ANTIARRHYTHMIC ACTION OF THE ANTIOXIDANT SD-6 ON THE ISCHEMIC AND REPERFUSED ISOLATED RAT HEART

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Restoration of the coronary blood flow after acute myocardial ischemia leads to the development of cardiac arrhythmias, which may change into potentially lethal fibrillation and to circulatory arrest [4, 14]. The development of arrhythmias is linked with the formation of nonhomogeneities of refractoriness of the myocardial tissue, shortening and dispersion of the duration of action potentials (AP), and the formation of cyclic excitation waves of the "reentry" type [1, 6, 7]. However, traditional prolongers of AP used to abolish reperfusion-induced arrhythmias, such as the antiarrhythmic amiodarone, have proved ineffective. There are likewise no reliable preparations among other classes of antiarrhythmics [9]. When studying the protective action of synthetic antioxidants in hypoxia and reoxygenation of isolated fragments of myocardium, we found that these preparations not only stabilize myocardial contractility, but also restore normal pacemaker function, evidence of the possible antiarrhythmic action of this class of compounds [2].

The aim of this investigation was to study the antiarrhythmic action of the antioxidant 2-ethyl-6-methyl-3-hydroxypurine-6-hydrochloride (SD06) in arrhythmias induced in the isolated rat heart by regional ischemia and reperfusion, and to compare these data with the effects of the compound on parameters of AP during hypoxia and reoxygenation of isolated papillary muscles.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 250-300 g. Before sacrifice the animals were given an intraperitoneal injection of hexabarbital (200 mg/kg) and an intravenous injection of heparin (400 U/kg). The heart was immersed in cold Tyrode solution, containing ice, of the following composition (in mM): NaCl 118.4, KCl 2.7, NaH_2PO_4 1.2, $MgCl_2$ 1.8, $CaCl_2$ 1.8, $NaHCO_3$ 25, glucose 11.2. The heart was then fitted on a cannula by the aorta, and retrograde perfusion was carried out throughout the experiment by Langendorff's method under a pressure of 115 mm Hg. The Tyrode solution was oxygenated with a gas mixture of 95% O_2 + 5% CO_2 and maintained at a temperature of 37°C and at pH 7.4. To keep the temperature of the heart mounted on the cannula steady, it was immersed in a vessel with a water jacket at the same temperature as the perfusion fluid. After adaptation for 15 min, regional ischemia was produced by ligating the descending branch of the left principal coronary artery [8]. The duration of ischemia was 10 min, which is the optimal time for the development of the longest-lasting arrhythmias and the onset of fibrillation [9]. After this time interval the ligature was removed and reperfusion carried out for 15 min. Throughout the experiment the ECG was recorded. To confirm that ischemia and reperfusion did in fact take place, the coronary blood flow was measured by determining the accumulation of perfusion fluid at the outlet from the heart. Only those experiments in which, in both the control and under the influence of SD-6, occlusion of the left coronary artery led to a sharp reduction of the coronary blood flow, and removal of the ligature was followed by an equally marked recovery and reperfusion, were taken into account (Fig. 1). The water-soluble antioxidant belonging to the 3-hydroxypyridine class was developed at the Chernogolovka Branch, Institute of Chemical Physics, Academy of Sciences of the USSR. The compound was added to the Tyrode solution 10 min before ischemia in concentrations of 10^{-6} and $5 \cdot 10^{-6}$ g/ml. In experiments on isolated papillary muscles AP were measured as described previously [2]. Hypoxia was induced by

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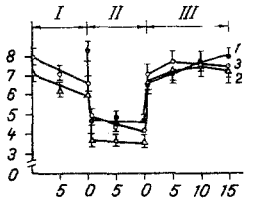

Fig. 1

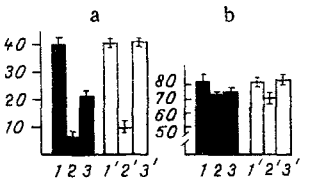

Fig. 2

Fig. 1. Changes in coronary blood flow of isolated rat heart following occlusion of left principal coronary artery and removal of the ligature. Abscissa, time counted from beginning of each procedure (in min); ordinate, coronary blood flow V_c (in ml/min). I) SD-6; II) ischemia; III) reperfusion. 1) Control (without SD-6); 2) 10^{-6} g/ml of SD-6; 3) $5 \cdot 10^{-6}$ g/ml of SD-6.

Fig. 2. Action of SD-6 on duration of AP and on resting potential (RP) of isolated rat papillary muscle during hypoxia and reperfusion. Ordinate: a) duration of AP, measured at level of 2/3 of repolarization (in msec); b) RP (in mV). Black columns — control (without compound); white columns — $5 \cdot 10^{-6}$ g/ml of SD-6. 1, 1') Original values of parameters; 2, 2') hypoxia; 3, 3') reperfusion.

stopping perfusion and exhausting the oxygen in the Tyrode solution from 700 to 150 mm Hg in the course of 60 min, after which the original conditions were restored and, for the next 60 min, reperfusion was carried out. The composition of the Tyrode solution was the same as in experiments on the whole heart; the temperature was 28°C. During the experiment the muscle contracted in response to stimulation by above-threshold pulses with a frequency of 1 Hz.

