Effect of SOD and NO Donor on Reperfusion-Induced Rhythm Disturbances in Rats

V. A. Kostyuk and G. S. Polyukhovich

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 126, No. 7, pp. 137-140, February, 1999
Original article submitted January 15, 1998

Individual infusion of SOD and NO donor produces an antiarrhythmic effect, although does not completely prevent reperfusion-induced arrhythmias, while the combination of the antiradical agent and NO donor provides the most efficient protection of reperfused myocardium due to summation of their effects.

Key Words: *myocardial reperfusion; oxygen radicals; nitric oxide; arrhythmias*

Active oxygen species (AOS) and free radical reactions induced by them are the main damaging factors in reperfusion of various organs and tissues. There is ample experimental evidence on AOS generation in reperfused myocardium [6] and potent cardioprotective effect of structurally different low-molecular antioxidants [1,2,9]. It is also established that antioxidant defense system enzymes superoxide dismutase (SOD) and catalase moderate AOS synthesis [11] and decrease the occurrence of severe cardiac rhythm disturbances provoked by reperfusion [5].

A certain contribution into the development of ischemia-reperfusion-induced damage is presumably made by nitric oxide (NO) produced by vascular endothelium and blood cells, which is capable to react at a high rate with oxygen anion radical. The most important physiological functions of NO are prevention of thrombosis [7] and maintenance of the necessary vascular dilation, in particular, rapid compensatory vasodilator reaction during the postocclusion period [10]. Utilization of NO in the reaction with superoxide anion radicals intensively produced in the postischemic period can delay blood flow recovery and cause secondary ischemia. Moreover, the highly toxic product of this reaction peroxynitrite and its catabolite hydroxyl radical probably play a key role in the reperfusion-induced damage [4].

Laboratory of Thermoregulation, Department of Human and Animal Physiology, Belorussian State University, Minsk

We assumed that if the damage caused by free radicals and NO deficiency resulting from excessive production of AOS are the molecular basis of reperfusion disturbances of the cardiac rhythm, the combination of antiradical agents and NO can provide the most efficient recovery of the myocardium from reperfusion damage and normalize the cardiac rhythm. Our aim was to test this hypothesis. SOD and 1,1-diethyl-2-hydroxy-2-nitrosohydrazine sodium salt (DEA/NO) were used as the antiradical agent and NO donor, respectively.

MATERIALS AND METHODS

Experiments were carried out on Thiopental-anesthetized (100 mg/kg intraperitoneally) and artificially ventilated outbred albino rats of both sexes weighing 150-250 g. Myocardial ischemia-reperfusion was modeled by 12- and 30-min occlusion of the descending branch of the left coronary artery 2 mm below its orifice [8] with subsequent removal of the ligature to restore the blood flow.

SOD (Orgotenin, Gruenental, specific activity 3000 U/mg) and DEA/NO (RBI) were injected intravenously 2 min prior to the start of reperfusion. SOD was injected in physiological saline in a dose of 4 mg/kg body weight, DEA/NO was injected in distilled water in a dose providing blood concentration about 4×10^{-7} mol/liter. Combined administration was performed by successive injections of the preparations. The control

0007-4888/99/0002-0123$22.00 ©1999 Kluwer Academic/Plenum Publishers

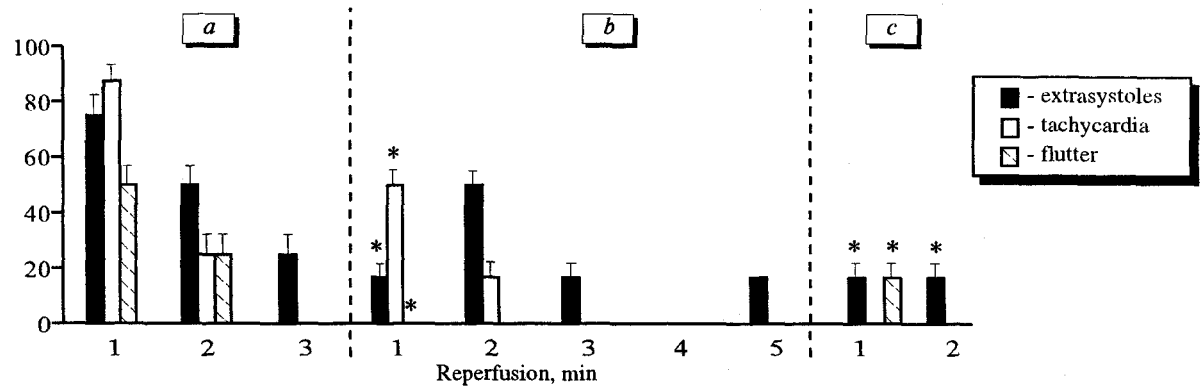

Fig. 1. Effect of SOD and DEA/NO on reperfusion-induced ventricular arrhythmias after 30-min coronary occlusion. *a*) control; *b*) SOD, 4 mg/kg; *c*) DEA/NO (blood level about 4×10^{-7} mol/liter). Here and in Fig. 2: ordinate, occurrence, %; $^{*}p<0.05$ in comparison with the control group.

rats were injected with physiological saline 2 min prior to reperfusion.

