Cardioprotective Effect of Histidine-Containing Dipeptides in Pharmacological Cold Cardioplegia

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The cardioprotective effect of cardioplegic solution based on histidine-containing dipeptides was evaluated on isolated rat heart under conditions of hyporthermia and long ischemia. The use of natural dipeptides in cardioplegic solutions promoted an increase in the buffer capacity of myocardial cells and creation of an additional anti-ischemic effect under conditions of long ischemia and hypothermia.

Key Words: *cardiosurgery; cardioplegic solution; histidine-containing dipeptides; carnosine; acetylacarnosine*

The main drawback of crystalloid cardioplegic solutions (CPS) in comparison with blood-based solutions is the absence of natural buffer systems for neutralization of acid metabolites forming in myocardial ischemia [4,5]. At present, special attention is paid to the possibility of using natural dipeptides in cardioplegia, for example, carnosine, consisting of L-histidine β-alanine, and its acetylated derivative acetylcarnosine possessing the characteristics of natural buffer, antioxidant, and membrane-protective effects [3]. Total content of histidine-containing dipeptides in the myocardium is about 10 mM; they are mainly presented by acetylated derivatives [1,3]. Carnosine and acetylcarnosine (natural components of cardiac muscles) function as cell antioxidants preventing myoglobin and adenine nucleoside release from cardiomyocytes [1,2,8].

We evaluated the possibility of using histidine-containing dipeptides (carnosine and its derivatives) in CPS and compared the cardioprotective effects of the most prevalent CPS.

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MATERIALS AND METHODS

Three series of experiments were carried out (a total of 60 experiments). In series I, cardioplegia was realized with St. Thomas Hospital potassium solution, in series II with custodiol (Franz Kehler Chemie GmbH), and in series III with experimental ACH solution (acetylcarnosine-carnosine-histidine; Table 1). Experiments were carried out on 5-6-month-old Wistar rats (300-350 g). The heart was fixed on a cannula of the isolated heart device. Perfusion was carried out by the Langendorff' method with Krebs—Henseleit solution at 37°C [6,7], which was followed by a 15-min period of adaptation to antegrade perfusion. Perfusion was then stopped and CPS (12-14°C) was infused for 5 min at a rate of 15-20 droplet/min. The heart in medium with Krebs—Henseleit solution was placed into a chamber for 480 min at 8°C for simulation of total ischemia.

Heart activity was evaluated by continuous registration of pressure curve in the left ventricle. To this end, a polyethylene catheter (0.75 mm) filled with fluid and connected to a pressure pickup was inserted into left-ventricular cavity through the aorta. Registration was carried out with the analog signal

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discretization frequency of 0.2 kHz, due to which the left-ventricular pressure curve was recorded sufficiently accurately. Systolic pressure (SAP), end diastolic pressure (EDP), AP amplitude (difference between SAP and EDP), and heart rate were calculated. In addition, maximum rate of pulse wave front-line in the left ventricle (+dp/dtmax), maximum rate of pulse wave fading (-dp/dtmax), and contractility were evaluated. After calculation of the parameters, analysis was carried out for each cardiac cycle using Pulsar software. Heart rates, SAP, EDP, SAP/EDP ±dp/dtmax, and contractility for each minute of the record were calculated as the medians. As parameters of functioning of isolated hearts varied from one experiment to another, the changes in each parameter after recovery were expressed in percent of the initial value of the parameter: $A_2/A_1 \times 100\%$, where A_1 is the initial value and A_2 the value after cardioplegia (during reperfusion) for a certain interval. Analysis included comparison of the initial values of isolated heart work with the parameters of cardiac activity during minutes 15, 30, and 45 of reperfusion in all three series. The mean amplitude before CPS was 78 mm Hg, heart rate 260 bpm, front-line rate 1580 mm Hg, and contractility 20,280 mm Hg/min. The significance of differences between them was evaluated using the Mann—Whitney test (Statistica 6.0).

The following biochemical parameters of myocardial protection adequacy were studied: pH, Pco_2, Po_2, and lactate level in perfusate flowing from the right heart. The studies were carried out on an ABL 800 FLEX gas analyzer (Radiometer Medical ApS). The results were processed by the method of variation statistics using Student's *t* test, evaluation of the means and standard deviation in the Biostat software.

Histochemical staining of the myocardium was carried out with hematoxylin and eosin, by Van Gieson's method, with oil red O for detection of triglycerides; SDH activity was detected by Nachlas' method.

