EFFECT OF THE SYNTHETIC ANTIOXIDANT IONOL (BHT) ON CARDIOMYOCYTE ELECTRICAL ACTIVITY AND ARRHYTHMIAS IN GLOBAL ISCHEMIA AND SUBSEQUENT PERFUSION OF THE ISOLATED RAT HEART

L. M. Belkina, V. I. Vovk, and F. Z. Meerson

UDC 616.127-005.4-085.272.4.014.425-036.8-07:616.127-018.1-073.97]092.9

KEY WORDS: ionol, cardiomyocytes, cardiac ischemia, reperfusion.

Activation of lipid peroxidation (LPO) plays an important role in the pathogenesis of arrhythmias and fibrillation of the heart [7, 12]. This is because LPO induces damage to cardiomyocyte membranes and a disturbance of ionic transport, as a result of which the generation and conduction of impulses are disturbed. Correspondingly it has been shown that antioxidants prevent injury to membranes and cardiac arrhythmias [7, 12]. The authors found previously that the natural antioxidant coenzyme Q_9 and the synthetic antioxidant ionol (butylated hydroxytoluene — BHT) prevent the development of arrhythmias in acute myocardial ischemia and reperfusion [3, 4]. However, the electrophysiological mechanisms lying at the basis of the antiarrhythmic action of antioxidants, and of BHT in particular, have not been studied.

The aim of this investigation was to study the effect of BHT on electrical activity of cardiomyocytes during transient global cardiac ischemia and subsequent reperfusion.

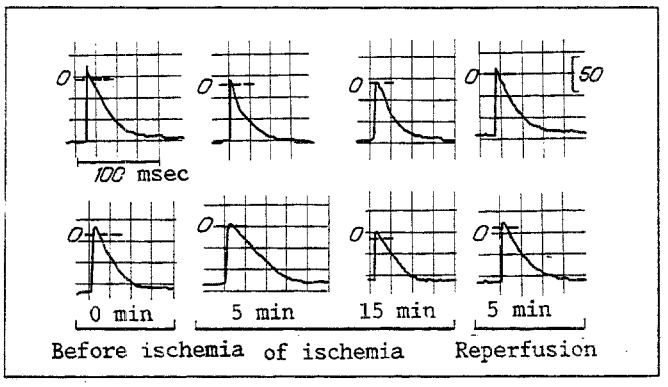

Fig. 1. Transmembrane potentials of cardiomyocytes before and during ischemia and reperfusion in control animals (top row) and in animals previously treated with BHT (bottom row).

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences, Moscow.
(Presented by Academician N. R. Paleev, Academy of Medical Sciences.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 4, pp. 367-370, April, 1992. Original article submitted September 12, 1991.

