6. F. Z. Meerson, Physiology of Adaptive Processes. Textbook of Physiology [in Russian], Moscow (1986), pp. 521-622.
7. V. M. Savov, V. V. Didenko, R. S. Dosmagambetova, et al., Nauch. Dokl. Vyssh. Shkoly, Biol. Nauki, No. 5, 30 (1985).
8. O. Desiderato and M. Testa, Physiol. Behav., 16, 67 (1976).
9. I. Fridovich, J. Biol. Chem., 245, 4053 (1970).
10. R. H. M. Julicher, L. B. M. Tijberg, L. Sterrenberg, et al., Life Sci., 35, 1281 (1984).
11. H. Luck, Methods of Enzymatic Analysis, Weinheim (1963), pp. 885-894.
12. H. Nohl, O. Hegner, and K.-H. Summer, Biochem. Pharmacol, 30, 1753 (1981).
13. M. E. Persy, Can. J. Biochem., 62, 1006 (1984).
14. S. L. Taylor, M. P. Lamden, and A. L. Tappel, Lipids, 11, 530 (1976).
15. P. Valk, P. Gille, A. B. Oosta, et al., Oxygen Radicals in Chemistry and Biology, New York (1984), pp. 695-697.

EFFECT OF DIBUNOL* AND VERAPAMIL ON SERUM CREATINE KINASE AND MYOGLOBIN LEVELS IN DOGS DURING POSTISCHEMIC CORONARY REPERFUSION

A. P. Golikov, O. A. Avilova, V. Yu. Polumiskov, A. A. Berestov, E. A. Konorev, S. P. Smotrov, and I. N. Sharipova

UDC 616.12-005.4-008.66-07:/616.153.962.42+616.153.1:577.152.273/-02:615.272.4.014.425

KEY WORDS: reperfusion of the ischemic myocardium; creatine kinase; myoglobin; dibunol*; verapamil.

Restoration of the coronary blood flow after ischemia accelerates the release of intracellular high-molecular-weight proteins from the heart [4, 14]. The mechanisms of these changes have not been fully elucidated. It has recently been shown that postischemic reperfusion aggravates structural damage to the cardiomyocyte membrane [9], and the most important role in the formation of this damage is played by intensification of lipid peroxidation (LPO) [1, 2] and a disturbance of calcium transport [3].

The aim of this investigation was to study the effect of the antioxidant dibunol and the calcium antagonist verapamil on postreperfusion release of myoglobin (MG) and creatine kinase (CK) from the heart in experimental myocardial infarction due to coronary occlusion.

EXPERIMENTAL METHOD

Experiments were carried out on 30 dogs weighing 7-20 kg. Myocardial infarction was produced by application of an atraumatic vascular clip to the anterior descending branch of the left coronary artery under pentobarbital sodium anesthesia (40 mg/kg, intraperitoneally) with artificial ventilation of the lungs by the RO-6-03 apparatus. The myocardium was revascularized by removal of the clip 3 h after its application. Dibunol was injected intraperitoneally in a dose of 30 mg/kg 2 and 3 h after coronary occlusion, in the form of an oily emulsion in Tween-80; verapamil was injected intravenously (a bolus dose of 0.1 mg/kg followed by 0.2 mg/(kg·h) by the drip method, starting with 2 h after application of the clip to the artery). The serum MG level of the dogs was determined by solid-phase enzyme immunoassay, using the sandwich technique. This method is based on detection of the MG–antibody complex with the aid of peroxidase-labeled γ-globulin against MG. Antiserum to MG was obtained by immunizing rabbits with a purified preparation of MG, isolated from dog skeletal muscles by the method in [12]. Antibodies to MG were isolated from the immune serum by affinity chromatography on CNBr-sepharose 4B (Sigma, USA). Peroxidase-labeled γ-globulin from rabbit antiserum was synthesized by the periodate method [7]. MG was determined by consecutive treatment of 9-well polystyrene plates (Labsystems, Finland) with sensitized antibodies, with the dog serum to be

*4-Methyl-2,6-di-tert-butylphenol.

Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 413-416, October, 1987. Original article submitted December 15, 1986.

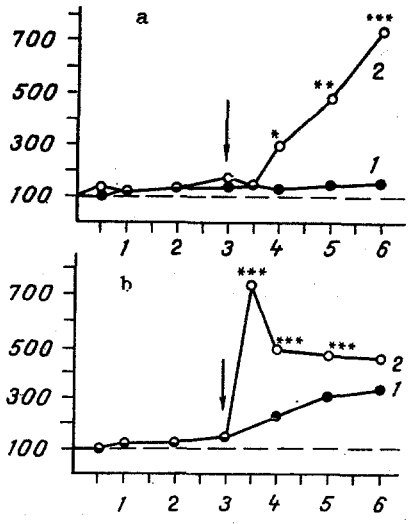

Fig. 1. Plasma MB-CK (a) and MG (b) levels in experimental myocardial infarction. Abscissa, duration of ischemia and reperfusion (in h); ordinate, MB-CK activity (MG concentration, in percent of initial level, shown by broken line). 1) Occlusion of coronary artery for 6 h (control), 2) occlusion for 3 h followed by reperfusion for 3 h. Arrow indicates beginning of reperfusion. Here and in Fig. 3, $^{*}p < 0.005$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with control.

analyzed, and with peroxidase-labeled anti-MG-γ-globulin. After addition of the substrate mixture containing o-phenylenediamine to the wells and incubation for 10 min the enzyme reaction was stopped with 50% sulfuric acid solution. The optical density of the solutions in each well was measured on an FP-9 biochemical analyzer (Labsystems) at 500 nm. The quantity of MG was calculated by means of a calibration curve plotted with the use of standard solutions of canine MG. Chromatographic fractionation of CK isozymes was carried out on analytical columns with DEAE-Sephadex A-50 (Pharmacia, Sweden), using a stepwise sodium chloride gradient [6]. Activity of the MB-isozyme of CK (MB-CK) was measured by a kinetic method, using standard kits (Boehringer, West Germany) [13], on the FP-9 analyzer at 37°C. For a more accurate quantitative assay of MB-CK, the background level, due to the presence of adenylate kinase in the sample, was subtracted from the measured activity. MB-CK activity was expressed in percentages of the initial level. The results were processed by HP-9815A calculator (USA) using appropriate standard programs. The animals as a whole were divided into five groups (six animals in each group): 1) occlusion of the coronary artery for 6 h; 2) occlusion for 3 h followed by reperfusion for 3 h; 3) coronary occlusion for 3 h followed by reperfusion for 3 h accompanied by injection of dibunol; 4) coronary occlusion for 3 h followed by reperfusion for 3 h accompanied by injection of verapamil; 5) coronary occlusion for 3 h followed by reperfusion for 3 h, accompanied by combined injection of dibunol and verapamil.

EXPERIMENTAL RESULTS

In the animals of group 1, with continuous occlusion of the descending branch of the left coronary artery, morphological investigation revealed an extensive transmural myocardial infarct, which was accompanied by elevation of the blood MG level and MB-CK activity (Fig. 1). Definite differences were found in the time course of these parameters during ischemia. For instance, plasma MB-CK activity after 3 h was 1.5 times higher than initially. However, in the latter stages of ischemia no further increase in the activity of this isozyme could be found. By contrast, changes in the MG level were more marked. This was shown by the progressive myoglobinemia, exceeding the initial level by 1.5 times after 3 h of ischemia and by 3.3 times after 6 h.