RESULTS

Infusion of custodiol and ACH CPS by the method described previously [7] arrested cardiac activity after 2.5-3 min, while St. Thomas Hospital hyperpotassium CPS arrested cardiac activity by the 4-th min of infusion. In series I, spontaneous recovery of heart work during minutes 6-7 of reperfusion was observed only in 2 cases. In 18 cases, contractions could be restored only by electrical stimulation. Low amplitude of contractions in parallel with EDP increase (28-32 mm Hg) was noted in all cases. The pulse wave front-line rate reached the maximum by min 15 of reperfusion and was 65-70% of its values in series II and III. Later the parameters in series I tended to decrease and constituted 50% of values in two other series, heart rate also decreased sharply, constituting 50% of the values in series II and III. Contractility was about 47% of the initial values. The ejection was realized mainly at the expense of extrasystole amplitude and rate.

In series II, heart work was restored 5 min after the start of normothermal reperfusion. The recovery was spontaneous in 12 of 20 preparations of this group, with a satisfactory amplitude, without arrhythmia. Electrical stimulation was used in 8 preparations (because of ventricular fibrillation). The maximum values of all heart work parameters in this group were restored only after 44 min of reperfusion. Electrogram showed dilatation of the complexes with the appearance of a compensatory extrasystole. Heart rats gradually increased during reperfusion and by minute 45 reached 93% of the initial value. The mean EDP in this series was no higher than 18-22 mm Hg.

In series III, spontaneous recovery of cardiac activity was observed in 13 preparations after 3-4 min of reperfusion, electrical stimulation was used in only 7 cases. Regular rhythm with the minimum conduction disorders was observed in all cases (the distance between the complexes, evaluated by minute 45, was 96% of the initial values). No conduction disorders were observed in the complexes (from the beginning of wave front-line rate till the end of pulse wave fading rate). The maximum rate of pulse wave front-line was recorded by minute 45 of reperfusion (85-90% of initial value). Solitary extrasystoles were detected only in the preparations in which cardiac activity had to be electrically stimulated for recovery.

A significant increase in oxygen consumption was noted in biochemical samples collected after 15 min of reperfusion, the maximum Po_2 values being observed in experimental series III (ACH solution). Reperfusion with ACH solution was associated with a significant reduction of lactate content. Analysis of the samples from the custodiol series showed an increase of oxygen consumption in comparison with that in the preparations perfused with St. Thomas Hospital solution, while the use of custodiol led to a significant reduction of oxygen consumption in comparison with that after perfusion with ACH solution. Comparative study of lactate content in the solution flowing from perfused preparation showed the most adequate values in experimental series III (ACH solution). Hence, aerobic oxidation associated with higher oxygen consumption and minimum content of lactate predominates during reperfusion wite ACH solution in comparison with

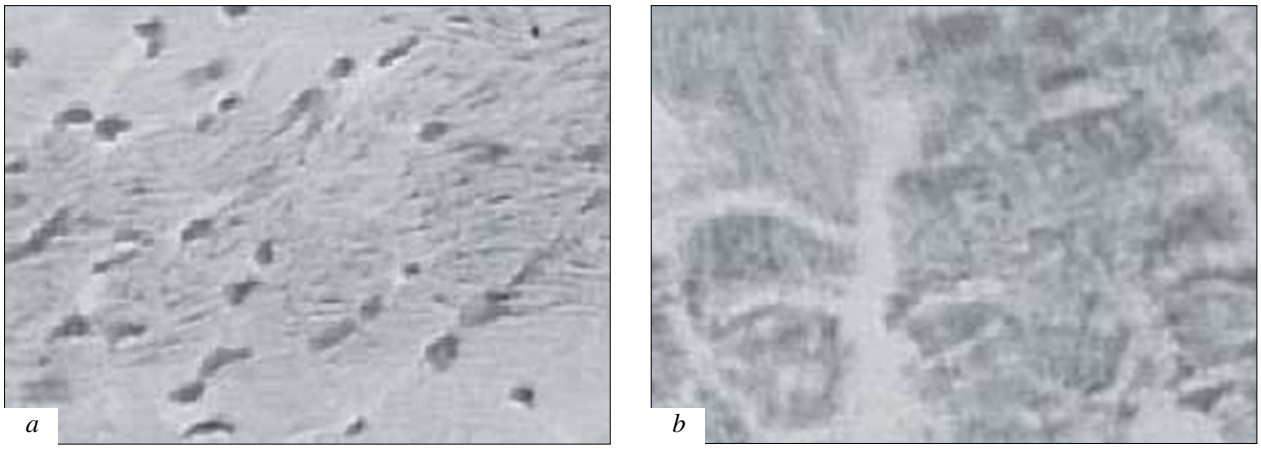

Fig. 1. Myocardium specimen: experimental series I. *a*) foci of fatty degeneration; *b*) multiple contractures, sharply reduced SDH activity.