0007-4888/92/0004-0481$12.50

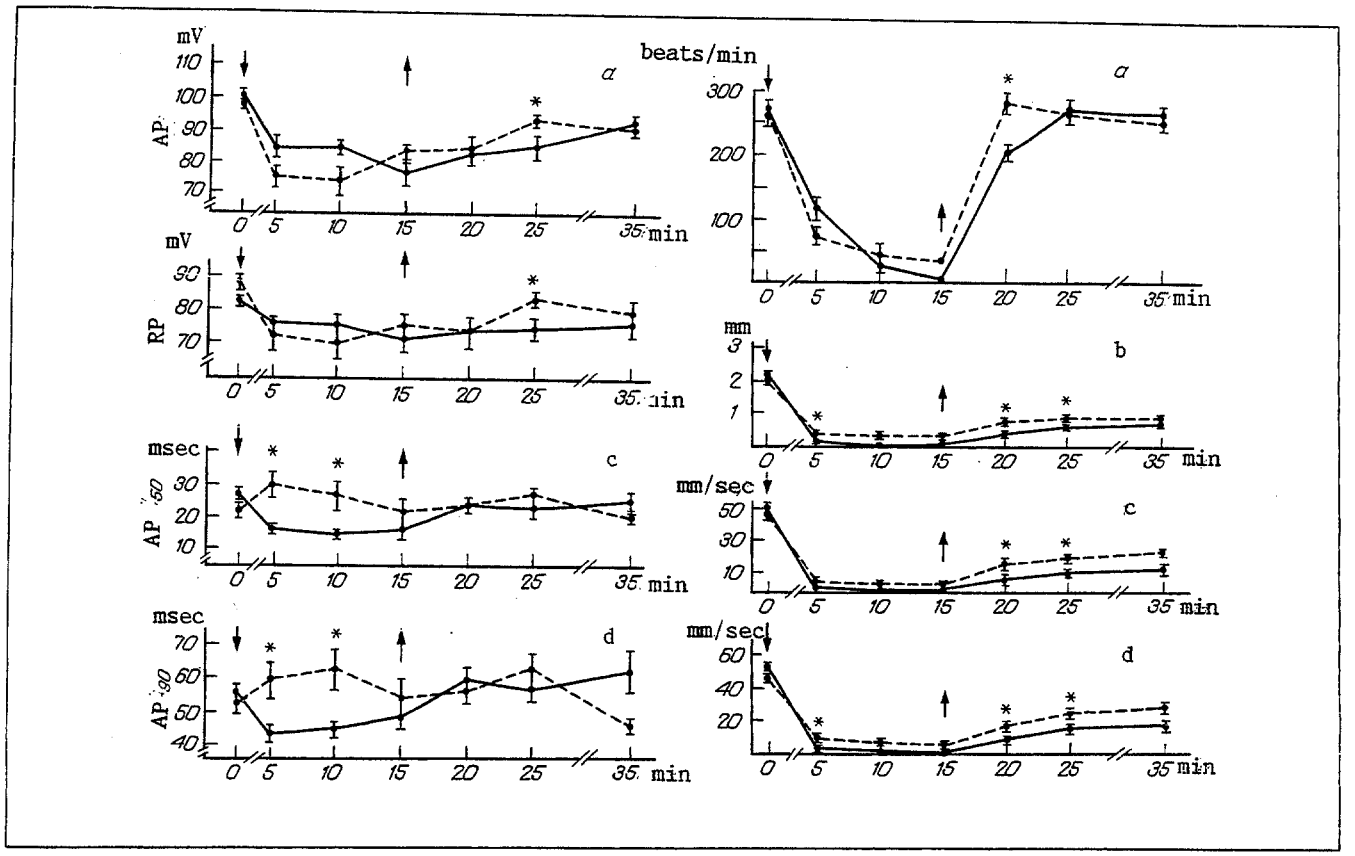

Fig. 2 Fig. 3

Fig. 2. Time course of parameters of cardiomyocyte electrical activity during ischemia and reperfusion in control animals (continuous line) and animals previously treated with BHT (broken line). Abscissa: time, min. Ordinate: a) amplitude of AP (mV); b) resting potential (mV); c, d) duration of AP at level of 50% and 90% repolarization (msec). Arrows indicate beginning and end of ischemia. *$p < 0.05$.

Fig. 3. Time course of parameters of cardiac contractility during ischemia and reperfusion in control animals (continuous line) and in animals previously receiving BHT (broken line). Abscissa: time, min. Ordinate: a) HR; b) amplitude of shortening (mm); c, d) velocity of contraction and relaxation of myocardium (mm/sec), respectively. Arrows indicate beginning and end of ischemia. *$p < 0.05$.

