ANTIARRHYTHMIC AND VASODILATOR ACTION OF THE ANTIOXIDANT PHENOSAN DURING ACUTE ISCHEMIA AND REPERFUSION

L. A. Vasilets, V. P. Mokh, and L. G. Plekhanova

UDC 616.12-005.4-092.9-06:616.12-008.318-085.272.014.425

KEY WORDS: reperfusion arrhythmias; free radicals; antioxidants; antiarrhythmics; coronary blood flow.

The study of the antiarrhythmic action of synthetic antioxidants is of great theoretical and practical interest in connection with the investigation of the molecular mechanisms of reperfusion arrhythmias, which are difficult to abolish by traditional antiarrhythmics, and with elucidation of the role of free radicals in the pathogenesis of these disturbances. The writers showed previously [3, 4] that the water-soluble antioxidant SD-6, belonging to the 3-hydroxypyridine class, and which effectively blocks post-ischemic fibrillation, can restore electrical activity and, in particular, the duration of action potentials (AP) in the ischemic myocardium during reperfusion, which suggested that compounds of this type can be regarded as class III antiarrhythmics. The present investigation continues the study of the antiarrhythmic properties of synthetic water-soluble antioxidants, for the use of such compounds seemed, in our view, to be most strongly indicated in acute ischemia and also, conveniently, experimentally.

The aim of this investigation was to study the dose-dependent antiarrhythmic action of phenosan, a water-soluble antioxidant belonging to the class of sterically hindered phenols, in acute regional ischemia and perfusion of the isolated rat heart, and also to study its effect on coronary vascular tone.

EXPERIMENTAL METHOD

Noninbred male rats weighing 250-300 g were used. Before sacrifice the animals were given hexobarbital intraperitoneally in a dose of 200 mg/kg and heparin intravenously in a dose of 400 U/mg. The heart was placed in cold Tyrode solution, then the aorta was fitted in to a cannula, and throughout the experiment retrograde perfusion was carried out under a pressure of 115 mm water. The Tyrode solution, circulating with the aid of a peristaltic pump, was oxygenated with carbogen (95% O_2, 5% CO_2) and maintained at a temperature of 37°C and at pH = 7.4. To maintain a constant temperature, the heart fitted on the cannula was lowered into a vessel fitted with a water jacket. After adaptation for 15 min regional ischemia was created by ligation of the descending branch of the left main coronary artery. The duration of ischemia was 10 min, the optimal time for development of the most severe arrhythmias and for the onset of fibrillations [8]. After this time interval the ligature was removed and reperfusion carried out for 15 min. The ECG was recorded by means of two Ag—AgCl electrodes. The velocity of the coronary blood flow (CBF) was determined by measuring the accumulation of perfusion fluid escaping from the heart (in ml/min), and these parameters were used to monitor both occlusion and reperfusion. Only those experiments in which, both in the control and under the influence of phenosan, occlusion led to a sharp decrease in CBF, whereas removal of the ligature led to its equally sharp recovery, were taken into account (Fig. 1). To study dose-dependence, phenosan, synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR in Professor V. V. Ershov's laboratory, was added to the Tyrode solution

Institute of Chemical Physics, Academy of Sciences of the USSR, Chernogolovka, Moscow Oblast. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 554-557, November, 1988. Original article submitted January 20, 1988.

