Comparison of Protective Effects of Carnosine and Acetylcarnosine during Cardioplegia

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Bioactive peptide carnosine (β-alanyl-L-histidine) and its acetylated derivative added to cardioplegic solution in physiological concentrations (2-10 mM) promote complete recovery of the contractile function of rat heart after cardioplegia. Carnosine prevented the release of myoglobin, while acetylcarnosine prevented the loss of both myoglobin and nucleotides. These data attest to membrane-protective effects of these compounds.

Key Words: *carnosine; acetylcarnosine; cardiac ischemia; cardioplegy*

At present, cardiac arrest with cardioplegic solutions containing high concentrations of K^+ and/or Mg^{2+} is widely used in cardiac surgery. However, cardiac contractility is often impaired during reperfusion period because of inadequacy of the anti-ischemic protection [8]. More than 50 different compositions of cardioplegic solutions have been proposed, but all of them remain far form perfect [3,4,8]. Recent attention has been focused on histidine-containing peptides, hydrophilic cell antioxidants possessing membrane-stabilizing and antioxidant properties [2]. Despite similar physicochemical properties of carnosine and acetylcarnosine (AC) their distribution in the organism is tissue specific: carnosine is predominantly localized in skeletal muscles (10-40 mM), while in the myocardium almost all carnosine is acetylated on its animo group. The total concentration of histidine-containing peptides in mammalian heart is about 10 mM [9]. The mechanism of this unequal distribution remains unclear. Both peptides exhibit a protective effect in subtotal myocardial ischemia [1]. In light of this we compared the effects of these compounds on ischemic heart using a cardioplegic solution (CPS).

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MATERIALS AND METHODS

Experiments were carried out on Langendorf-perfused hearts from albino random-bred rats. The rats were decapitated under ether narcosis, the chest was opened, and the heart was removed and placed into cool perfusion solution containing (in mM): 140 NaCl, 0.5 NaH_2PO_4, 5 KCl, 5 Trizma Base (pH 7.4), 11 glucose, and 2 $CaCl_2$. The aorta was cannulated and the heart was perfused with warm (37°C) oxygenated solution at a rate of 10 ml/min/g wet tissue.

In all experiments, the hearts were perfused for 15 min with the initial solution until stabilization of the contractile function and parameters of heart function at the end of this stabilization period were taken as 100%. Cardiac arrest was induced by 2-min perfusion with CPS containing (in mM): 110 NaCl, 16 KCl, 20 $NaHCO_3$ (pH 7.8), 16 $MgCl_2$, and 1.2 $CaCl_2$ and the coronary flow was stopped for 30 min for total ischemia modeling. Test agents were added to CPS in concentrations from 2 to 10 mM corresponding to their concentrations in native heart [2,9]. Reperfusion was performed with the initial solution. Temperature was maintained at 37°C throughout the experiment. In special experimental series hypothermic conditions were created. To this end CPS was cooled to 21±2°C before perfusion. When CPS containing 10 mM AC was used, 3-min perfusion was required for cardiac arrest (in

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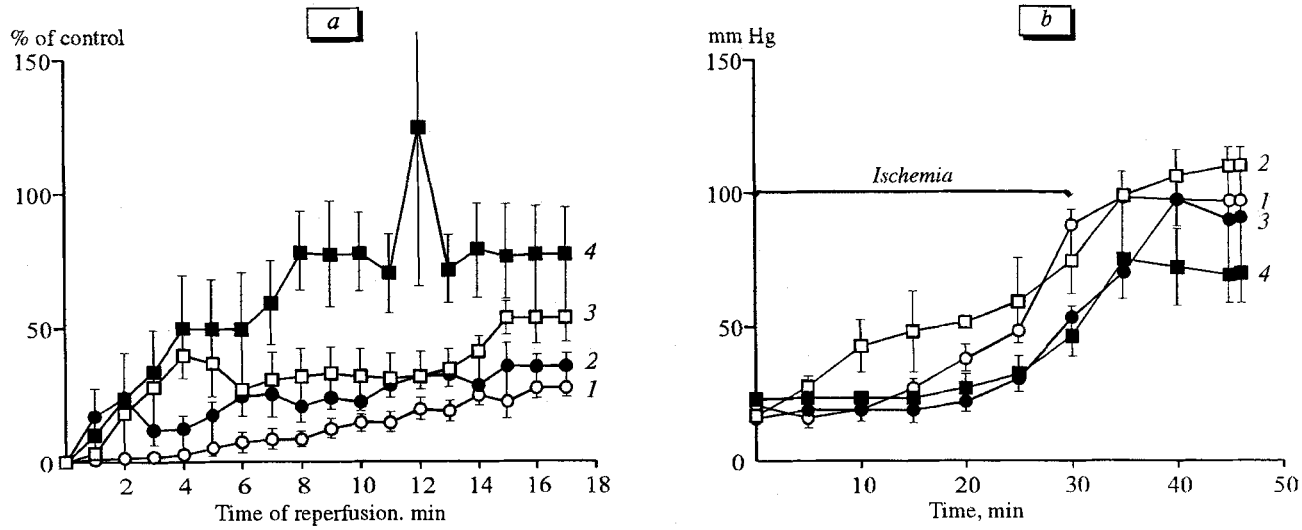