EXPERIMENTAL RESULTS

During ischemia the 20 animals of the control group developed arrhythmias on average 8.7 min after occlusion. In 90% of experiments ventricular extrasystoles were observed and tachycardia appeared in 75% of experiments, but fibrillation developed in only two experiments (Table 1) and lasted 75 and 4 sec, respectively. The arrhythmias during reperfusion were much more severe than in ischemia. In 100% of experiments rhythm disturbances changed during the first minute after removal of the ligature into total fibrillation, which was long-lasting (about 10-15 min) in 13 experiments. In seven experiments the fibrillation ceased spontaneously and its duration varied from a few seconds to 2 min. Tachycardia was present in 90% of experiments and complexes of ventricular extrasystoles in 75%. It will be clear from Table 1 that the use of the antioxidant significantly reduced the frequency of fibrillation and the severity of the ischemic and reperfusion-induced arrhythmias. In the case of ischemia this was reflected in a significant reduction of the incidence of tachycardia and the complete absence of fibrillation. The antifibrillatory action of the antioxidant during reperfusion must be particularly mentioned, for it is under those conditions, when fibrillation responds least to traditional antiarrhythmics [3], that the compound proved effective. Under the influence of a compound in concentrations of 10^{-6} and $5 \cdot 10^{-6}$ g/ml the frequency of fibrillation fell to 71 and 60%, respectively, but these periods of fibrillation were prolonged, and their duration in nearly all experiments was about 10-15 min. At the same time, the frequency but not the duration of tachycardia was reduced, and a tendency was observed for the time of onset of fibrillation to move toward the beginning of reperfusion. The antioxidant thus prevented the onset of fibrillation and tachycardia but did not make it more difficult to emerge from them. The observed increase in the percentage of extrasystoles was evidently the result of the decrease in frequency of the much more serious arrhythmias, i.e., of fibrillation.

As already mentioned, the onset of cyclic excitation waves of re-entry type and the development of arrhythmias take place at electrical nonhomogeneities of heart tissue as the result of shortening of AP and depolarization of the ischemic myocardium. The effect of the antioxidant on the parameters of AP during hypoxia and reperfusion of the isolated papillary muscle is shown in Fig. 2. In the control experiments the duration of AP and the amplitude of the resting potential (RP) were reduced and remained low throughout reoxygenation. Shortening of AP was the clearest and most reproduceable effect. The effect of a compound on these

TABLE 1. Antiarrhythmic Action of SD-6 during Ischemia and Reperfusion of the Isolated Rat Heart

Experimental conditions	Type of arrhythmia						
	fibrillation, %	duration of fibrillation, sec	extrasystoles, %	number of extrasystoles	tachycardia, %	duration of tachycardia, sec	time of onset of arrhythmias, sec
			Ischemia				
Control (n = 20)	10	40±6	90	21±10	75	16±2	521±6
SD-6:							
10^{-6} g/ml (n = 15)	0	0	57	41±12	36	14±3	468±15
$5 \cdot 10^{-6}$ g/ml (n = 20)	0	0	80	26±7	25	26±13	505±60
			Reperfusion				
Control (n = 20)	100	577±79	75	33±9	90	64±15	12±2
SD-6:							
10^{-6} g/ml (n = 15)	71	480±103	100	34±9	64	124±57	8±1
$5 \cdot 10^{-6}$ g/ml (n = 20)	60	636±90	100	20±6	80	86±39	9±2

parameters during hypoxia was not significant, but during reperfusion complete recovery both of RP and of the duration of AP took place.

Ischemia and reperfusion are known to lead to activation of free-radical processes in the cell and to the formation of toxic, membrane-damaging, peroxide radicals of lipids [3, 10, 13]. Membrane voltage clamping experiments have shown that the cause of the shortening of AP during hypoxia and reoxygenation is activation of the outward potassium current [5] and, to some degree also, inactivation of the outward calcium current [12]. Since potassium and calcium channels are ATP-dependent and since changes in the currents may take place in response to a fall of the ATP level comparable with that in hypoxia [11], O_2 insufficiency under these conditions will also bring about the observed shortening of AP. During hypoxia, the action of the antioxidant, acting as an interceptor of free-radical metabolites, will not be so effective. During reperfusion, when the fall of ATP is no longer limited by the O_2 deficiency, the antioxidants can restore AP both by protecting the mitochondrial membrane against damage by lipid peroxidation products and against the action of superoxide-dependent lysosomal enzyme systems, normalizing ATP, and also by inhibition of free-radical processes, which damage the surface membrane and affect the operation of ion channels. Comparison of these data with the results of experiments on the whole heart shows that restoration of the duration of AP and the amplitude of RP probably also accounts for the antiarrhythmic action of the antioxidant during reperfusion. The antioxidant is evidently not a "true" antiarrhythmic in the sense that, under these conditions, it does not interact directly with the ion channel, but affects it through the lipid matrix of the membrane and through the stabilization of cellular metabolism.

The results of this investigation give direct proof of the properties of this antioxidant as a class III antiarrhythmic, discovered previously [1]. The results are evidence of the important role of the formation of active forms of molecular oxygen in the development of electrical instability of the myocardium during ischemia and perfusion, and they indicate that synthetic antioxidants of the free-hydroxypyridine class can be used as antiarrhythmic drugs.

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