Reperfusion of the myocardium after ischemia for 3 h not only accelerated the release of intracellular proteins considerably, but also led to the appearance of marked myoglobinemia and high blood enzyme levels (Fig. 1). For instance, after reperfusion for 30 min the peak level of MG was observed, more than 7 times higher than the initial value, and differing

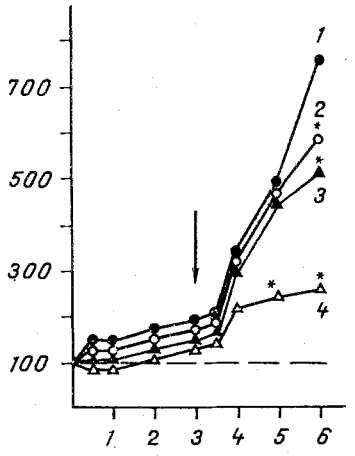

Fig. 2

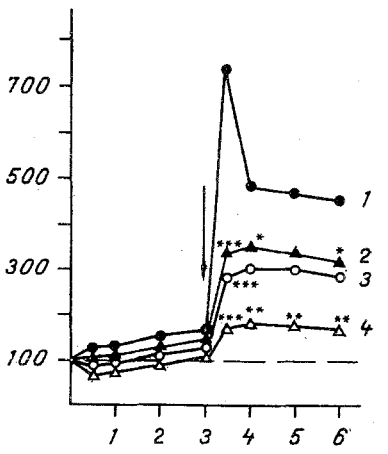

Fig. 3

Fig. 2. Effect of dibunol and verapamil on plasma MB-CK level in experimental myocardial infarction. Abscissa, duration of ischemia and reperfusion (in h); ordinate, MB-CK activity (in percent of initial level, indicated by broken line). 1) Occlusion for 3 h followed by reperfusion for 3 h (control), 2) occlusion for 3 h followed by reperfusion for 3 h, accompanied by injection of verapamil, 3) occlusion for 3 h followed by reperfusion for 3 h, accompanied by injection of dibunol, 4) occlusion for 3 h followed by reperfusion for 3 h, accompanied by combined injection of dibunol and verapamil. Arrow indicates beginning of reperfusion. *$p < 0.01$ compared with control.

Fig. 3. Effect of dibunol and verapamil on plasma MG level in experimental myocardial infarction. Legend as to Fig. 2.

significantly from the value of the corresponding parameter after the same period of ischemia ($p < 0.001$). After 180 min of reperfusion, MB-CK activity was 5 times higher than in the group with coronary occlusion for 6 h.

Injection of verapamil and dibunol 60 min before restoration of the blood flow caused the blood MG level and MB-CK activity to decline (Figs. 2 and 3). The most marked effect was obtained during postischemic renewal of the blood flow after preliminary combined injection of dibunol and verapamil. Under these circumstances, after 180 min of reperfusion the blood levels of MB-CK and MG were almost two-thirds below the "reperfusion" values (Figs. 2 and 3).

Consequently, on restoration of the coronary blood flow the degree of myoglobinemia and blood enzyme activity was considerably higher than that observed during continued ischemia; in the opinion of several workers, this may be due to a regular process of "flushing out" of proteins from a previously unperfused region of myocardium [11]. The increase in the rate of arrival of intracellular enzymes in the blood stream considerably reduces the degree of their inactivation, which takes place in the lymphatic system [8]. However, since the rate of outflow of MG and MB-CK from a focus of necrosis into the blood stream differs [10], and since changes in their levels during reperfusion were identical in direction, it can be postulated that elevation of the MG and MB-CK levels, which is an established fact, is not only the result of "flushing out."

According to another point of view, an increase in the calcium permeability of membranes during postischemic perfusion, as a result of LPO activation changes the stability of the sarcolemma and stimulates the loss of intracellular enzymes by cardiomyocytes [5]. This view is confirmed by data obtained in the present investigation. Preliminary injection of dibunol and verapamil, i.e., of drugs acting simultaneously on the key stages in the mechanism of reperfusion changes, had in fact a marked protective action on the myocardium, manifested by a considerable fall of the blood MG and MB-CK levels compared with their values in the control experiments with restoration of the coronary blood flow. The protective effect of these drugs is probably connected with their membrane-stabilizing effect on cardiomyocytes, as a result of a decrease in the intensity of LPO processes in the membranes and inhibition of the influx of calcium into the sarcolemma.