Fig. 1. Effect of acetylcarnosine and carnosine on recovery of myocardial contractility (*a*) and diastolic pressure (*b*) after normothermic cardioplegia. *1*) control; acetylcarnosine: 2 (*2*), 5 (*3*), and 10 mM (*4*).

comparison with 2-min perfusion in other cases). Reperfusion was performed at 37±0.1°C.

Cardiac contractility was assessed under isovolumic conditions with a latex balloon introduced into the left ventricle. The left ventricular developed pressure was measured as the difference between systolic and diastolic pressures. Index of contractility was calculated as the product of developed pressure and heart rate. A Bentley Lab. Europe electrical manometer, analog-to-digital converter for IBM PC (constructed by A. I. Zharkikh) and special software (created by A. I. Glotov) were used.

The degree of cardiomyocyte damage was assessed by the release of myoglobin and nucleosides (adenosine+hypoxanthine+xanthine) into perfusion solution during 12 min. The concentrations of myoglobin and nucleosides were measured as described previously [1]. The data were processed statistically using ANOVA and Student *t* test.

RESULTS

Normothermic cardioplegia led to cardiac arrest after 2-min perfusion with CPS. The left ventricle diastolic pressure increased during ischemia and ischemic contracture developed (Fig. 1). During subsequent reperfusion with the initial Ringer—Locke solution we observed further increase in diastolic pressure and only partial recovery of the contractile function accompanied by the release of myoglobin and adenine nucleosides to extracellular medium (Table 1).

Addition of 5 or 10 mM AC promoted the recovery of myocardial contractility in a dose-dependent manner. Under these conditions the contractile function and left ventricular developed pressure during reperfusion returned to the baseline levels (10 mM AC), while the increase in diastolic pressure during the ischemia period was considerably less pronounced. At the same time, AC added during ischemia pre-

TABLE 1. Effect of AC (10 mM) added to CPS (37°C) on Left Ventricular Developed Pressure, Index of Cardiac Contractility, and Myoglobin and Nucleoside Release from Cardiomyocytes during Reperfusion (*M*±*m*)

Concentration of agents, mM		Index of cardiac contractility	Developed pressure	Diastolic pressure on reperfusion minute 15, mm Hg	Release during reperfusion	
		reperfusion minute 15, % of preischemic level			nucleosides, mmol/g dry tissue	myoglobin, μg/g dry tissue
Control		22.3±4.6	22.6±4.2	97.0±7.2	1.19±0.06	244.04±23.25
AC,	10	76.2±19.6**	127.8±17.9**	69.9±15.3	1.02±0.09	241.64ë9.7
	5	41.1±6.1*	72.1±9.1**	89.8±6.2	1.01±0.11*	175.08±6.71**
	2	27.3±5.2	41.2±9.2	110.3±8.9	0.81±0.17	316.43±79.51
Carnosine,	10	35.6±10.0	28.7±7.6	115.4±10.4	1.02±0.17	177.09±2.49*

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$ compared with the control.