METHODS

Experiments were carried out on male Wistar rats weighing 250-270 g. The animals were divided into two groups. One group received BHT (50 mg/kg per os), dissolved in sunflower oil (0.05 ml/100 g body weight) daily for 3 days and on the 4th day, 2 h before the experiments. Animals of the other group, which served as the control, received the same volume of sunflower oil. The animals were anesthetized with pentobarbital (50 mg/kg) and heparinized (200 U/100 g body weight), and the heart was quickly removed and attached to a Langendorff perfusion system. After a period of stabilization, global ischemia of the heart was produced by stopping perfusion for 15 min, after which reperfusion was carried out for 20 min. Parameters of electrical activity of the cardiomyocytes and contractility of the heart were studied as described previously [5], using the TD-112 isotonic transducer of specialized modules of the RM-6000 polygraph (Nihon Kohden, Japan). The transmembrane potential of the cardiomyocytes was recorded on the subepicardial surface of the left ventricle by means of floating microelectrodes, filled with KCl solution (3M) and an MEZ-8201 amplifier (from the same firm). From the amplifier block the signal was led to a VC-9 oscilloscope and to an RAT-1100 memory unit for subsequent analysis. The resting potential (RP), the amplitude of the action

potential (AP), and its duration at levels of 50% and 90% repolarization, were measured. To record parameters of RP at the height of ischemia, when spontaneous electrical activity disappeared, brief (20-25 sec) electrical stimulation of the heart by square pulses with a frequency of 0.5 Hz and duration 5 msec (SEN-3201 stimulator from the same firm) was used. During the study of disturbances of the rhythm of the isolated heart during reperfusion (on this particular model ischemic arrhythmias are virtually absent) the heart rate (HR), the frequency and duration of ventricular fibrillation, tachycardia, and extrasystoles, and also the total duration of severe arrhythmias, calculated for the whole group and on average per animal, were evaluated. The contractility of the heart was estimated from the amplitude of shortening during apical—basal displacement of the contracting heart, and the velocity of contraction and relaxation. The results were subjected to statistical analysis by Student's test.

RESULTS

Traces of bioelectrical potentials of cardiomyocytes before and during total ischemia and reperfusion of the heart are shown in Fig. 1. Clearly, both in the control animals and in animals previously treated with BHT, ischemia induced a decrease in the values of AP and RP characteristic of its action in this situation. However, the time course of the duration of AP in the groups of animals compared differed qualitatively. Whereas in the control the usual shortening of the duration of AP was observed, after administration of BHT, on the contrary, there was a significant increase in the duration of AP. This effect was preserved in BHT-treated animals until the 10th minute of ischemia, and by the 15th minute of ischemia the duration of AP in these animals had returned to its initial level. During reperfusion the duration of AP in the control was partly restored, but against the background of BHT it remained stable at the same level as initially.

Quantitative assessment of the changes described above is illustrated in Fig. 2. Clearly, in the control animals at the 5th minute of ischemia, RP was reduced by 14%, the amplitude of AP by 25%, and the duration of AP at the level of 50% and 90% repolarization was reduced by 45% and 15%, respectively. Later, during 15 min of ischemia, these parameters continued to fall. In the course of 20 min of reperfusion almost total restoration of RP and of the duration of AP took place in control animals, whereas the amplitude of AP still remained reduced at the end of perfusion compared with its initial value of 9% ($p < 0.01$).

The original parameters of electrical activity were altered by BHT: RP was increased a little (by 6%) and the duration of AP was reduced at 50% and 90% repolarization by 21% and 23%, respectively, compared with the control. It must be pointed out that toward the end of the period of stabilization HR was similar in the groups compared (270 ± 6.2 and 268 ± 5.9 beats/min, respectively. As has already been pointed out, BHT had no significant effect on the time course of RP or the amplitude of AP during ischemia, but it led to qualitative changes in the time course of the duration of AP. For instance, 5 min after the beginning of ischemia the duration of AP at 50% and 90%

TABLE 1. Effect of Synthetic Antioxidant BHT on Reperfusion Arrhythmias of Isolated Rat Heart