 0007-4888/88/0011-1558$12.50

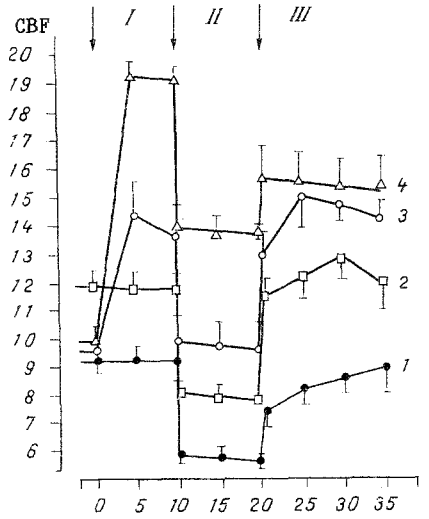

Fig. 1. Changes in CBF during ischemia and reperfusion under the influence of various doses of phenosan. Arrows: addition of phenosan to perfusion fluid (I), beginning of ischemia (II), and beginning of reperfusion (III). Changes in CBF in control without phenosan (1) and during its action in concentrations of 3.6×10^{-6} M (2), 3.6×10^{-5} M (3), and 3.6×10^{-4} M (4). Abscissa, time (in min); ordinate, CBF (in ml/min).

(composition, in mM: NaCl — 118.4; KCl — 2.7; $NaHCO_3$ —25; NaH_2PO_4 — 1.2; $MgCl_2$ — 1.8; $CaCl_2$ — 1.8; glucose — 11.2) 10 min before occlusion in concentrations of $3.6 \cdot 10^{-4}$, $3.6 \cdot 10^{-5}$, 3.6×10^{-6} M. The average of the above-mentioned concentrations is equimolar to the concentration of the antioxidant SD-6 studied previously (5×10^{-6} g/ml), so that the effectiveness of these compounds could be compared.

EXPERIMENTAL RESULTS

The results of the study of the antiarrhythmic action of phenosan during ischemia and reperfusion are given in Table 1. During ischemia, in the control group of 20 animals arrhythmias developed on average 8.8 min after occlusion. Ventricular extrasystoles were observed in 90% of experiments, and tachycardia developed in 75%. Fibrillation developed in only two experiments and was of short duration. Rhythm disturbances during reperfusion were much more marked than during ischemia. In all the experiments, the rhythm disturbances changed into total fibrillation in the first minute after removal of the ligature, and in 13 experiments the effect was long-lasting (about 10-15 min). In seven experiments the fibrillations ceased spontaneously, and their duration varied from a few seconds to 2 min. In 90% of cases tachycardia was present, and in 75% series of ventricular extrasystoles occurred.

It will be clear from Table 1 that the use of the antioxidant substantially reduced frequency of onset and severity of the ischemic and reperfusion arrhythmias. During ischemia this was expressed as a decrease in the frequency and duration of fibrillations and tachycardias with an increase in the dose of the compound. With phenosan in a concentration of 3.6×10^{-4} M, moreover, ischemic tachycardias were not observed during ischemia in any experiment. The increase in the percentage of extrasystoles and in their number in this series of experiments is evidence that under the influence of phenosan tachycardias were replaced by less serious forms of disturbances of the cardiac rhythm.

The antifibrillatory action of phenosan during reperfusion must be particularly noted (Fig. 2), for it is under those conditions that a disturbance of the cardiac rhythm is difficult to block by traditional antiarrhythmics. In the control long-term fibrillations (lasting longer than 10 min) developed in 70% of experiments, but under the influence of 3.6×10^{-5} M phenosan their frequency fell to 27%. In 40% of experiments, when this dose was used the fibrillations were short-lasting and disappeared spontaneously after 16 ± 4 sec, and their total percentage fell to 67. Thus phenosan not only reduced the frequency of appearance of fibrillations, but also facilitated their disappearance. This distinguishes it from SD-6,

TABLE 1. Antiarrhythmic Action of Phenosan during Regional Ischemia and Reperfusion of Isolated Rat Heart

