Effect of Sarcolemmal Anion Transport System Blockers on the Development of Ischemia-Induced Myocardial Edema and Recovery of Contractile Function during Reperfusion

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Subtotal 30-min ischemia leads to myoglobin release and increases water content in the heart. Reperfusion partially restores the developed pressure. Addition of furosemide (a $Na^+,K^+,2Cl^-$-symport blocker) or NMA (inhibitor of Na^+/H^+-exchange) to perfusate decreases myocardial water content, reduces myoglobin loss, and completely restores myocardial contractile function. The low-rate perfusion of isolated heart and its reperfusion with solutions containing DIOA (inhibitor of K^+,Cl^--co-transport) or IAA-94 (Cl^- channel blocker) increases water accumulation and myoglobin release from the myocardium, and deteriorated its contractile function during reperfusion.

Key Words: *myocardial ischemia; edema; $Na^+,K^+,2Cl^-$-symport; Na^+/H^+-exchange; K^+,Cl^--co-transport; Cl^- channels; amiloride derivatives*

Similar to other cells, cardiomyocytes possess a system regulating their volume [4,6,7,10]. The volume regulation system is activated by swelling or shrinkage of cardiomyocytes [10,13,14]. An increase in the cardiomyocyte volume stimulates efflux of the osmotically active substances (osmolytes) from the cells, which decreases the amount of intracellular water. An important role in this process is played by Cl^- channels and $K^+,2Cl^-$-symport. The decrease of the cell volume is accompanied by opposite processes: entry of Cl^-, Na^+, and K^+ into the cytoplasm via Na^+,K^+, $2Cl^-$-uniport and Na^+ via Na^+/H^+-exchange [7,10,13].

A decrease in the coronary blood flow modulates activity of proteolytic and lipolytic enzymes, induces accumulation of metabolic products in the ischemic region, and activates Na^+/H^+-exchange [2,8,9,15]. These changes may form the basis for an increase in the intracellular osmotic pressure and water entry into the cytoplasm [1,5,11]. It is supposed that extreme cardiomyocyte swelling may be a cause of mechanical rupture of the sarcolemma [6,12].

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The role of sarcolemmal anion-transporting mechanisms (Cl^- channels, K^+,Cl^-- and $K^+,Na^+,2Cl^-$-symport, and Na^+/H^+-exchange) in the development of cardiomyocyte edema and their ischemic and reperfusion damage is not clear. Our aim was to study the effects of these ion-exchange sarcolemmal systems on myocardial edema during subtotal ischemia and reperfusion.

MATERIALS AND METHODS

Experiments were performed on hearts perfused according to the Langendorff technique. Random-bred albino rats were decapitated under ether narcosis, the hearts were isolated and placed into cold Ringer—Lock solution. The initial solution contained (in mM): 140 NaCl, 0.5 NaH_2PO_4, 5 KCl, 5 Tris-OH (pH 7.4), 11 glucose, and 2 $CaCl_2$. The heart was perfused with oxygenated solution through cannulated aorta at the rate of 10 ml/min/g wet tissue at 37°C. To stabilize the contractile activity, the heart was perfused with initial solution for 15 min. The cardiac parameters during this period were considered as control (100%). Subtotal ischemia was modeled by reducing the perfusion

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rate to 0.1 ml/min for 30 min, followed by reperfusion with initial solution at a control rate.

When $NaHCO_3$ (20 mM) was added to perfusion solutions, the concentration of NaCl was decreased to 120 mM to maintain Na^+ activity at a constant level.

The studied agents were added to perfusion solution 15 sec before and during the entire ischemic and reperfusion periods. The following amiloride derivatives were used to selectively inhibit the ion-transporting systems: 3-amino-6-chloro-N-diaminomethylen-5-(1-homopiperidyl)-pyrazinecarboxamide (HMA); [(dihydroindenyl)oxy]alkanoic acid (DIOA); R(+)-[(5,6-dichloro-2,3,9,9a-tetrahydro-3-oxo-9a-propyl-1H-fluoren-7-il)oxy]acetate (L-644,711); R(+)-[(2-cyclopentyl-6,7-dichloro-2-methyl-1-oxo-1,2-dihydro-indenyl)-5-oxy]acetate (R(+) IAA-94). The inhibitors were used in concentrations, which produced at least 50% selective inhibition of the corresponding ion-transporting system.