Parameter	Control, n = 10	BHT, n = 10	p
Ventricular tachycardia (VT)			
Number of animals with VT	9	4	
Total duration, sec			
– Per group	629	52	
– Per animal	63±42,0	5±4	>0,1
Number of animals with VF	5	2	
Total duration, sec			
– Per group	296	36	
– Per animal	30±11,6	4±3,6	<0,05
Extrasystoles (ES) – number of animals with ES	10	9	
Total duration, sec			
– Per group	792	105	
– per animal	79±15,6	10±5,7	<0,01
Total duration of severe arrhythmias, sec			
–Per group	925	89	
-Per animal	92±26,8	9±6,5	<0,01

repolarization was increased compared with initially by 36% ($p < 0.05$) and 13% ($p < 0.1$), respectively, but after 10 min it was increased by 27% ($p < 0.1$) and 29% ($p < 0.05$), respectively. Toward the 15th minute of ischemia the duration of AP in these animals had returned to its initial value. During reperfusion, the duration of AP in animals receiving BHT remained close to the pre-ischemic level, whereas RP and the amplitude of AP were restored more rapidly than in the control: after 10 min of reperfusion, in BHT-treated animals RP and AP were almost completely restored, exceeding the control values by 9% ($p < 0.05$). Toward the end of reperfusion the absolute values of the parameters of electrical activity are similar in the groups compared.

Thus BHT had a significant effect on parameters of cardiomyocyte electrical activity, shown during ischemia as an increase in the duration of AP, i.e., the duration of repolarization, whereas during reperfusion it took the form of more rapid recovery of RP and of the amplitude of AP. This effect of BHT was accompanied by marked antiarrhythmic activity during reperfusion. Data on the effect of BHT on arrhythmias are given in Table 1. They show that the antioxidant reduced the total duration of severe arrhythmias (ventricular tachycardia and fibrillation), whether calculated for the group as a whole or on average per animal, almost tenfold, whereas the frequency of tachycardia and fibrillation was increased by 2.2 and 2.5 times, respectively, compared with the control.

Besides its antiarrhythmic action, BHT also had a marked protective action on the contractile function of the heart. It will be clear from Fig. 3 that in the control animals there was an abrupt depression of mechanical activity after 10 min of ischemia, and the heart stopped after 15 min. Reperfusion only partially restored the contractile function of the heart, as a result of which the parameters of its function remained several times lower in value than initially. Meanwhile, if BHT was given beforehand, residual mechanical activity was still preserved 15 min after the beginning of ischemia, and during reperfusion restoration of function took place more rapidly. For instance, if HR was the same in the groups of animals compared, in those receiving BHT the amplitude of shortening at the 10th minute of reperfusion was 1.5 times greater ($p < 0.05$) than in the control, and the rates of contraction and relaxation were 1.8 ($p < 0.05$) and 1.5 ($p < 0.05$) times greater, respectively. Similar data on the cardioprotective action of BHT were obtained by the writers previously on intact animals [4]. The results show that in the presence of ischemic and reperfusion injuries the protective action of BHT relative both to electrical activity and the contractile function of the heart is clearly manifested on isolated hearts also. This is evidence that BHT acts directly on the myocardium.

Data on the effect of BHT on cardiomyocyte electrical activity showed that the action of the antioxidant was exhibited most clearly as an increase in the duration of AP, i.e., of the refractory period. Factors increasing refractoriness of the myocardium are known to possess an antiarrhythmic action, for they increase the length of the excitation wave and thereby prevent the circulation of excitation, which lies at the basis of severe arrhythmias. Our data suggest that the increase in duration of repolarization of the cardiomyocytes during ischemia induced by BHT is one mechanism of its antiarrhythmic action, not only in acute ischemia and reperfusion, but also in myocardial infarction [3] and postinfarction cardiosclerosis [2].

When the mechanisms of the increase in duration of AP under the influence of ionol during ischemia are analyzed it must be recalled that ischemia itself causes shortening of AP [8], which is mainly linked with activation of the potassium current [14] and leakage of potassium ions from the cell [9]. Activation of this current is known to be connected with increased entry of calcium ions into the cell [10] and with ATP deficiency in the region of sarcolemma [13], arising when ischemic damage to the myocardium is present. It can be tentatively suggested that ionol increases the duration of AP on account of blockade of the potassium current, which is effected through the limitation of excessive accumulation of calcium ions and preservation of the energy reserves of the cell. The "anticalcium" effect of BHT may be associated with its membrane-protective action, thanks to which the antioxidant prevents disturbance of the Ca-transport function of the sarcolemma and sarcoplasmic reticulum. This hypothesis is supported by the fact that BHT increases the resistance of the myocardium to calcium-induced damage, as shown by our separate experiments. The ability of the antioxidant to maintain the energy balance in the cell is confirmed by the fact, which we discovered previously, that the antioxidant limits exhaustion of glycogen during hypoxia and reoxygenation of the myocardium [1].