Thus the marked myoglobinemia and high blood enzyme levels observed during postischemic reperfusion are largely determined by damage to cardiomyocytes during reperfusion. Prophylactic injection of the antioxidant dibunol and of the calcium antagonist verapamil, however, inhibits the reperfusion component of myocardial damage, and this is accompanied by prevention of loss of enzymes and myoglobin by the cardiomyocytes.

LITERATURE CITED

1. M. V. Bilenko, V. N. Otverchenko, and V. V. Astaf'ev, Grudn. Khir., No. 6, 20 (1983).
2. P. F. Litvitskii, A. Kh. Kogan, A. N. Kudrin, et al., Byull. Éksp. Biol. Med., No. 3, 271 (1981).
3. E. Carmeliet, Inform. Cardiol., 9, No. 2, 103 (1985).
4. R. Ferrari, F. D. Lisa, Q. Raddino, et al., Myocardial Ischemia and Lipid Metabolism, New York (1984), p. 135.
5. C. Ganote, S. Liu, S. Safari, et al., J. Mol. Cell. Cardiol., 13, 93 (1981).
6. D. W. Mercer, S. Y. Surtees, M. D. Bastable, et al., Lancet, 2, 1283 (1977).
7. P. K. Nakane and A. Kawaoi, J. Histochem. Cytochem., 22, 1084 (1974).
8. H. Nordbeck, H. Kahles, C. J. Preusse, et al., J. Mol. Med., 2, 255 (1977).
9. K. A. Reimer and R. B. Jennings, Eur. Heart J., 6, Suppl. E, 97 (1985).
10. R. Roberts, Heart Lung, 10, No. 3, 486 (1981).
11. W. E. Shell, H. J. Swan, and D. Canz, Clin. Res., 29, 241 (1981).
12. H. R. Strausser, E. L. Rothfeld, and R. A. Bucsi, Proc. Soc. Exp. Biol. Med. (New York), 122, 621 (1966).
13. G. Szacz, Y. Gruber, and E. Bernt, Clin. Chem., 22, 650 (1976).
14. S. T. Vatner, H. W. Baig, S. W. T. Mander, et al., J. Clin. Invest., 61, 1048 (1978).

Na^+/H^+ EXCHANGE IN ERYTHROCYTES OF SPONTANEOUSLY HYPERTENSIVE RATS

N. I. Pokudin, S. N. Orlov, and Yu. V. Postnov

UDC 616.12-008.331.1-07.616.155.1-008.923.3-07

KEY WORDS: Na^+/H^+ exchange; rat erythrocytes; spontaneous hypertension; valinomycin.

The membrane concept of the pathogenesis of primary hypertension, which was formulated more than 10 years ago [5], has been confirmed experimentally many times [7, 10]. Most of these investigations have been conducted on blood cells from patients with essential hypertension and from rats with spontaneous hypertension, which is regarded as an adequate model of human primary hypertension (essential hypertension). Research aimed at discovering the molecular mechanisms of formation of membrane disturbances is currently in progress in several laboratories. Particular attention is being paid to the study of the state of proteins forming the cytoskeleton of the membrane [1, 6, 11]. We know that some cytoskeletal proteins participate directly in the regulation of the shape and volume of cells, including erythrocytes.

It was shown previously that if valinomycin is added to rat erythrocytes the cells are compressed and H^+-dependent sodium inflow is induced [2, 3]. It was therefore decided to compare the velocity of Na^+/H^+ exchange in the erythrocytes of spontaneously hypertensive rats (SHR) and rats of a control group, with different degrees of cell contraction.

EXPERIMENTAL METHOD

Male SHR (spontaneous hypertensive Kyoto-Wistar rats) and control WKY (normotensive Kyoto-Wistar rats), acting as the control, and whose ages and blood pressure (BP) are indicated in Table 1, were used. The procedures of taking blood and obtaining erythrocytes were described by the writers previously. The value of Na^+/H^+ exchange was judged from the amiloride-inhibited component of the velocity of ^{22}Na inflow [3]. For this purpose 200 µl of erythrocytes

Central Research Laboratory, Ministry of Health of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 10, pp. 416-418, October, 1987. Original article submitted April 3, 1987.

0007-4888/87/0010-1364$12.50