The myocardial contractile activity (MCA) was studied in the isovolumetric mode using a small latex cylinder inserted into the left ventricle. The developed pressure was calculated as the difference between systolic and diastolic pressures measured with an electromanometer (Bentley Lab. Europe) connected to an IBM PC via a digitizer.

The degree of cardiomyocyte damage was assessed by myoglobin release during 30-min ischemic and 12-min reperfusion periods. Myoglobin concentration in perfusate was measured spectrophotometrically at 410 nm [3].

Water content in the myocardium was determined as the difference between wet and dry tissue weights. To this end, the hearts were dried at 100°C for 24 h.

The data were statistically analyzed using AWPE software (A. I. Glotov) and by variation statistics with Student's *t* test.

RESULTS

Decrease in the rate of coronary perfusion to 0.1 ml/min and its subsequent restoration to the preischemic level resulted in accumulation of water in the myocardium and myoglobin release into perfusate (Table 1). Left ventricle diastolic pressure (LVDP) increased, while MCA did not return to normal during the reperfusion period (Table 1).

Furosemide (10 μM) decreased myocardial water content and myoglobin release in comparison with the control. At the same time, almost complete restoration of MCA occurred together with an insignificant change in LVDP (Table 1). The Na^+/H^+-exchange blocker HMA (1 μM) considerably reduced myoglobin release from the heart during ischemia, decreased water content in the myocardium, and contributed to a better (compared with the control) recovery of pressure developed by the myocardium during reperfusion. It also produced an insignificant increase in LVDP (Table 1).

Therefore, the inhibition of sarcolemmal mechanisms increasing cardiomyocyte volume (Na^+,K^+, $2Cl^-$-symport and Na^+/H^+-exchange) prevents ischemia-induced swelling and the release of myoglobin

TABLE 1. Effect of Anion Transport System Blockers on Water Content in the Myocardium, Myoglobin Release from the Heart, Recovery of Developed Pressure and LVDP during 30-min Subtotal Ischemia and 10-min Reperfusion (*M±m*)

Experimental series	Water content, ml/g dry weight	Myoglobin, μg/g dry weight		MCA parameters at the 10th minute of reperfusion	
		ischemia	reperfusion	developed pressure, % of preischemic level	LVDP, mm Hg
Before ischemia	3.22±0.08	0	0	100	17.5±1.7
After 30-min ischemia					
Control	5.76±0.14**	42.05±5.3***	119.42±4.62	39.0±6.0**	50.0±6.0**
Furosemide, 10 μM	4.12±0.075*	6.05±0.6***	18.45±4.0	111.0±19.0	29.0±3.8
HMA, 1 μM	4.37±0.17	8.85±0.4***	62.71±4.9	81.5±2.2	39.9±1.0**
DIOA, 10 μM	7.14±0.15**	202.41±7.8***	134.58±3.95	12.3±1.6***	75.0±12.0**
IAA-94, 1.5 μM	7.41±0.24**	193.52±12.0***	187.05±15.02	12.6±2.5***	60.9±12.0**
HCO_3^-, 20 mM	4.88±0.26	7.37±0.14***	36.99±0.77	78.3±1.2*	41.1±0.6**
HCO_3^-, 20 mM+					
L-644,711, 0.8 μM	5.92±0.26*	33.48±1.40***	123.89±11.1	29.0±4.0	62.5±3.0**
Tris-HCl, 20 mM	5.50±0.18*	28.69±1.68***	113.97±7.9	53.0±6.0	65.0±3.0**

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the control.

