Heart Sensitivity to Electrolyte Composition of Perfusion Solution of Rats after Severe Brain Injury

V. V. Rusakov and V. T. Dolgikh

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 6, pp. 639-642, June, 2006
Original article submitted September 2, 2005

The *in vitro* model of isovolumic beating heart revealed enhanced sensitivity of the hearts from rats survived severe brain injury to concentration of Ca^{2+}, Na^{+}, and H^{+} ions in the perfusion solution. Inhibition of myocardial contractile function after trauma was accompanied by destruction signs in cardiomyocyte sarcolemma and mitochondrial dysfunction.

Key Words: *brain injury; heart; functional and metabolic disturbances*

The conception of primary and secondary lesions in the brain after brain injury (BI) is widely accepted. The secondary lesions are not directly related to the effect of mechanical impact on the brain, but rather reflect the reactions of the brain and the organism to the trauma and related phenomena caused by respiratory obstruction, disturbances caused by transportation of the patient, inadequate treatment, *etc*. The important pathogenic factors leading to secondary cerebral abnormalities are hemodynamics disturbances [1,4,8] caused by imbalance of autonomic nervous regulation during the acute post-traumatic period [2,3]. Our aim was to evaluate direct damage to the heart after severe BI. To this end, we studied function of isolated isovolumically contracting hearts from rats subjected to BI in perfusion solution of different electrolyte compositions.

MATERIALS AND METHODS

The experiments were carried out on random-bred male albino rats (n=85) weighing 160-250 g. Severe BI was inflicted with a load of certain weight freely falling onto the midline parietal region of the skull of ether-narcotized rats. One hour after BI, the heart was isolated, and its contractile function was examined on the model of isolated heart contracting in an isovolumetric mode [5]. The heart was perfused with Krebs—Henseleit solution saturated with carbogen at 37°C and containing (in mM): 120 NaCl, 4.8 KCl, 2.0 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20 $NaHCO_3$, 10 glucose, pH 7.4. We recorded diastolic and systolic pressure in the left ventricle, and the maximum contraction and relaxation rates of the left-ventricular myocardium. Heart sensitivity to electrolyte composition of the perfusate was examined using low-Ca^{2+}, high-Ca^{2+}, high-Na^{+}, and acidic tests. The concentrations of glucose and lactate and AST activity (Hospitex reagents) in the perfusate passed via the coronary bed was measured on a Mars Automatic Biochemical Analyzer. The measurements were made after a 30-min stabilization period, after termination of each electrolyte test, and after the recovery period. Glucose consumption by 1 g dry myocardium over 1 min, release of lactate, and the loss of AST by cardiomyocytes were calculated per 1 mm Hg developed pressure. The data were processed statistically using Student's t test.

RESULTS

Assessment of contractile function of the isolated hearts 30 min after the stabilization period (Table 1) revealed decreased relaxation rate of the left

Department of Pathophysiology with Clinical Pathophysiology Course, Omsk State Medical Academy. ***Address for correspondence:*** vvr@omsk-osma.ru. V. V. Rusakov

0007-4888/06/1416-0691

TABLE 1. Effect of BI on Dynamics of Pressure and Rate Indices of Isolated Rat Hearts Measured during High-Ca^{2+} Test and Recovery Period (*M*±*m*)

