BIOPHYSICS AND BIOCHEMISTRY

Protein Kinase C Inhibitors Block Acetylcarnosine-Induced Contractions of Ischemic Myocardium

A. A. Vinokurov, A. A. Boldyrev,* D. N. Chesnokov, A. I. Dmitrashchuk, and V. V. Alabovskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 9, pp. 305-307, September, 1999
Original article submitted January 21, 1999

Protein kinase C inhibitors chelerythrine and Ro31-8220 blocked acetylcarnosine-induced myocardial contractions during subtotal ischemia, which was associated with an increase in ischemic and reperfusion myocardial contractures and a decrease in the developed pressure and cardiac contractility index during subsequent reperfusion. Neither chelerythrine nor Ro31-8220 decreased the membranoprotective effect of acetylcarnosine determined by its antioxidant properties. The results indicate the involvement of protein kinase C in acetylcarnosine stimulation of myocardial contractions in ischemia.

Key Words: *protein kinase C; acetylcarnosine; myocardium*

Histidine dipeptides are characterized by a unique capacity of restoring myocardial contractions stopped by ischemia [1]. Acetylcarnosine, a natural myocardial metabolite, is characterized by the most pronounced effect among previously studied compounds of this group (acetylanserine, ofidin, acetylcarnosine, anserine, homocarnosine). The mechanism of this effect is unknown.

Activation of protein kinase C (PKC) is one of the mechanisms of cardiac protection from ischemia [2,4, 6,7]. We suggested that the effect of acetylcarnosine on the myocardium is mediated via PKC activation. Here we investigated the effects of PKC inhibitors on the protective and positive inotropic effects of acetylcarnosine during subtotal myocardial ischemia.

MATERIALS AND METHODS

Experiments were carried out on isolated hearts of outbred albino rats perfused according to Langendorff's method with oxygenated solution of the following composition (in mM): 140 NaCl, 0.5 NaH_2PO_4, 5 KCl, 5 Tris-OH (pH 7.4), 11 glucose, and 2 $CaCl_2$. Rats were decapitated under ether narcosis, the chest was opened, and the heart was put in cold Langendorff's solution. A cannula was inserted into the aorta, oxygenated solution (37°C) was infused for 15 min at a rate of 10 ml/min/g wet tissue for stabilization of the contractile function. The parameters of the isolated heart during this period were taken as 100%.

Ischemia was induced by decreasing the rate of perfusion to 0.1 ml/min for 40 min. Acetylcarnosine and PKC inhibitors were added to the solution 15 sec before and throughout ischemia. Reperfusion was carried out with the standard solution.

Myocardial contractility was measured in the isovolumic regimen with a latex balloon inserted into the left ventricle. The efficiency of contractions was evaluated by the magnitude of developed pressure (difference between systolic and diastolic pressure) and index of cardiac contractile function (ICF, product of the rate of myocardial contractions and developed pressure). Myocardial ICF during perfusion was expressed in percent of preischemic value (100%).

Department of Biochemistry, N. N. Burdenko State Medical Academy, Voronezh; *Department of Neurochemistry, Institute of Neurology, Russian Academy of Medical Sciences, Moscow

0007-4888/99/0009-0922$22.00

An electromanometer (Bentley Lab. Europe) and an IBM PC analog-to-digital converter were used. The severity of cardiomyocyte injury was evaluated by the levels of myoglobin and nucleosides (adenosine+hypoxanthine+xanthine) in the perfusate. Myoglobin concentration was measured spectrophotometrically at 410 nm [3]. The content of nucleosides and bases (adenosine+hypoxanthine+xanthine) in perfusion solution was measured as described previously [5].

Chelerythrine chloride (CC) was used in a concentration of 100 μM [8] and Ro31-8220 in a concentration of 1.5 μM equal to the inhibition constant of bovine heart PKC [7]. Sigma salts, trisamine, and CC and Roche Products Ro31-8220 were used.