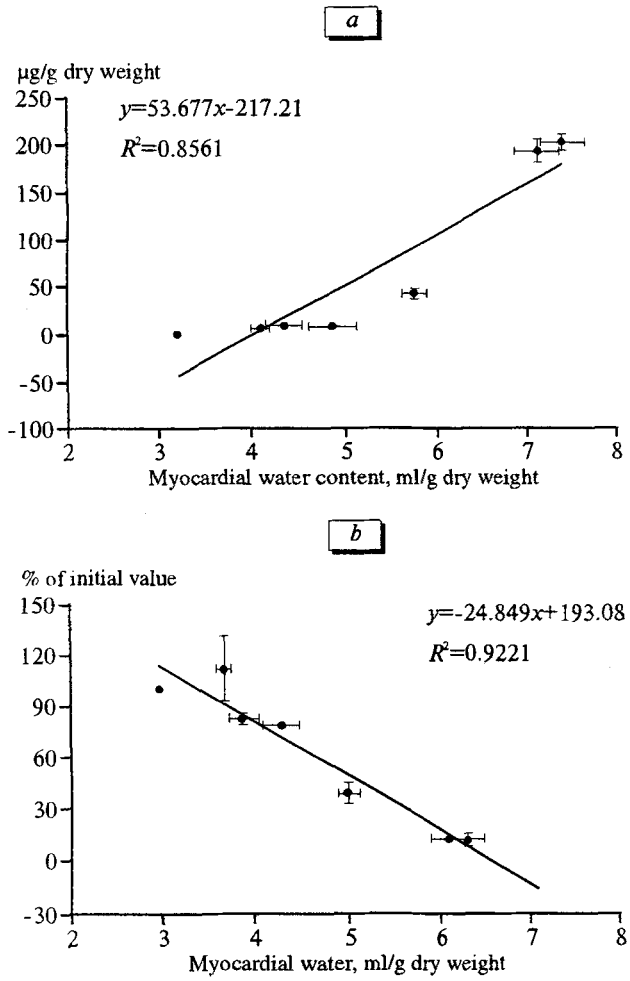

Fig. 1. Myoglobin release from ischemic heart (*a*) and recovery of developing pressure 30 min after ischemia (*b*) as a function of water content in the myocardium.

from the myocardium and considerably promotes recovery of MCA during reperfusion.

The low-rate perfusion of isolated heart followed by reperfusion with solutions containing DIOA and IAA-94 increased water accumulation in the myocardium and myoglobin release during ischemia. It also impaired MCA and elevated LVDP (Table 1).

Thus, the inhibition of the mechanisms that regulate the content of intracellular water (blockade of Cl^- channels with IAA-934 or K^+,2Cl^--symport by DIOA) in the ischemic heart aggravates damage to cardiomyocytes and impairs MCA recovery during reperfusion.

Activation of HCO_3^-/Cl^--antiport by $NaHCO_3^-$ (20 mM) had practically no effect on myocardial water content, but improved MCA during reperfusion. This may indicate not only activation of the anion-transporting mechanism, but also stabilization of intracellular pH due to an increase in the buffer capacity of the perfusion solution. Replacing $NaHCO_3$ by corresponding amount of Tris-HCl (at the same concentration) also improved MCA recovery, but to a lesser degree (Table 1). In addition, the HCO_3^-/Cl^--exchange inhibitor L-644,711 completely blocked the positive effect of $NaHCO_3$. This suggests the important role of HCO_3^-/Cl^- exchange in the maintenance of sarcolemmal integrity and MCA recovery in ischemic heart.

It should be noted that all studied agents can prevent myoglobin release from the heart not only during ischemia, but also during reperfusion period (Table 1).

We found a strong correlation between the cell water content and myoglobin release in ischemic heart (Fig. 1, *a*). Maintenance of a low (i.e. physiological) water content contributes to more efficient recovery of MCA during reperfusion, which is evidenced from the negative correlation between the myocardial water content and developed pressure after ischemia (Fig. 1, *b*).

Our data demonstrate the potency of the agents modulating the volume and anion transport in cardiomyocytes to change the state of ischemic heart. This phenomenon may serve as the basis for developing a new class of preparations, which could protect the heart from ischemic and reperfusion damage.

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