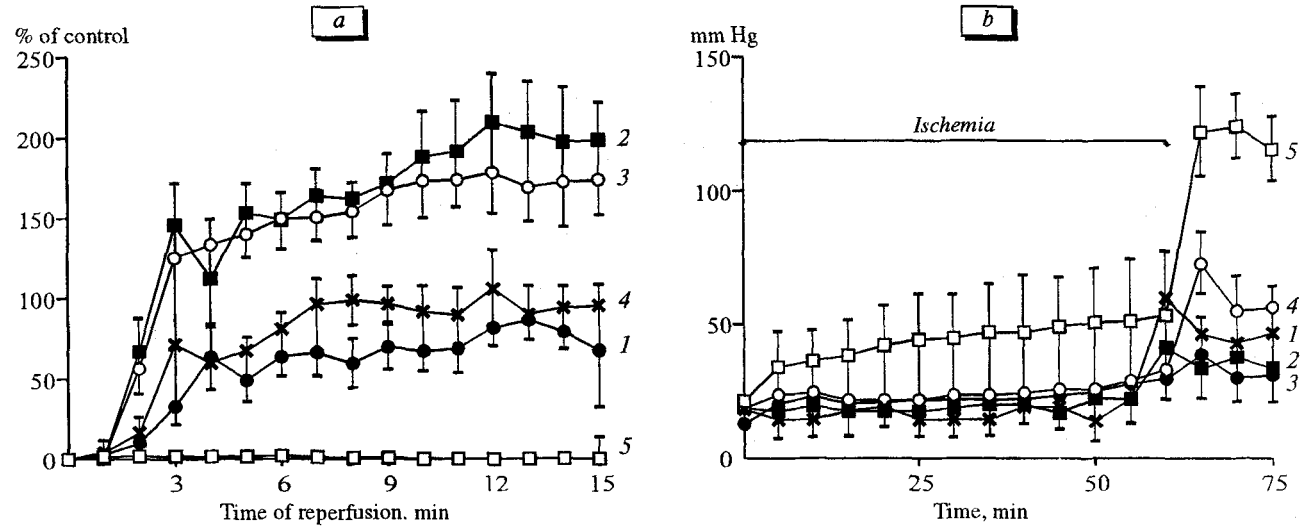

Fig. 2. Effect of acetylcarnosine and carnosine on myocardial contractility (*a*) and diastolic pressure (*b*) under conditions of hypothermic cardioplegia. *1*) control; acetylcarnosine: 5 (*2*) and 10 mM (*3*), carnosine 5 (*4*) and 10 mM (*5*).

vented the loss of myoglobin and adenine nucleotides during subsequent reperfusion. Low concentration of AC (2 mM) had no protective effect.

Addition of carnosine (10 mM) to CPS accelerated the development of ischemic myocardial contracture in comparison with the control. Diastolic pressure in the left ventricle during both ischemia and reperfusion considerably surpassed the control values, while the index of heart contractile function only slightly increased during reperfusion (Fig. 2). Carnosine reduced the loss of myoglobin but not adenine nucleosides.

In contrast to normothermic cardioplegia, diastolic pressure did not increased during hypothermic ischemia. During subsequent reperfusion with Ringer—Locke solution the left ventricular developed pressure returned to the control value, while the index of cardiac contractility recovered by 60-70% (Fig. 2, Table 2). This was accompanied by the release of adenine nucleosides and myoglobin from cardiomyocytes.

Acetylcarnosine (5 mM) added to CPS considerably reduced the loss of myoglobin and adenine nucleosides from cardiomyocytes and inhibited the development of reperfusion myocardial contracture. Left ventricular developed pressure and the index of cardiac contractile function were fully restored. A high concentration of AC (10 mM) had no protective effect (Fig. 2, Table 2).

Unlike AC, carnosine (5 mM) had no effect on the contractile function and diastolic pressure under conditions of hypothermic cardioplegia and reperfusion and did not prevent the release of myoglobin and nucleosides from cardiomyocytes, while in a concentration of 10 mM it even impaired functional state of the heart during both cardioplegia and reperfusion and aggravated ischemic and reperfusion myocardial contracture. Solitary heart contractions were observed during reperfusion, but to the end of the experiment the index of cardiac contractility did not exceed 5%

TABLE 2. Effect of AC (10 mM) added to CPS on Left Ventricular Developed Pressure, Index of Cardiac Contractility, and Myoglobin and Nucleoside Release from Cardiomyocytes during Reperfusion after Hypothermic Cardioplegia (21°C, *M*±*m*)