Ventricular arrhythmias were identified from the electrogram (monopolar lead from epicardiac surface of the left ventricle) and ECG (standard lead I). The incidence of different types of arrhythmias was statistically analyzed [3].

RESULTS

The maximum number of transient ventricular arrhythmias was observed during the 1st min of reperfusion after 30-min coronary occlusion. These arrhythmias disappeared to the 4th min of reperfusion (Fig. 1, *a*).

SOD injected 2 min prior to removal of the coronary ligature had a positive effect on the reperfusion recovery of the myocardium: ventricular flutter disappeared, while the occurrence of ventricular tachycardia and extrasystoles during the 1st min of reperfusion decreased. However, at least in 50% cases tachycardia was observed during the 1st min and extrasystoles during the 2nd min (Fig. 1, *b*). Injection of DEA/NO at the end of 30-min ischemia stabilized electrical activity of the left ventricle: solitary extra-

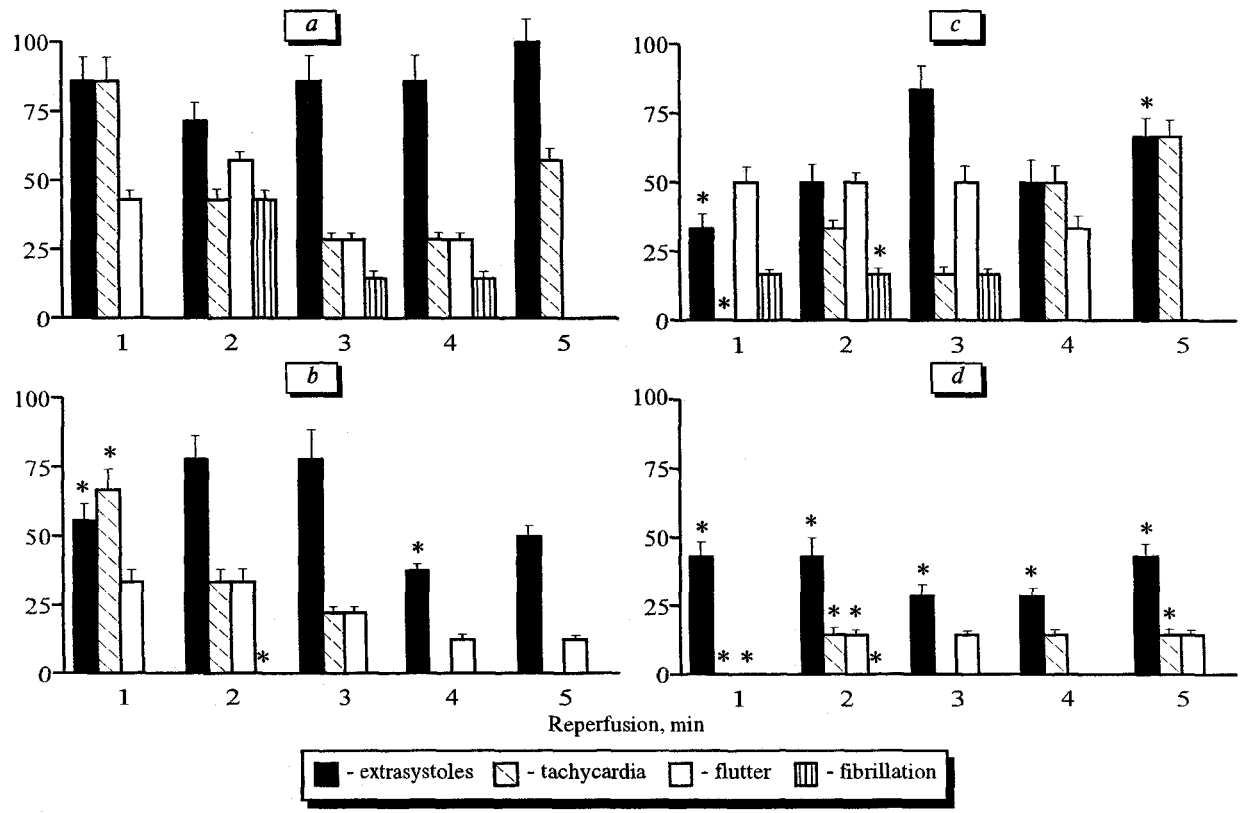

Fig. 2. Effect of SOD and DEA/NO injected individually or in combination on the development of ventricular arrhythmias after 12-min coronary occlusion. *a*) control; *b*) SOD, 4 mg/kg; *c*) DEA/NO (blood level about 4×10^{-7} mol/liter); *d*) SOD+DEA/NO.

systoles or flutter were noted only in 1 out of 6 rats (Fig. 1, *c*).