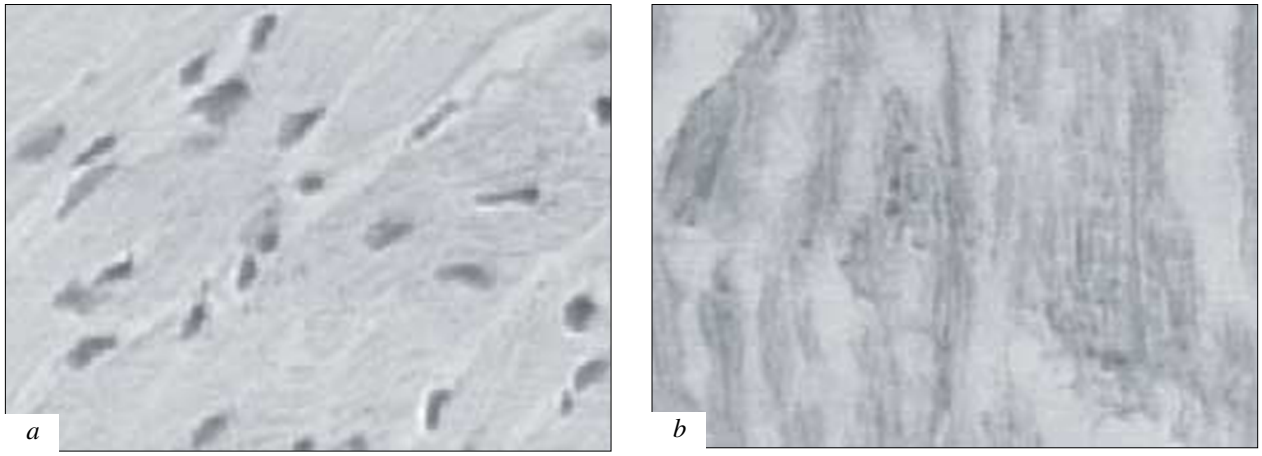

Fig. 2. Myocardium specimen: experimental series II. *a*) diffuse pulverized fatty degeneration; *b*) solitary contractures, moderately reduced SDH activity.

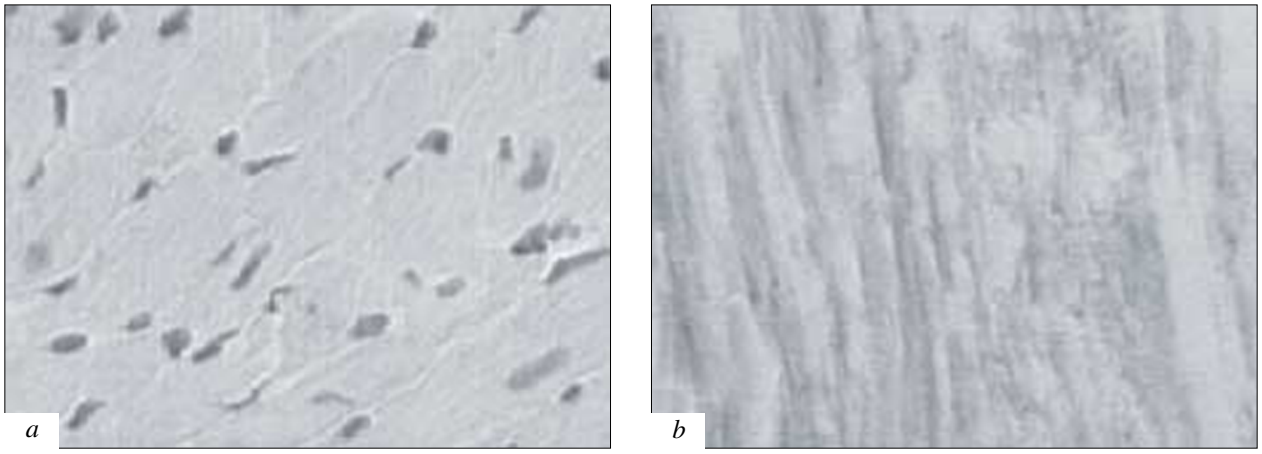

Fig. 3. Myocardium specimen: experimental series III. *a*) no injuries; *b*) no injuries, normal SDH activity.

those during perfusion with other solutions ($p<0.05$). It is noteworthy that after 45 min of reperfusion with ACH solution, oxygen debt was absent and lactate level was minimum ($p<0.05$; Table 2). The pH and osmolarity values corresponded to normal during CPS perfusion (Table 1).

