg, and the left ventricle ejected against a pressure of 70 cm water. Perfusion was stopped after 15 min, and the heart was allowed to stand for 30 min at 20°C. Reperfusion with the same solution was carried out alternately for 15 min by Langendorf's method and 15 min by Neely's method. To assess cardiac function, the pressure developed by the left ventricle (P), its rate of rise (dP/dt), and the end-diastolic pressure (EDP) were recorded. The volume velocity of coronary perfusion (VCP) was determined by measuring outflow. In the experiments of series II and III a model of heterotopic transplantation of the rat heart to the recipient's abdominal vessels by means of cannulas was used. In series II (12 experiments) the heart removed from the donor was kept for

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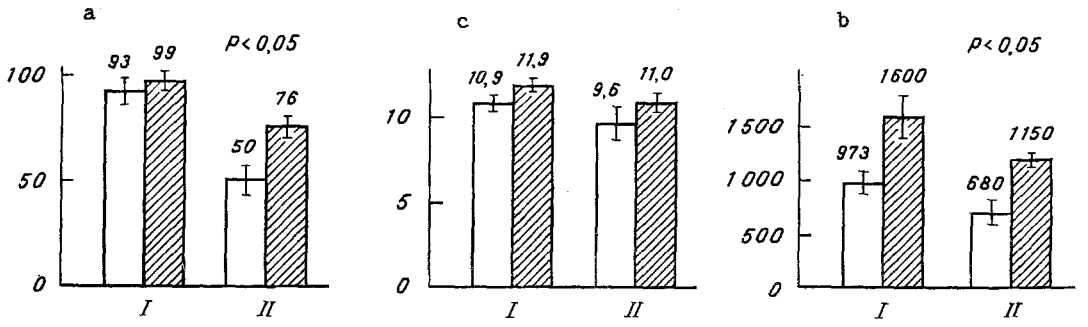

Fig. 1. Effect of ionol on contractile function (a, b) and volume VCP (c) of isolated rat heart (perfused by Neely's method). Ordinate: a) P (in mm Hg), b) dP/dt (in mm Hg/sec), c) VCP (in ml/min). I) Before ischemia, II) after ischemia lasting 30 min. Unshaded columns — control, shaded — ionol. Significance of differences shown by comparison with control.

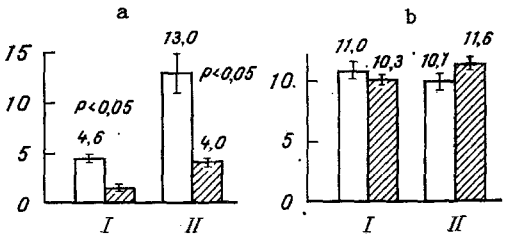

Fig. 2. Effect of ionol on EDP and volume VCP of rat heart (perfusion by Langendorf's method). Ordinate: a) EDP (in mm Hg), b) VCP (in ml/min). Remainder of legend as to Fig. 1.

60 min at 20°C. Function of the graft was estimated by the isovolumetric balloon technique, which was developed for use with this particular model [10]. Indices of contractility were calculated by the method in [15, 16] and compliance was characterized by the reciprocal of EDP. In series III (28 experiments) the heart was kept for 60 min at 37°C. Since, after such harsh conditions of ischemia, contractile function was absent or extremely weak, the following parameters were used as criteria of the protective effect of ionol: the time of development of total ischemic contracture, the number of hearts whose contraction was restored, the time of appearance of contractions after the beginning of reperfusion, and the duration of the contractions. The development of ischemic contracture was recorded as the volume of physiological saline displaced from the left ventricle into a graduated capillary tube. The solution was injected into the left ventricle through a thin (diameter 0.5 mm) catheter, and the time when the level of fluid in the capillary tube ceased to rise was taken as the development of total contracture. The pressure parameters in all series were recorded on the "Mingograf-82" instrument (Elema–Schönander, Sweden). The results were subjected to statistical analysis by Student's test for small samples.

EXPERIMENTAL RESULTS

Administration of ionol had virtually no effect on parameters of the contractile function of the nonischemic myocardium, but significantly increased them in the heart reperfused by Neely's method after ischemia lasting 30 min (Fig. 1). During perfusion of the heart by Langendorf's method (Fig. 2), in experiments in which ionol was used, significantly lower values of EDP were observed even before ischemia than in experiments without ionol. In the postischemic period EDP in both groups of experiments of series I was increased, but significant differences remained between the experiment and control. The volume VCP under these conditions of ischemia did not change significantly and no effect of ionol on it could be found, when perfusion was carried out by either Landendorf's or Neely's method (Figs. 1 and 2).