Index, experiment		Initial values	High-Ca^{2+} test			Recovery period		
			30 sec	7 min	10 min	30 sec	7 min	20 min
Diastolic pressure, mm Hg	control	2.40±0.18	3.60±0.46^{+}	16.60±2.67^{+}	27.50±2.93^{+}	31.20±3.27^{+}	24.30±3.22^{+}	18.5±3.3^{+}
	BI	2.30±0.32	2.80±0.37	36.80±7.38^{+*}	45.40±7.33^{+*}	47.5±6.8^{+*}	43.20±6.57^{+*}	38.00±5.68^{+*}
Systolic pressure, mm Hg	control	51.80±3.84	59.80±5.12	43.50±3.06	43.40±2.87	38.80±2.04^{+}	41.90±2.97	40.10±2.88^{+}
	BI	50.30±3.14	67.00±3.71^{+}	56.7±4.1*	58.70±3.31*	53.70±4.38*	54.50±3.36*	53.40±3.25*
Developed pressure, mm Hg	control	49.90±3.01	56.20±4.22	26.90±2.03^{+}	15.90±1.47^{+}	7.50±0.69^{+}	17.60±1.54^{+}	21.50±1.81^{+}
	BI	48.00±3.32	64.20±4.14^{+}	19.90±1.62^{+*}	13.30±1.27^{+}	6.3±0.5^{+}	11.40±1.04^{+*}	15.40±1.43^{+*}
Contraction rate, mm Hg/sec	control	1081.0±97.2	1280.0±118.7	586.0±49.6^{+}	328.0±22.7^{+}	188.0±15.2^{+}	332.0±28.4^{+}	419.0±36.7^{+}
	BI	1054±77	1414.0±99.2^{+}	417.0±30.7^{+*}	257.0±17.6^{+*}	134.0±13.3^{+*}	212.0±16.3^{+*}	302.0±28.6^{+*}
Relaxation rate, mm Hg/sec	control	879±69	921.0±82.6	391.0±36.2^{+}	235.0±21.8^{+}	101.0±9.0^{+}	179.0±16.5^{+}	251.0±20.2^{+}
	BI	709.0±37.4*	1080.0±98.2^{+}	279.0±23.2^{+*}	160.0±14.6^{+*}	67.0±6.6^{+*}	122.0±11.2^{+*}	179.0±15.5^{+*}

Note. $p<0.05$ compared to $^{+}$initial and *control values.

ventricular myocardium in rats subjected to BI (709.0± 37.4 *vs*. 879.0±69.0 mm Hg/sec in the control, $p<0.05$). At the same time, the efficiency of substrate utilization by the myocardium decreased by 36.4% ($p<0.05$) as attested by the growth of glucose consumption per 1 mm Hg developed pressure. Cardiomyocyte membrane permeability also increased, which manifested in 43.1% ($p<0.05$) increase in AST activity in the perfusate passed via the coronary bed.

The decrease in Ca^{2+} concentration to 1.25 mM during low-Ca^{2+} test sharply impaired pumping function of the left ventricle even in intact rats. After 30 sec, the left ventricular pressure and the myocardium contraction and relaxation rates decreased by 2.5, 2.4, and 3.7 times, respectively ($p<0.001$), while the diastolic pressure increased by 2.1 times ($p<0.001$). After termination of the electrolyte test, the recovery period was characterized by a rapid reverse dynamics. After 10 min, the parameters of cardiac contractility did not differ from the initial values. The subsequent high-Ca^{2+} perfusion produced a short-term increase in myocardial contractility followed by a pronounced drop of pumping function (Table 1). After 10 min, the developed pressure and myocardial contraction and relaxation rates of the left ventricle decreased to 31.9, 30.3, and 26.7% of the initial values, respectively ($p<0.001$), against the background of diastolic pressure rise. The following 20-min reperfusion with Krebs—Henseleit solution did not normalize the indices of cardiac contractility.

In the experimental hearts (isolated from the rats subjected to BI), restoration of Ca^{2+} concentration in the perfusion solution after low-Ca^{2+} test induced a more pronounced response: the developed pressure increased by 36.6% (p<0.02), while the rate of myocardial contraction and relaxation increased by 24.0% ($p<0.05$) and 33.5% ($p<0.02$) compared to the control values. The subsequent high-Ca^{2+} perfusion induced a more pronounced increase of the diastolic pressure in the experimental group: on perfusion minute 10 it surpassed the control value by 65% ($p<0.05$). This increase was combined with a more pronounced decrease in myocardial force and, especially, rate indices of the left ventricle, where the rates of contraction and relaxation were below the control values by 21.6% ($p<0.05$) and 31.9% ($p<0.02$), respectively. During 20-min recovery period, the indices of cardiac pumping function of experimental hearts were below the control values. At the end of this period, the left ventricular pressure and contraction and relaxation rates were below the control by 28.4% ($p<0.02$), 27.9% ($p<0.05$), and 28.7% ($p<0.02$), respectively.

