ELECTROPHYSIOLOGICAL INVESTIGATION OF THE ANTIARRHYTHMIC ACTION OF THE ANTIOXIDANT IONOL

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Antioxidants have been shown to prevent lowering of the fibrillation threshold and an increase in ectopic activity of the heart during stress, and also in experimental myocardial infarction [6, 8]. This paper describes a study of the action of the antioxidant ionol on postdepolarization oscillations of membrane potential, and also on the duration of the excitation wave spreading over the myocardium, on isolated preparations of the heart.

EXPERIMENTAL METHOD

Experiments were carried out on isolated papillary muscles of the right ventricle and on isolated preparations of the auricle of the left atrium of New Zealand rabbits. The technique of isolation of the papillary muscles was described in detail in [2]. The method of isolation of the auricle of the left atrium was similar to that suggested in [12]. Isolated muscle preparations were perfused with modified Tyrode solution of the following composition (in mM: NaCl — 137; $CaCl_2$ — 2.7; KCl — 4; $MgCl_2$ — 1; NaH_2PO_4 — 1.8; $NaHCO_3$ — 10; glucose — 10; the pH of the solution was 7.4 ± 0.1 and its temperature 36.5 ± 0.5°C. The solution was oxygenated with a gas mixture of 96% O_2 + 4% CO_2. The method used to measure the transmembrane potential and the strain of isometric contraction was the same as in [2]. To record the spread of the excitation wave over the heart tissue, a technique of multiple-electrode mapping was used: electrical activity was recorded simultaneously by means of 32 surface electrodes, after which a computer (using an assigned program) distinguished the times when the wave passed beneath each electrode, and on the basis of these times reconstructed a map of spread of the wave [1, 3-5, 9].

EXPERIMENTAL RESULTS

Postdepolarization fluctuations of membrane potential are known to arise in response to stimulation of a preparation kept in potassium-free physiological saline with an increased calcium concentration by short pulses of current [13]. In the present experiments, after stimulation of isolated preparations for 1 h with short (5 msec) pulses of current with a frequency of 1 Hz, the original control perfusion fluid was replaced by potassium-free solution with an increased calcium concentration (5.4 mM). As a result, stable postdepolarization waves appeared after 5-7 min (Fig. 1a).

Addition of ionol to this potassium-free solution caused no change in the characteristics of the electromechanical responses. However, after preliminary injection of ionol (10^{-5} M) postdepolarization waves were absent in the control physiological saline for 20-25 min in all nine experiments (Fig. 1b). By simultaneously recording electrical activity of the myocardium at 32 points, we were able to observe changes in the wave patterns at times of stimulation: a) in the control, b) on the addition of adrenalin to the perfusion fluid (1 mg/liter), and c) after simultaneous addition of adrenalin and ionol

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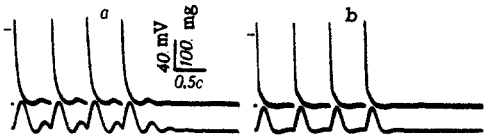

Fig. 1. Effect of ionol on postdepolarization fluctuations of transmembrane potential (top trace) and isometric strain of contraction (bottom trace) in rabbit papillary muscles: a) postdepolarization waves induced by replacement of control perfusion fluid by potassium-free solution with increased Ca^{2+} ion concentration (5.4 mM) (frequency of stimulation 1.7 Hz); b) postdepolarization fluctuations induced by potassium-free solution after preliminary addition of ionol (10^{-5} M) to the control solution.

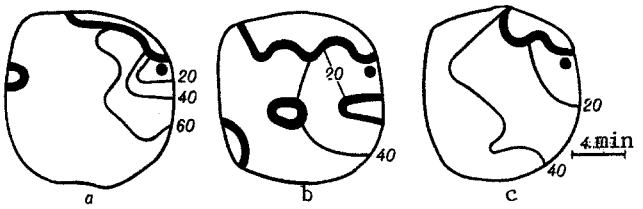

Fig. 2. Effect of adrenalin and ionol on spread of excitation over atrial myocardium. Black circle indicates location of stimulating electrode. Numbers near isochrones (wave fronts) indicate times of activation of corresponding myocardial regions (in msec). Bold lines denote zones of functional block to conduction of excitation. a) Control, b) after addition of adrenalin, c) after addition of adrenalin and ionol.

(in the above-mentioned concentrations) to the perfusion solution. Examples of these wave patterns are given in Fig. 2 (in this case, the period of steady–state stimulation T was 110 msec). Clearly, against the background of adrenalin, the area of spread of the zones of the functional block to conduction of the myocardial activation wave increased (Fig. 2b). Under these circumstances, arrhythmias appeared more frequently than in the control, and the range of linkage intervals, inducing arrhythmias, increased significantly. On simultaneous addition of adrenalin and ionol to the perfusion fluid (Fig. 2c), arrhythmias appeared less frequently. Changes in myocardial electrical activity of this type were observed in three of the five preparations tested.