The data were processed using AWPE software (designed by A. I. Glotov, Ph. D.) by ANOVA Student's *t* test.

RESULTS

The decrease in coronary perfusion of isolated rat heart to 0.1 ml/min leads to a progressive decrease in the left-ventricular developed pressure and cardiac arrest by the 3rd min of ischemia (Fig. 1). Starting from the 15th min of ischemia, diastolic pressure in the left ventricle increased indicating the formation of ischemic myocardial contracture. By the 40th min of ischemia the contracture attained 45 mm Hg (Fig. 2). The decrease in coronary perfusion induced the release of myoglobin, a marker of cardiomyocyte sarcolemma destruction, in the perfusate (Table 1). Ischemia promoted the release of nucleotide degradation products (adenosine, inosine, and hypoxanthine) from cardiomyocytes. Reperfusion of the heart with the initial solution partially restored developed pressure (Fig. 1) and myocardial ICF (Table 1).

Addition of acetylcarnosine (10 mM) to perfusion solution during ischemia decelerated the development of ischemic contracture of the myocardium (Fig. 2). Starting from the 20th min of ischemia, acetylcarnosine stimulated myocardial contractures. The developed pressure reached 50-60% of the preischemic level

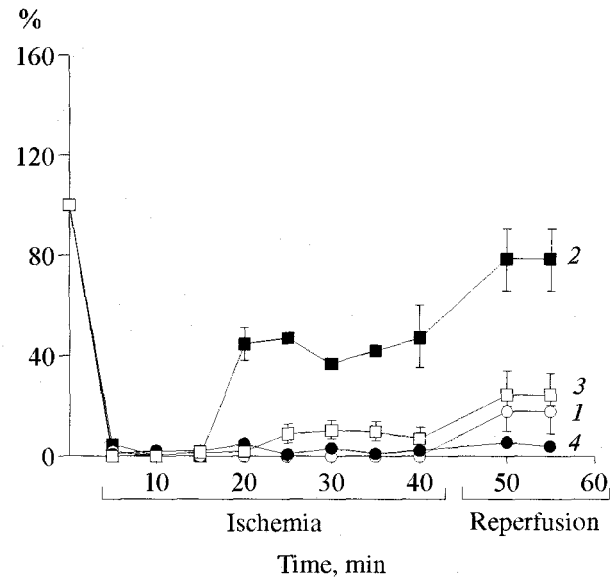

Fig. 1. Effects of acetylcarnosine, chelerythrine, and Ro31-8220 on left-ventricular developed pressure during subtotal ischemia and reperfusion (% of preischemic value). Here and in Fig. 2: *1*) control; *2*) acetylcarnosine (10 mM); *3*) acetylcarnosine (10 mM)+chelerythrine chloride (100 μM); *4*) acetylcarnosine (10 mM)+Ro31-8220 (1.5 μM).

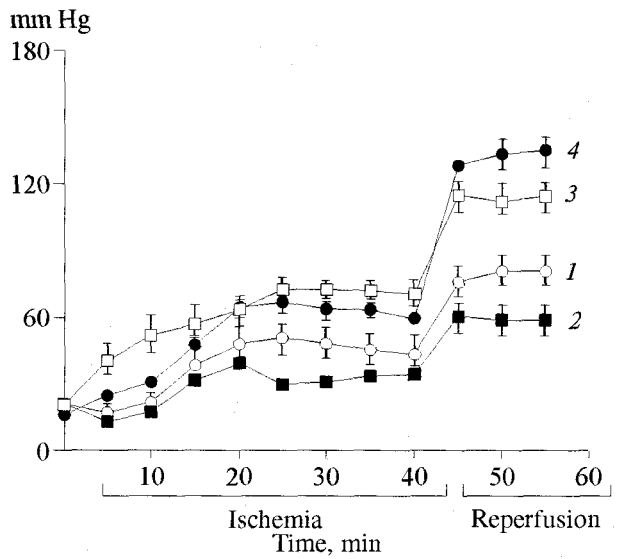

Fig. 2. Effects of acetylcarnosine, chelerythrine, and Ro31-8220 on left-ventricular diastolic pressure during subtotal ischemia and reperfusion.

