EFFECT OF VERAPAMIL ON ACTIVITY OF Ca^{2+},Mg^{2+}-DEPENDENT ENDONUCLEASES IN DOG LIVER NUCLEI IN LONG-TERM HYPOVOLEMIC SHOCK

V. L. Kozhura, N. V. Smirnova, and I. S. Novoderzhkina

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KEY WORDS: verapamil; Ca^{2+},Mg^{2+}-dependent endonucleases; liver nuclei; hypovolemic shock

Disturbance of calcium homeostasis is one of the most important mechanisms of ischemic damage to the hepatocyte in various pathological states [5, 7]. There is conclusive evidence that massive intake of Ca^{2+} into the cell cytosol causes activation of lipolysis with accumulation of free fatty acids, activation of proteolysis, and disaggregation of components of the cytoskeleton [3, 7]. It has been shown [1, 4, 5] that an increase in the Ca^{2+} concentration in the cytosol accelerates activation of nuclear endonucleases and fragmentation of DNA of the haptocyte nuclei, leading to their death [1, 7]. There is no information in the literature on the role of calcium channel blockers in the regulation of the Ca^{2+},Mg^{2+}-dependent endonucleases in the liver nuclei in hypovolemic shock. Nevertheless, the elucidation of this causal connection could play an important role in the study of the molecular mechanism of irreversible damage to hepatocytes in lethal blood loss and in the development of ways of preventing these disturbances.

The aim of this investigation was to study the action of the calcium channel antagonist verapamil on activity of Ca^{2+},Mg^{2+}-dependent endonucleases of the liver nuclei during long-term hypovolemic shock.

EXPERIMENTAL METHOD

Experiments were carried out on 24 mongrel male and female dogs weighing 8-15 g. After anesthesia with trimeperidine (8 mg/kg) and pentobarbital (10-15 mg/kg) the heparinized dogs (500 IU/kg) were bled from the femoral artery, lowering the blood pressure on average to 40 mm Hg, at which level it was maintained with fractional intraarterial injection of blood for a period of 4 h (group 1). Group 2 comprised animals receiving an intravenous injection of verapamil (Finoptin, from "Orion," Finland) in a dose of 0.1 mg/kg 30 min before the beginning of bleeding. Animals of group 3 received verapamil in the above dose, but did not develop hypovolemic shock. Group 4 (control) comprised animals not receiving verapamil and not subjected to hypovolemic shock. The animals of groups 1 (6 dogs) and 2 (6 dogs), after 4 h of arterial hypotension, the animals of group 3 (6 dogs) 30 min after intravenous injection of verapamil, and animals of group 4 (6 dogs) were anesthetized with pentobarbital and killed by electrocution by the 127-V supply grid. The liver tissue was excised from the central lobe in all four series of experiments and kept in ice.

The liver cell nuclei were isolated by the detergent method. The liver tissue after removal was placed in cold buffer A, containing 50 mM Tris-HCl, pH 8.0, 0.25 M sucrose, 10 mM $CaCl_2$, and 5 mM 2-mercaptoethanol. All procedures were carried out at 4°C. The tissue was ground in a Teflon-glass homogenizer in buffer A. The homogenate was filtered through four layers of gauze and centrifuged at 1500*g* for 10 min. The nuclear residue was washed in buffer B, containing 0.5% Triton X-100 in buffer A. The washing was repeated twice. To measure DNA the cell nuclei were suspended in water, after which EDTA was added up to 20 mM and sodium dodecylsulfate (SDS) to 1%, after which optical absorption was measured at 260 nm. One optical unit was taken to correspond to a DNA concentration of 40 μg/ml.

Laboratory of Experimental Therapy of Terminal States, Institute of General Resuscitation, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. A. Negovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 12, pp. 583-584, December, 1991. Original article submitted February 5, 1991.

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TABLE 1. Effect of Verapamil on Activity of Nuclear Ca^{2+},Mg^{2+}-Dependent Endonucleases of Dog Liver during Hypovolemic Shock for 4 h (M ± m; n = 6)

Activity	Group of animals			
	1-	2-	3-	4-
	hypotension 4 h		intact animals	
	verapamil not given	verapamil given	verapamil not given	verapamil given
Activity of Ca^{2+}, Mg^{2+}-dependent liver endonucleases, U/mg protein	4679±435	1022±155*	3062±151	2905±182

Legend. *p < 0.001: significant differences between groups 1 and 2.