The results of five experiments are given in Table 1. They show that against the background of adrenalin, values of V_0, V_∞, and R may both rise and fall compared with the control. However, the wavelength $\lambda = V_0 \cdot R$ was always reduced by adrenalin. If both adrenalin and ionol were added to the physiological saline surrounding the preparation, the wavelength increased again (in four of five cases).

There is the highest degree of probability that acute ischemia of the heart is complicated by arrhythmias when this ischemia is combined with a stress reaction [15]. In turn, sustained stress reactions can induce or activate ischemic heart disease, accompanied by injury to cell membranes and their depolarization [6, 8, 11]. Such a situation was modeled in our experiments by perfusion of an isolated papillary muscle with potassium-free physiological saline containing an increased calcium concentration. This experimental model reproduces sufficiently adequately the conditions essential for generation of an ectopic trigger source in the damaged myocardium [13]. As our experiments showed, addition of the antioxidant ionol to the perfusion fluid lengthened the period of time until the appearance of postdepolarization waves of membrane potential three-fourfold, thereby delaying the time of appearance of arrhythmias initiated by ectopic focal sources. Since in our experiments ionol had this type of action, not on the whole body, but on an isolated muscle, this supports the hypothesis that the antiarrhythmic effect of antioxidants is realized not only through the CNS [6-8]. Ionol evidently can also act on the myocardium directly. This conclusion is confirmed by our experiments on atrial tissue. The fact that ionol counteracts

TABLE 1. Action of Ionol on Length of Myocardial Excitation Wave

Velocity of wave near stimulating electrode V, m/sec			Velocity of wave at a distance from stimulating electrode V, m/sec			Refractory period R, m/sec			Length of wave, mm		
C	A	A+I	C	A	A+I	C	A	A+I	C	A	A+I
0,10	0,11	0,09	0,45	0,41	0,64	87	75	91	8,7	8,2	8,2
0,12	0,12	0,16	0,70	1,05	0,70	102	82	103	12,2	9,8	16,5
0,14	0,05	0,53	1,05	1,40	1,40	138	84	132	19,4	4,2	70,0
0,50	0,12	0,62	0,60	1,05	1,40	112	136	127	56,2	16,3	78,7
0,16	0,07	0,11	0,40	0,20	1,00	84	88	91	13,5	6,1	10,0

Legend. C) Control, A) adrenalin, A + I) adrenalin + ionol.

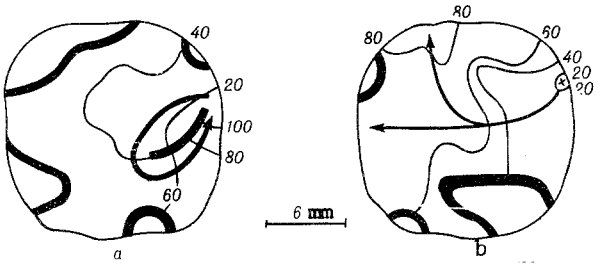

Fig. 3. Two types of sources of arrhythmias arising against the background of adrenalin: re-entry (a) and focal source (b — indicated by a cross). Arrows indicate directions of spread of excitation. Remainder of explanation as to Fig. 2.

the decrease in wavelength against the background of adrenalin, thereby preventing the development of re-entry and the arrhythmias connected with it, points to a possible antiarrhythmic action of the antioxidants on re-entry. It must be pointed out that in our experiments, besides re-entry (35% of cases, Fig. 3a) focal sources of arrhythmias were observed frequently (in 65% of cases; Fig. 3b). However, these sources are evidently places where the excitation waves circulating in the depth of the heart tissue come out onto the surface [3, 4, 10, 14], i.e., they are a special case of re-entry.

Estimation of the length of the excitation wave, as undertaken in this study, was not free from defects. First, the refractory period R was determined at the point of stimulation, whereas velocity V was determined at the site of the recording electrode. These points are situated very close together (only 3-5 mm apart), and during estimation of wavelength it was assumed that the refractory periods at these points were closely similar in value. However, at points remote from the site of stimulation, where the refractory period was not measured, estimation of the wavelength (λ_∞) could not be carried out correctly. If, however, it is assumed that the refractory period at these points changes by the same number of times as the refractory period changes at the site of stimulation, it becomes possible to assess the relative change of wavelength Λ_∞ under the influence of ionol: $\Lambda_\infty = \lambda_{\infty 1}/\lambda_\infty$ ($\lambda_{\infty 1}$ denotes the wavelength in the presence of ionol, λ_∞ in its absence). We found that $\lambda_\infty > 1$ in four of five experiments. Consequently, these estimations do not contradict the hypothesis that the length of the excitation wave is increased by the action of ionol.

Ionol thus has an inhibitory effect both on arrhythmias induced by ectopic focal sources and also on disturbances of the cardiac rhythm caused by re-entry.

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