Concentration of agents, mM		Index of cardiac contractility	Developed pressure	Diastolic pressure on reperfusion minute 15, mm Hg	Release during reperfusion	
		reperfusion minute 15, % of preischemic level			nucleosides, mmol/g dry tissue	myoglobin, μg/g dry tissue
Control		67.4±9.7	90.3±17.7	56.6±4.4	0.87±0.04	109.39±4.68
Carnosine,	5	92.12±7.7	82.29±4.72	47.00±12.2	0.88±0.02	113.28±5.4
	10	0.1±0.1**	0**	115.7±12.2*	1.04±0.09*	194.74±7.65**
AC,	5	198.58±37.7*	113.97±13.7	33.79±12.8	0.37±0.1**	37.40±7.60**
	10	172.5±26.9**	118.3±15.2	31.2±10.3*	0.37±0.1*	37.38±7.62**
Carnosine,	10					
+acetate,	10	15.9±6.5**	11.4±4.7**	130.5±20.4*	0.75±0.14	35.09±1.63**

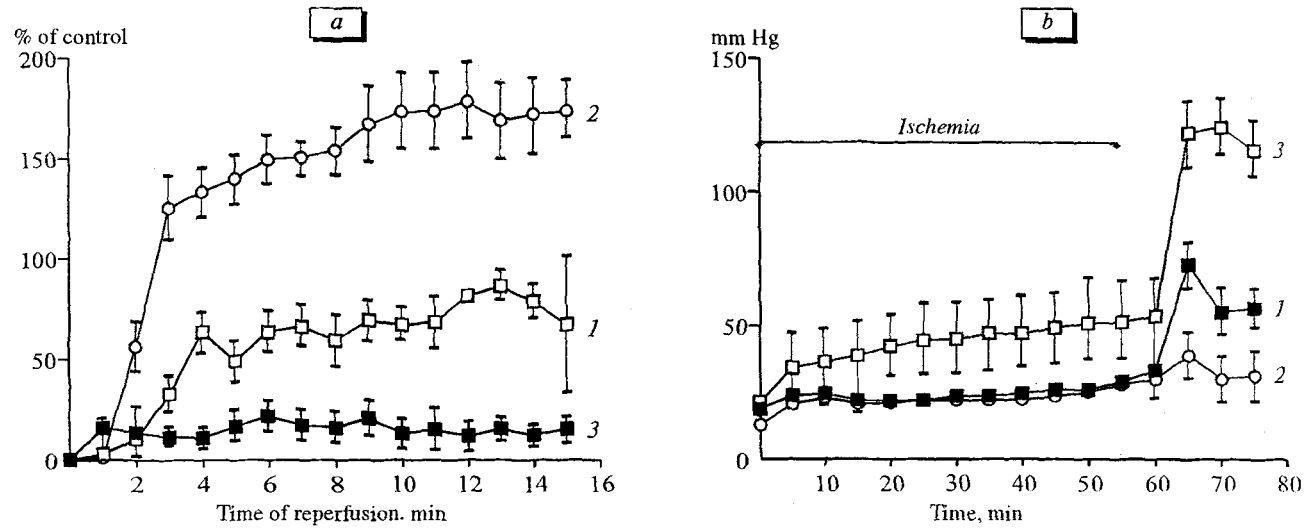

Fig. 3. Effect of acetylcarnosine, carnosine, and acetate on myocardial contractility (*a*) and diastolic pressure (*b*) under conditions of hypothermic cardioplegia. *1*) control, *2*) acetylcarnosine, 10 mM, *3*) carnosine, 10 mM and acetate, 10 mM.

of its initial level and the release of adenine nucleotides and myoglobin increased (Table 2).

Our findings suggest that AC effectively protects the myocardium during cardioplegia, while carnosine similar to AC by its physicochemical characteristics, in particular, buffer effect, possesses no protective properties. Bearing in mind that enzymes metabolizing carnosine and its derivatives are present in the myocardium, we assumed that AC hydrolysis products (carnosine and acetate) are responsible for its protective effect. To verify this assumption in special experimental series we added 10 mM carnosine and 10 mM sodium acetate to CPS (the content of NaCl was reduced to 100 mM). AC hydrolysis products possess no protective activity and similarly to carnosine alone (10 mM) impair cardiac function. At the same time, this mixture in contrast to carnosine alone prevent the loss of myoglobin and adenine nucleosides (Fig. 3, Table 2).

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