Group	Long-lasting fibrillations		Short fibrillations		Tachycardias		Extrasystoles		Time before beginning arrythmias, sec
	%	duration, sec	%	duration, sec	%	duration, sec	%	number	
Ischemia									
Control (n = 20)	—	—	10	40±6	75	16±2	90	21±10	532±6
Phenosan:									
$3{,}6 \cdot 10^{-6}$ M (n=13)	—	—	15	4,0±1,4**	77	41±17*	92	77±36	467±20
$3{,}6 \cdot 10^{-5}$ M (n=15)	—	—	13	36±8	40	14±3	60	20±7	495±25
$3{,}6 \cdot 10^{-4}$ M (n=15)	—	—	7	1,5±0**	—	—	100	48±19	590±0
Reperfusion									
Control (n = 20)	70	803±24	30	40±6	90	64±15	75	33±9	12,0±1,6
Phenosan:									
$3{,}6 \cdot 10^{-6}$ M (n=13)	46	631±100	39	14±3*	100	123±59	69	19±4	7,0±1,4*
$3{,}6 \cdot 10^{-5}$ M (n=15)	27	826±36	40	16±4*	60	38±19	73	14±4*	5,0±1,3**
$3{,}6 \cdot 10^{-4}$ M (n=15)	67	811±83	—	—	27	10±6*	60	33±9	1,2±0,3**

* — $p < 0{,}05$.
** — $p < 0{,}01$.

which prevented the onset of reperfusion fibrillations but had no effect on their duration. Meanwhile phenosan, in a dose of 3.6×10^{-4} M, led to a marked reduction in the frequency of onset (to 27%) and the duration (to 10 ± 6 sec) of reperfusion tachycardias. The percentage and number of extrasystoles during reperfusion did not change significantly.

In the presence of phenosan the extremal dose-dependence of changes in the parameters of the ischemic and reperfusion arrhythmias, characteristic of the biological action of antioxidants [6], could be clearly seen. For antioxidants of phenolic type, this is associated with a fall in the level of antioxidative activity of the cellular structures with an increase in dose of the preparation [1]. However, optimal doses of phenosan differ for each of these parameters, as Table 1 shows. Blocking of ischemic and reperfusion tachycardias takes place more effectively under the influence of comparatively high doses of the preparation, of the order of 10^{-4} M. Suppression of reperfusion fibrillations, however, was maximal in the presence of 3.6×10^{-5} M phenosan. An increase in its concentration by an order of magnitude was accompanied by the appearance of long-lasting fibrillations but did not affect their total percentage. However the optimum of suppression of ischemic (but not reperfusion) extrasystoles lies within this same dose range (about 5×10^{-5} M).

It is a noteworthy fact that under the influence of phenosan fibrillations began much earlier than in the control. With phenosan in concentrations of 3.6×10^{-5} and 3.6×10^{-6} M the time before their appearance decreased by 2.4 and 10 times respectively. A similar tendency (the beginning of fibrillation coming closer to the time of reperfusion) also was found for the action of SD-6 [3]. This evidently indicates that a free radical inhibitor facilitates the discovery of yet another, faster component of the initiation of arrhythmias, which is connected with changes in the ionic composition of the extracellular medium when the flow of perfusion solution irrigating the intercellular spaces is "switched on." Our investigations showed that one such mechanism is Na/H exchange, which is activated during perfusion and causes a decrease in the duration of AP and the development of heterogeneity with respect to this parameter in cells of the normal and acidified zone [5].

Addition of phenosan to the perfusion fluid also was accompanied by a marked increase in CBF. The kinetics of changes in CBF in the course of the experiment is shown in Fig. 1. It can be seen that 3.6×10^{-5} M and 3.6×10^{-4} M phenosan, added 10 min before occlusion, led to an increase of 1.5 and 1.7 times in CBF. The level of CBF remained high in the course of its subsequent changes corresponding to occlusion and removal of the ligature. The lowest of the doses studied had no effect on CBF. The increase in the rate of flow through the vessels