The antiarrhythmic action of BHT is evidently manifested even more strongly during reperfusion, when during the first few seconds there is an explosive generation of oxygen radicals [15] and, consequently, sudden activation of LPO [6]. Oxygen damage to the membranes at this period gives rise to severe arrhythmias, arising during the same

first few seconds. Recording electrical activity at the beginning of reperfusion could therefore give much information for use in the analysis of the mechanism of the antiarrhythmic action of ionol.

Meanwhile, data on parameters of cardiomyocyte electrical activity obtained 5 min after the beginning of reperfusion provide a basis for discussion of the effect of BHT on yet another factor which plays an important role in the dispersion of restoration of the parameters of cardiomyocyte electrical activity during reperfusion [11]. Our data are evidence that ionol abolished the increase in dispersion of the duration of repolarization during reperfusion, and thereby removed one of the factors necessary for the circulation of excitation. Thus, whereas in the control animals 5 min after the beginning of reperfusion the duration of AP at 50% and 90% repolarization was restored, after an increase of 44% ($p < 0.05$) and 23% ($p < 0.05$) respectively compared with the preperfusion level, in animals receiving BHT the duration of AP did not undergo any significant changes on the resumption of perfusion. These findings show that BHT smooths the sudden change of refractoriness of the myocardium on the switch from ischemia to reperfusion.

An increase in the refractoriness of the cardiomyocytes is thus one of the mechanisms of the antiarrhythmic action of the antioxidant BHT.

LITERATURE CITED

1. L. Yu. Golubeva, L. M. Belkina, V. A. Santykova, et al., *Bull. Éksp. Biol. Med.*, No. 6, 685 (1989).
2. F. Z. Meerson, L. M. Belkina, S. S. Dyusenov, et al., *Bull. Éksp. Biol. Med.* (in press).
3. F. Z. Meerson, L. M. Belkina, S. S. Dyusenov, et al., *Kardiologiya*, No. 8, 19 (1986).
4. F. Z. Meerson, V. I. Vovk, L. M. Belkina, et al., *Kardiologiya*, No. 7, 61 (1991).
5. F. Z. Meerson, I. Yu. Malyshev, and T. G. Sazontova, *Kardiologiya*, No. 8, 69 (1989).
6. R. Barsacchi, G. Pelosi, P. Camici, et al., *Biochim. Biophys. Acta*, **804**, 356 (1984).
7. M. Bernier, D. Hearse, and A. Manning, *Circulat. Res.*, **58**, 331 (1986).
8. E. Carmeliet, *Circulat. Res.*, **42**, 577 (1978).
9. J. L. Hill and L. S. Gettes, *Circulation*, **61**, 768 (1980).
10. G. Isenberg, J. Verecke, G. van der heiden, et al., *Pflügers Arch.*, **397**, 251 (1983).
11. E. Kaplinsky, S. Ogawa, E. Michelson, et al., *Circulation*, **63**, 333 (1981).
12. F. Z. Meerson, L. M. Belkina, T. G. Sazontova, et al., *Basic Res. Cardiol.*, **82**, 123 (1987).
13. A. Noma, *Nature*, **305**, 147 (1983).
14. A. Vleugels, J. Verecke, and E. Carmeliet, *Circulat. Res.*, **47**, 501 (1981).
15. J. L. Zweier, J. T. Flaherty, and M. L. Weisfeldt, *Proc. Natl. Acad.Sci. USA*, 1404 (1987).