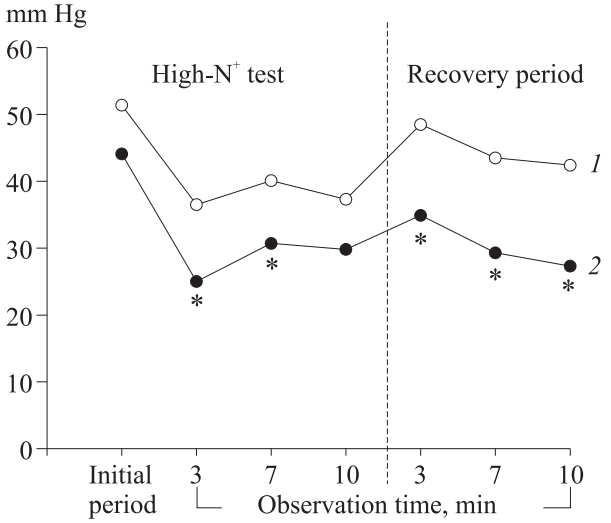

Fig. 1. Effect of brain injury (BI) on the dynamics of pressure developed by the left ventricle during high-Na^+ test. Here and in Fig. 2: *1*) control; *2*) BI. **p*<0.05 compared to the control.

In this (experimental) group the diastolic pressure remained elevated by 2.1 times (p<0.02), which reflected more intensive contracture formation after BI.

Biochemical analysis of the perfusate passed via the coronary bed of experimental rats revealed more pronounced changes during the recovery period after low- and high-Ca^{2+} perfusion compared to those observed immediately after the termination of these tests. After 10- and 20-min recovery periods, the hearts of experimental rats consumed more glucose (by 49.7%, $p<0.02$ and by 35.3%, $p<0.05$) and released more lactate (by 57.1%, $p<0.001$ and by 64.0%, $p<0.001$, respectively) per 1 mm Hg developed pressure than control hearts. This can be explained by more pronounced disturbances in Ca^{2+} balance in cardiomyocytes during the tests, because the increase in intracellular Ca^{2+} leads to intensive uptake of these ions by mitochondria and block of oxidative phosphorylation. This can decrease the efficiency of substrate utilization by the heart. At the end of the recovery period after high-Ca^{2+} perfusion, the hearts of experimental rats released more AST (by 41.4%, $p<0.05$) than control hearts. Probably, pronounced activation of solubilized and membrane-bound proteases, lipases, and phosphorylases triggered by Ca^{2+} overload in cardiomyocytes [7] of experimental rats intensified "delipidization" and "deproteinization" of cardiomyocyte membranes [6], which increased their permeability.

The increase in Na^+ concentration in the perfusion solution produced a greater (compared to control hearts) drop of developed pressure on minutes 3 (by 31.5%, $p<0.02$) and 7 (by 23.4%, $p<0.05$) of testing (Fig. 1). Changes in the rate parameters were more pronounced: the relaxation rate of the left ventricular myocardium was below the control at all stages of the experiment. During recovery after the electrolyte test, changes in the dynamics of developed pressure were most persistent: on recovery minutes 1 and 10 it was 74.8% ($p<0.05$) and 64.4% ($p<0.02$) of the control value. Lactate concentration in the perfusate passed via the coronary bed of experimental hearts was elevated during the entire experiment. During recovery, the glucose consumption and AST release increased by 34.5% ($p<0.05$) and 39.0% ($p<0.05$), respectively.