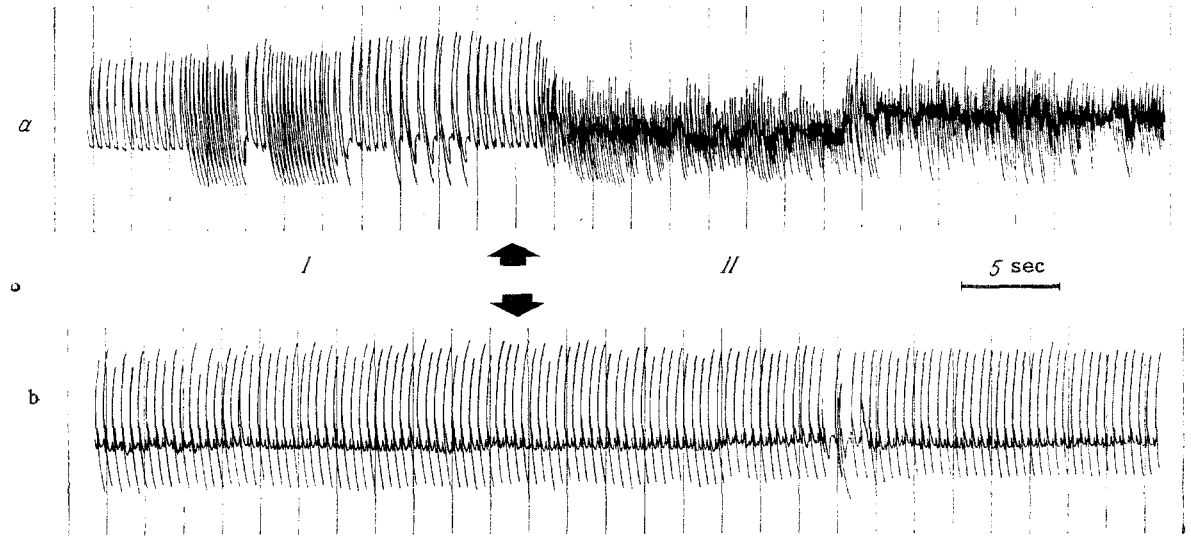

Fig. 2. Antiarrhythmic action of phenosan during ischemia (I) and reperfusion (II) of isolated rat heart. ECG in control (a) and under influence of 3.6×10^{-5} M phenosan (b).

of the heart is evidence of lowering of their tone and relaxation, which may help to reduce the severity of ischemia during the action of the antioxidant.

Evidence is now available to explain the vasodilator effect of phenosan. It can be tentatively suggested that phenosan, as an inhibitor of free radicals (including $O_2^{\cdot}$), can induce relaxation of smooth muscle on account of stabilization of the endothelial relaxing factor of the vessels which, as has been shown [7], is unstable and is destroyed by superoxide anion-radicals. In this connection it is worth noting that another antioxidant (SD-6), which has very low constant of interaction with $O_2^{\cdot}$, $K_7 = 26 \pm M^{-1}/sec$, and it therefore virtually does not interact with superoxide [2], does not induce relaxation of the coronary vessels or increase CBF [3].

The data given above are evidence that water-soluble antioxidants belonging to the sterically hindered phenol class possess a vasodilator action and are also effective blockers of ischemic and reperfusion arrhythmias and can be used to terminate them.

LITERATURE CITED

1. E. B. Burlakova, G. V. Arkhipova, A. N. Goloshchapov, et al., Bioantioxidants in the Regulation of Metabolism under Normal and Pathological Conditions [in Russian], Moscow (1982), pp. 74-83.
2. L. S. Vartanyan, A. I. Kozachenko, and N. M. Émanuél', Stud. Biofiz., 83, 188 (1981).
3. L. A. Vasilets and V. P. Mokh, Byull. Éksp. Biol. Med., No. 5, 593 (1987).
4. L. A. Vasilets, V. P. Mokh, G. N. Bogdanov, and T. I. Guseva, Kardiologiya, 27, No. 5, 83 (1987).
5. L. A. Vasilets, V. P. Mokh, and B. I. Khodorov, Intracellular Signalization and Its Use to Control the Bodily Functions and in Biotechnology [in Russian], Moscow (1988).
6. I. G. Krapova, Biofizika, 22, No. 3, 436 (1973).
7. R. J. Gryglewski, R. M. Palmer, and S. Moncada, Nature, 320, No. 3, 454 (1986).
8. A. S. Manning and D. J. Hearse, J. Mol. Cell. Cardiol., 16, No. 6, 497 (1984).