Determination of Endonuclease Activity. DNA of the nuclear extract was sedimented by the addition of 0.5 volume of 18% polyethylene-glycol-6000 in lytic buffer. The residue was separated by centrifugation at 10,000*g* for 15 min. The extract was dialyzed for 12 h against 10 mM Tris-HCl, pH 8.0, 0.1 M EDTA, 0.5 M dithiothreitol, 50% glycerol, and kept at −20°C. To determine endonuclease activity, DNA of plasmid pIS, obtained by the method described in the textbook [2], was used as substrate. Ca^{2+},Mg^{2+}-dependent endonuclease was determined in an incubation sample with a volume of 20 μl, containing 2 μg DNA, 10 mM Tris-HCl, pH 7.4, at 37°C, 25 μg/ml of bovine serum albumin, fraction V, 0.5 mM dithiothreitol, 1 mM EDTA, nuclear extract in dilutions, 2 mM $CaCl_2$, and 5 mM $MgCl_2$.

The samples were incubated for 30 min at 37°C. The reaction was stopped by cooling the samples and adding 5 μl of a mixture of 1% SDS, 0.2 M EDTA (pH 8.0), 0.25% bromphenol blue, and 50% glycerol. Electrophoresis was carried out in 0.8% agarose, with electrode buffer: 0.04 M Tris-acetate, pH 8.3, 0.02 M Na acetate, 2 mM EDTA, with a voltage of 10 V/cm for 1 h. The gel was developed in ethidium bromide solution (0.5 μg/ml). The unit of endonuclease activity was taken to be the quantity of enzyme required to hydrolyze 1 μg DNA (50% DNA of the sample) in 30 min at 37°C. The protein content in the preparations was determined by Sedmark's method [6].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that in the animals of group 1, which received verapamil (0.1 mg/kg) intravenously 30 min before the beginning of bleeding, activity of Ca^{2+},Mg^{2+}-dependent endonuclease was significantly lower than that in the animals of group 2, which were subjected to hvpovolemic shock for 4 h without preliminary administration of verapamil. Consequently, prophylactic injection of verapamil, by blocking the entry of Ca^{2+} into the cell cytosol, inhibits activation of DNA-endonucleases. We know that if the blood flow in ischemic liver cells is low as a result of increased formation of active free radicals, permeability of the hepatocyte membranes for Ca^{2+} is altered and there is a significant increase in entry of Ca^{2+} into the cell cytosol [3, 7]. A direct connection also has been found between the elevation of the Ca^{2+} level in the cytosol and loss of viability leading to death of the cells [1, 5]. Under these circumstances the degree of destruction of components of the cell nucleus and, in particular, DNA, is the determining factor in preservation of the viability of the cells.

Attention is drawn to the fact, which we discovered for the first time, that the level of activity of the nuclear endonucleases studied was significantly lower than their value in the intact animals of groups 3 and 4, i.e., verapamil has a marked inhibitory effect on the activity of these enzymes in hypovolemic shock. The experiments on the animals of groups 3 and 4 showed that injection of verapamil into intact animals does not block activity of Ca^{2+},Mg^{2+} dependent endonucleases, for there was no difference in the activity of these enzymes in the two groups. The experimental data show that the action of verapamil prevents activation of the DNA-endonucleases studied, and effectively depresses their activity under conditions of enhanced permeability of the hepatocytes membranes, whose integrity is disturbed in prolonged hypovolemic shock [7]. It can be tentatively suggested that verapamil, a calcium channel blocker, has a stabilizing action on liver cell membranes in hypovolemic shock.

The investigation described above thus established an important molecular mechanism of protection of hepatocytes against the damaging action of a pathogenetic factor of the terminal process, namely increased activity of nuclear Ca^{2+},Mg^{2+}-dependent liver endonucleases.

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EXPERIMENTAL EVALUATION OF THE HEMODYNAMIC EFFICIENCY OF DYNAMIC CARDIOMYOPLASTY IN THE SURGICAL TREATMENT OF SEVERE LEFT VENTRICULAR FAILURE

V. S. Chekanov, K. V. Shatalov, V. Yu. Merzlyakov, M. V. Tolmachev, and R. R. Movsesyan

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Autologous muscle tissue was used for the first time in heart surgery for indirect revascularization of damaged myocardium in 1935 [8]. Since the end of the 1950s, autologous muscle tissue has been widely used in heart surgery for indirect revascularization and also for plastic replacement of injured myocardium. Progress in the development of electrophysiology and integrated electronic circuitry has led to the development of new trends in the use of skeletal muscles for surgical treatment of the heart. In 1960 [11] it was suggested that stimulated skeletal muscle be used not only for plastic, but also for dynamic replacement of the infarcted myocardium. In different countries of the world more than 100 operations have now been performed by the method of dynamic cardiomyoplasty (DCMP), including more than 15 such operations in the USSR [1, 2, 4, 7, 9, 12].

The aim of this investigation was a comprehensive experimental study of the hemodynamic status of the circulatory system in the immediate and late stages after performance of DCMP on the damaged heart.

A. N. Bakulev Institute of Cardiovascular Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. I. Burakovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 12, pp. 584-588, December, 1991. Original article submitted June 10, 1991.

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