The decrease of perfusate pH attests to more pronounced depression of contractility of experimental hearts at the start of testing and at the end of the recovery period, when the developed pressure and contraction and relaxation rates of the left ventricle were below the control values by 22.6, 26.3, and 26.6% ($p<0.05$), respectively. The acidic perfusion more severely affected heart metabolism in experimental rats, which manifested in increased glucose consumption during testing and recovery period (by 31.1 and 54.4%, respectively, $p<0.02$), increased lactate release (by 43.4 and 50.9%, $p<0.05$), and more pronounced enzyme loss in experimental hearts compared to the control (Fig. 2).

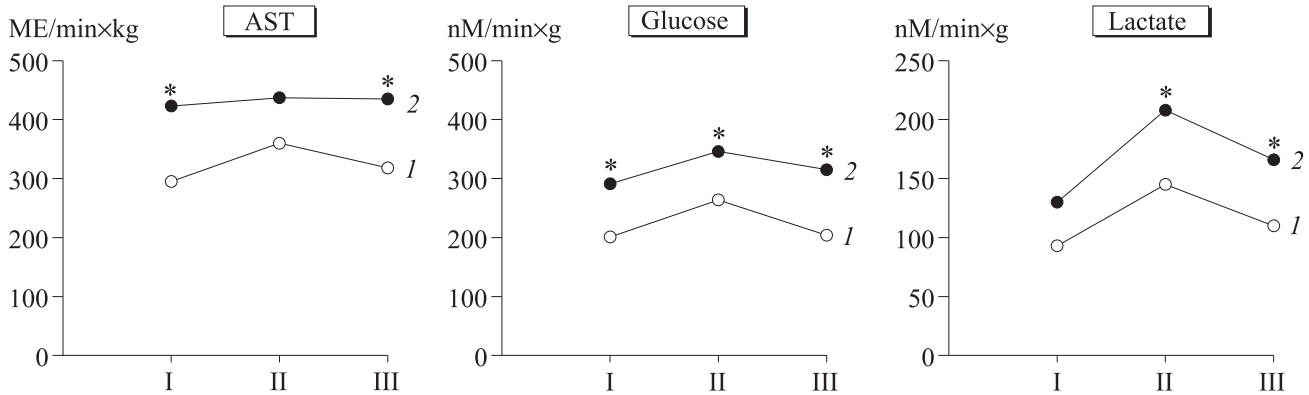

Fig. 2. Effect of BI on glucose consumption and release of lactate and AST by rat hearts during acidic test. *I*: 30-min stabilization period of the heart work; *II*: 15-min acidic perfusion; *III*: 15-min recovery period.

Thus, enhanced sensitivity to electrolyte composition of the perfusion solution was revealed in hearts of traumatized rats by low-Ca^{2+}, high-Ca^{2+}, high-Na^{+}, and acidic tests despite some differences in the dynamics of pressure and rate indices of myocardial contractility accompanied by differences in biochemical shifts detected in perfusion solution during testing. This enhanced sensitivity was more pronounced in changes of the rate indices, especially during the recovery period after the tests. Inhibition of myocardial contractility was accompanied by signs of destruction of cardiomyocytic sarcolemma and mitochondrial dysfunction.

REFERENCES

1. I. V. Bratishchev, *Vestn. Intens. Terap.,* No. 4, 17-21 (2003).
2. I. V. Molchanov, *Anest. Reanimatol.*, No. 3, 12-17 (2002).
3. D. V. Sadchikov, V. N. Kolesov, and A. G. Lezhnev, *Anest. Reanimatol.,* No. 2, 49-51 (2003).
4. M. Carrel, O. Moescher, P. Ravussin, *et al., Ann. Fr. Anesth. Reanim*., **13**, 326-335 (1994).
5. E. T. Fallen, W. G. Elliott, and R. Gorlin, *J. Appl. Physiol*., No. 22, 836-839 (1967).
6. P. Libby, *Circulation,* **94***,* 2844-2860 (1995).
7. H. Massaeri and G. N. Pierce, *Cardiovasc. Res.,* **29,** No. 5, 597-603 (1995).
8. J. R. M. White, Z. Farukhi, C. Bull, *et al., Crit. Care Med.,* **29**, No. 3, 534-540 (2001).