More adequate recovery of cardiac activity during perfusion with ACH solution promoted norma-

TABLE 1. Components of CPS

Solution component, mmol/liter	Custodiol	St. Thomas Hospital solution	ACH solution
Sodium	15	110	110
Potassium	10	15	15
Calcium	0.02	0.09	0.03
Magnesium	4	16	16
Mannitol	30	104	25
Histidine	180/18	—	5
Carnosine	—	—	100
Acetylcarnosine	—	—	40
Glucose	—	5.6	—
Ketoglutarate	1	—	—
Tryptophane	2	—	—
$NaHCO_3$	—	24	—
pH	7.02-7.2	7.7-7.8	7.2-7.4
Osmolarity, mosm/liter	310	340-345	320

lization of capillary bloodflow, which was directly linked with normalization of the redox processes in the myocardium and predominance of aerobic oxidation (judging from lactate level). In experimental series III, the content of lactate in the solution flowing from perfused preparation was minimum. In addition, this series was characterized by more effective recovery of heart work with minimum disorders in the rhythm and conduction. End diastolic pressure as a rule did not surpass 10 mm Hg, while maximum rate of pulse wave front-line, amplitude, and heart rate were higher than in series I and II, when arrhythmias and conduction disorders were observed.

Histochemical studies of heart tissues in specimens from series I showed diffuse dust-like fatty degeneration in all preparations. Foci of small droplet fatty degeneration were detected in 6 preparations (Fig. 1, *a*). In series II, multiple contracture injuries were detected in all samples: diffuse in subepicardial compartments of the myocardium and focal in intramural and subendocardial compartments of left-ventricular myocardium (Fig. 2, *b*). Diffuse dust-like fatty degeneration was present in all compartments of the left ventricle; foci of small droplet fatty degeneration were detected in 2 preparations (Fig. 2, *a*). In series III, just minor contracture injuries in the subepicardial compartment were detected in 13 of 20 preparations (Fig. 3, *b*) and a focus of small droplet fatty degeneration was detected in only 1 preparation (Fig. 3, *a*).

The predominance of aerobic oxidation presumably had a positive effect on the metabolic processes in cardiomyocytes, as a result of which no foci of fatty degeneration formed in experimental series III. Myocardial contracture injuries were less pronounced in comparison with those in series I and II, in which irreversible changes of the myocardium (contracture injuries in all myocardial compartments with foci of small droplet fatty degeneration) developed.

Comparative study of various methods for myocardial protection showed that the use of ACH solution was the optimal method. Its use during the

TABLE 2. Biochemical Criteria of the Rat Myocardium Protection ($M \pm m$)

Duration of reperfusion, min	Parameter		St. Thomas hospital solution	ACH solution	p
	CPS	custodiol			
15	Po_2, mm Hg	9.00±1.15	2.00±0.94	26.00±1.41	<0.0001
	Lactate, mmol/liter	0.30±0.04	0.370±0.016	0.15±0.015	
45	Po_2, mm Hg	11.00±1.76	7.00±1.76	3.00±1.67	<0.05
	Lactate, mmol/liter	0.15±0.03	0.25±0.03	0.05±0.02	

postischemic period was associated with predominance of aerobic oxidation, with the minimum oxygen debt and lactate levels. The results of histochemical studies of the myocardium also confirm its least injuries in this case.

REFERENCES

1. V. V. Alabovskii, A. A. Boldyrev, A. A. Vinokurov, *et al.*, *Byull. Eksp. Biol. Med.*, **127**, No. 3, 290-294 (1999).
2. V. V. Alabovskii, A. A. Boldyrev, A. A. Vinokurov, and V. Kh. Shavratskii, *Biokhimiya*, **62**, No. 1, 91-102 (1997).
3. A. A. Boldyrev, *Functional Activity of Histidine-Containing Dipeptides. Carnosine* [in Russian], Ed. T. M. Turpaev, Moscow (1998), pp. 83-110.
4. A. I. Malashenkov, *Lectures in Cardiovascular Surgery* [in Russian], Ed. L. A. Bokeriya, Vol. II, Moscow (2001), pp. 185-199.
5. R. R. Movsesyan, *Ibid.*, Vol. I, pp. 203-217.
6. G. Dunphy, H. W. Richter, M. Azodi, *et al.*, *Am. J. Physiol.*, **276**, No. 5, Pt. 2, H1591-H1598 (1999).
7. C. Schroder, A. Heintz, A. Pexa, *et al.*, *Eur. J. Cardiothorac. Surg.*, **31**, No. 5, 821-826 (2007).
8. K. Nagai, A. Niijima, T. Yamano, *et al.*, *Exp. Biol. Med.*, **228**, No. 10, 1138-1145 (2003).