In the experiments of series II, as a result of the use of the isovolumetric balloon technique, it was possible to evaluate the contractile function and diastolic properties of the

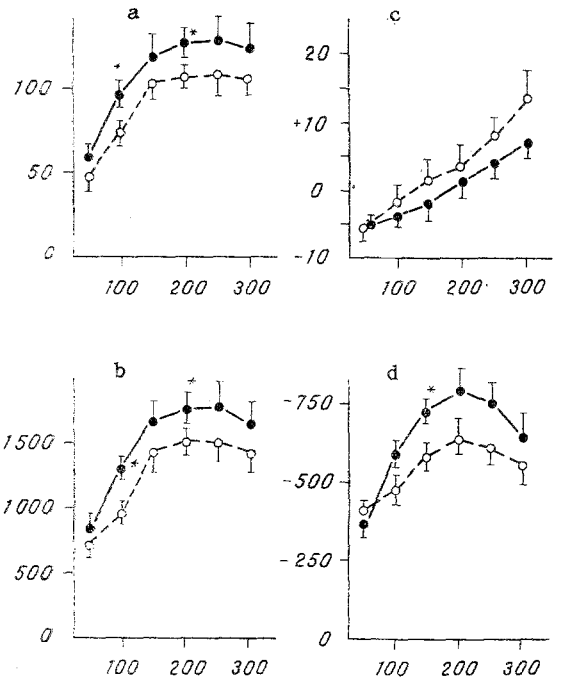

Fig. 3. Effect of ionol on contractile function (a, b), compliance (c), and relaxation (d) of transplanted rats' hearts subjected to ischemia at 20°C for 60 min. Abscissa, end-diastolic volume (in μl); ordinate: a) P (in mm Hg), b) $+dP/dt_{max}$ (in mm Hg/sec), c) EDP (in mm Hg), d) $-dP/dt_{min}$ (in mm Hg/sec). Empty circles — control, filled circles — ionol. *$p < 0.05$ compared with control.

left ventricle of a rat heart transplanted into a heterotopic position in detail. Administration of ionol improved the contractile function and rate of relaxation of the ischemic heart (Fig. 3a, b) and a tendency for EDP to fall also was observed (Fig. 3c), i.e., a tendency toward an increase of myocardial compliance. In the experiments with ionol Siegel's contractility index was significantly higher, although a tendency toward an improvement of contractility and relaxation under the influence of ionol also was observed relative to the other parameters (Table 1).

In the experiments of series III with the severest degree of ischemic damage to the myocardium, administration of ionol caused no significant lengthening of the time of development of ischemic contracture (30.9 ± 0.7 min compared with 28.3 ± 0.6 min in the control), nor did it increase the number of hearts whose contractile function was restored (1/3 in both groups of experiments of this series); however, contractions began sooner in the experiments with ionol (after 47 ± 17 sec compared with 106 ± 7 sec after the beginning of perfusion; $p < 0.05$) and lasted longer (throughout the 30-min period of observation compared with 12 ± 3 min in the control).

The results given above are evidence that prophylactic injection of ionol improves the functional capacity of a rat heart subjected to prolonged total ischemia. The effectiveness of ionol in this case is largely determined by the severity of ischemic damage to the myocardium. The greatest effect (an increase of 20-30% in P) was obtained in the experiments of series I, in which the heart was subjected to ischemia for 30 min at 20°C. With lengthening of the period of ischemia to 60 min (experiments of series II) the increase in P was 10-15%. In the experiments of series III, when the rat heart was subjected to prolonged (60 min) ischemia at 37°C, the effect of ionol could be detected significantly only with respect to the time of beginning of its contractile function and its duration.

It is well known that the rat heart is more sensitive to ischemia than the heart of any other animal. For instance, the time of development of ischemic contracture of the rat heart at 37°C is 17-20 min [11] or 28 min (our own data), whereas for the dog heart it is 70-90 min [12, 13]. It can accordingly be concluded that ionol has a protective action against both moderately severe and severe ischemic damage and is ineffective only in extremely severe degrees of ischemia. The protective effect of ionol on the ischemic heart was demonstrated pre-

TABLE 1. Effect of Ionol on Parameters of Function of Rat Heart Transplanted after Ischemia Lasting 60 min at 20°C

Experimental conditions	Veragut's index of contractility	Siegel's index of contractility	$\frac{dP/dt\ (min)}{dP/dt_{max}}$
Control	23,8±1,7	167±6	0,45±0,06
Ionol	26,4±2,6	193±4	0,49±0,05
	—	$p<0,05$	—

viously only during hypoxic perfusion of the heart with salt solutions or in the presence of a focus of myocardial ischemia of short duration [7, 9].

The possibility of using the protective action of ionol to pressure not only the contractile, but also the diastolic properties of the myocardium, which play an important role in maintenance of the adequate pumping function of the heart, deserves attention. In the experiments of series II, when ionol was used, a tendency was observed toward better preservation of myocardial compliance (Fig. 3c) and the rate of relaxation of the myocardium was significantly increased (Fig. 3d). The lower values of EDP when ionol was used were observed in both the nonischemic and the ischemic heart, and also during perfusion of the heart with saline by Langendorf's method (Fig. 2a), and this result can probably be explained not only by improvement of the contractile properties of the myocardium, but also by its higher compliance. The effect of ionol on the diastolic properties of the myocardium and the possibility of using it to prevent the development of contractural changes also have been demonstrated in stress-induced and anoxic damage to the heart [7].

Thus ionol, given prophylactically, has a protective action on the contractile function and diastolic properties of the myocardium after long-term, total ischemia. The protective effect of ionol is exhibited during reperfusion of the ischemic rat heart not only with saline, but also with blood, i.e., under conditions closely similar to those arising in open heart and heart transplant operations. This affords good prospects for the use of ionol as an anti-ischemic agent in clinical practice.

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