These findings indicate that ventricular arrhythmias during reperfusion after a 30-min occlusion of the coronary artery result mainly from NO deficiency and secondary local disturbances in the myocardium blood supply. The positive effect of SOD under these conditions is due to prevention of NO utilization in the reaction with superoxide radicals.

Reperfusion ventricular arrhythmias after a 12-min ischemia were longer and more frequent than after a 30-min ischemia (Fig. 1, *a* and 2, *a*): reperfusion ventricular arrhythmias persisted during the entire observation period, the incidence of extrasystoles varied from 71.4 to 100%, the most dangerous arrhythmias (tachycardia, flutter, and fibrillation) were primarily observed during the first 2 min of reperfusion (Fig. 2, *a*).

SOD (4 mg/kg) injected at the end of 12-min ischemia reduced the incidence of ventricular extrasystoles and tachycardia during the 1st min of reperfusion to 55.6 and 66.7%, respectively, and completely prevented ventricular fibrillation (Fig. 2, *b*). The protective effect of DEA/NO after a 30-min ischemia was much stronger then after a 12-min ischemia (Fig. 1, *c* and 2, *c*).

It can be hypothesized that more severe reperfusion disturbances after 12-min ischemia are caused not only by NO deficiency, but also by a direct damaging effect of AOS and can be prevented by a complex pharmacological intervention normalizing NO level and inhibiting AOS-induced reactions. Indeed, the combination of DEA/NO and SOD produced considerable antiarrhythmic effect in the postischemic period (Fig. 2, *d*): the occurrence of extrasystoles decreased to 28.6-42.9%, the incidence of tachycardia and flutter was 0-14.3%, while fibrillation was not observed at all.

It can be concluded that preliminary injection of the antiradical enzyme SOD produces the antiarrhythmic effect in the postischemic period of myocardium recovery, but does not completely prevent the appearance of reperfusion arrhythmias. After a 30-min ischemia, DEA/NO almost completely prevents reperfusion ventricular arrhythmias, while in the case of more severe reperfusion disturbances (provoked by a 12-min ischemia) it only decreases the occurrence of arrhythmia.

Our findings indicate that the protective effect of the antiradical compounds scavenging superoxide radicals in the postischemic myocardium is added by the effect of NO. Under given conditions, NO not utilized in the reaction with AOS can normalize the coronary blood flow in the damaged zone and promote reperfusion recovery of the myocardium.

REFERENCES

1. F. Z. Meerson, L. M. Belkina, A. A. Ugolev, *et al.*, *Kardiologiya*, **20**, No. 10, 81-86 (1980).
2. G. S. Polyukhovich, L. P. Vasil'eva, G. T. Maslova, *et al.*, *Vopr. Med. Khim.*, **37**, No. 4, 54-57 (1991).
3. P. F. Rokitskii, *Biological Statistics* [in Russian], Minsk (1967).
4. J. S. Beckman, T. W. Beckman, J. Chen, *et al.*, *Proc. Natl. Acad. Sci. USA*, **87**, 1620-1624 (1990).
5. M. Bernier, D. J. Hearse, and A. S. Manning, *Circ. Res.*, **58**, 331-340 (1986).
6. J. L. Flaherty and M. L. Weisfeldt, *Free Radic. Biol. Med.*, **5**, 409-419 (1988).
7. S. Moncada, M. W. Radomski, and R. M. J. Palmer, *Biochem. Pharmacol.*, **37**, 2495-2498 (1988).
8. H. Selye, E. Bajusz, and F. Grasso, *Angiology*, No. 11, 398-408 (1960).
9. S. Tanguy, F. Boucher, B. Malfroy, and J. G. Leiris, *Free Radic. Biol. Med.*, **21**, No. 7, 945-954 (1996).
10. J. L. Unthank, J. C. Nixon, and M. C. Dalsing, *Am. J. Physiol.*, **268**, H2523-H2530 (1995).
11. J. L. Zweier, B. K. Rayburn, J. T. Flaherty, and M. L. Weisfeldt, *J. Clin. Invest.*, **80**, 1728-1734 (1987).