Table 1. Effects of Acetylcarnosine, CC, and Ro31-8220 on Myoglobin Release (μg/g Dry Weight) from the Heart during Ischemia and Reperfusion and ICF during Reperfusion (*M*±*m*)

Experimental series	Myoglobin release		ICF
	ischemia, 40 min	reperfusion	reperfusion
Control, (*n*=9)	65.06±5.60	546.31±27.85	12.68±3.50
Acetylcarnosine, 10 mM (*n*=15)	44.05±5.45*	470.69±62.76	81.6±11.68**
+CC, 100 μM (*n*=9)	67.89±6.28^{+}	471.26±33.21	1.14±1.14***$^{++}$
+Ro31-8220, 1.5 μM (*n*=9)	46.54±3.73	503.68±37.54	1.53±0.64***$^{++}$

Note. *p<0.05, **p<0.01, ***p<0.001 *vs.* the control; ^{+}p<0.01, ^{++}p<0.001 *vs.* acetylcarnosine.

Table 2. Effects of Acetylcarnosine, CC, and Ro31-8220 on Adenine Nucleoside Release (mmol/g Dry Weight) from the Heart during Ischemia and Reperfusion (*M±m*)

Experimental series	Ischemia, 40 min	Reperfusion
Control, (*n*=9)	1.25±0.10	4.95±0.27
Acetylcarnosine, 10 mM (*n*=15)	0.51±0.04*	1.72±0.09*
+CC, 100 μM (*n*=9)	0.46±0.03*	1.63±0.20*
+Ro31-8220, 1.5 μM (*n*=9)	0.35±0.04*	1.62±0.21*

Note. *$p<0.01$ *vs.* the control.

(Fig. 1). Simultaneously, the release of nucleotide hydrolysis products and myoglobin from cardiomyocytes decreased (Tables 1 and 2). Postischemic reperfusion completely restored developed pressure (90-110%) and myocardial ICF (Fig. 1, Table 1).

PKC inhibitors CC and Ro31-8220 blocked cardiac contractions during ischemia (Fig. 1), recovery of developed pressure, and myocardial ICF during reperfusion (Fig. 1, Table 1). At the same time, the diastolic pressure surpassed the control values (Fig. 2) and ischemic and reperfusion contractures were more pronounced. Despite negligible recovery of the cardiac contractile function, PKC inhibitors did not stimulate the release of myoglobin and adenine nucleosides from cardiomyocytes, apparently due to antioxidant effect of acetylcarnosine [2].

Hence, PKC is involved in the stimulation of cardiac contractions by acetylcarnosine during ischemia.

The authors are grateful to Dr. D. Bradhsaw (Roche Products) for his gift of PKC inhibitor Ro31-8220.

REFERENCES

1. V. V. Alabovskii, A. A. Boldyrev, and V. Kh. Shavratskii, *Biokhimiya,* **62,** 91-102 (1997).
2. A. A. Boldyrev, *Carnosine* [in Russian], Moscow (1998).
3. J. M. Downey and M. V. Cohen, *Basic Res. Cardiol.,* **92,** Suppl. 2, 37-39 (1997).
4. M. Hori, *Methods Enzymol.,* **12,** 381-390 (1967).
5. R. B. Jennings, *Basic Res. Cardiol.,* **92,** Suppl. 2, 40-42 (1997).
6. Y. V. Ladilov, C. Balser, and H. M. Piper, *Circ. Res.,* **82,** No. 4, 451-457 (1998).
7. M. E. Speechly-Dick, G. J. Grover, and D. M. Yellon, *Ibid.,* **77,** 1030-1035 (1995).
8. K. Yoshida, S. Kawamura, Y. Mizukami, and M. Kitakaze, *J. Biochem.* (Tokyo), **122,** 506-511 (1997).