Activation of μ-Opiate Receptors as a Factor of Regulation of Heart Resistance to Ischemia-Reperfusion and Oxidative Stress

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Intravenous injection of the selective μ-opiate receptor agonist DAMGO (0.1 mg/kg, 15 min before isolation of the heart) improved resistance of isolated perfused rat heart to ischemia (45 min) and reperfusion (60 min) damages. *In vivo* administration of DAMGO prevented reperfusion-induced damages to cardiomyocytes and decreased the content of conjugated dienes in the myocardium during ischemia-reperfusion *in vitro*. Furthermore, stimulation of μ-opiate receptors promoted recovery of myocardial contractility during reoxygenation, but had no effect on heart resistance to free radical-induced damages during perfusion of isolated heart with a solution containing Fe^{2+} and ascorbic acid.

Key Words: *ischemia; myocardium; oxidative stress; opiate receptors; reperfusion*

Ischemia and reperfusion of the heart are accompanied by activation of lipid peroxidation (LPO) [1]. Free radical reactions damage cardiomyocyte membranes and, therefore, impair functional properties of the myocardium. The use of antioxidants in clinical practice does not completely prevent reperfusion-induced impairment of myocardial contractility [9]. The search for new preparations increasing myocardial resistance to free radical processes is of considerable importance.

In this respect, cardioprotective properties of opiate receptor (OR) ligands are of particular interest. It was shown that *in vivo* activation of OR during ischemia-reperfusion produces a cardioprotective effect [15]. Our previous experiments on isolated heart demonstrated *in vitro* antioxidant action of nonselective μ- and δ-OR agonist dalargin during oxidative stress [6]. We studied the effects of prestimulation of μ-OR on the resistance of isolated rat heart to LPO activation. The goal of this work was to evaluate the role of μ-OR in the regulation of the resistance of isolated rat heart to ischemia-reperfusion damages and oxidative stress.

MATERIALS AND METHODS

Experiments were performed on 163 male Wistar rats weighing 250-300 g. Perfusion of the heart was performed by the method of Langendorff at 37.0±0.5°C [3]. The perfusate was oxygenated with the mixture containing 95% O_2 and 5% CO_2.

Free radical processes in the myocardium were activated by adding Fe^{2+} and ascorbate to Krebs—Henseleit perfusion solution. The $O_2^{\cdot-}$ generating system consisted of 0.2 mmol/liter $FeSO_4$ and 0.5 mmol/liter ascorbic acid [6].

Ischemia-reperfusion damages were modeled by termination of perfusate supply after a 20-min stabilization and recording of the initial parameters of contractility. Total ischemia and reperfusion lasted 45 and 60 min, respectively.

The selective μ-OR agonist DAMGO (H-Tyr-D-Ala_2-Gly-N-Me-Phe_4-Gly_5-ol)-enkephalin was injected intravenously in a dose of 0.1 mg/kg 20 min before isolation of the heart. The following experimental se-

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ries were carried out on isolated hearts: 120-min normoxic perfusion (normoxic control, series I), 45-min ischemia followed by 5-min reperfusion (ischemic control, series II), 45-min ischemia followed by 60-min reperfusion (reperfusion control, series III), ischemia-reperfusion after DAMGO administration (series IV), 20-min perfusion with Fe^{2+}-ascorbic acid mixture (series V), and 20-min perfusion with Fe^{2+}-ascorbic acid mixture after DAMGO administration (series VI).

Mechanical activity of the myocardium was recorded using a strain transducer connected to a latex balloon introduced through the atrium into the left ventricle. The intraventricular pressure curve was recorded for further analyses of the maximum left ventricular pressure (MLVP) and end-diastolic pressure (EDP). MLVP was calculated as the difference between systolic and diastolic pressures [13]. Damage to cardiomyocyte sarcolemma was evaluated by creatine phosphokinase activity in the effluent using enzyme immunoassay kits (Sigma). After the experiment, the heart was frozen in liquid nitrogen for further assays of conjugated dienes (CD) [4].

The results were analyzed by Student's t test.

RESULTS

Total ischemia (45 min) considerably disturbed contractile functions of isolated rat heart: MLVP decreased by 50% by the 5th min of reoxygenation and remained practically unchanged to the end of observations (Fig. 1, *a*). EDP increased by 2.5 times due to ischemic contracture and did not change during re-

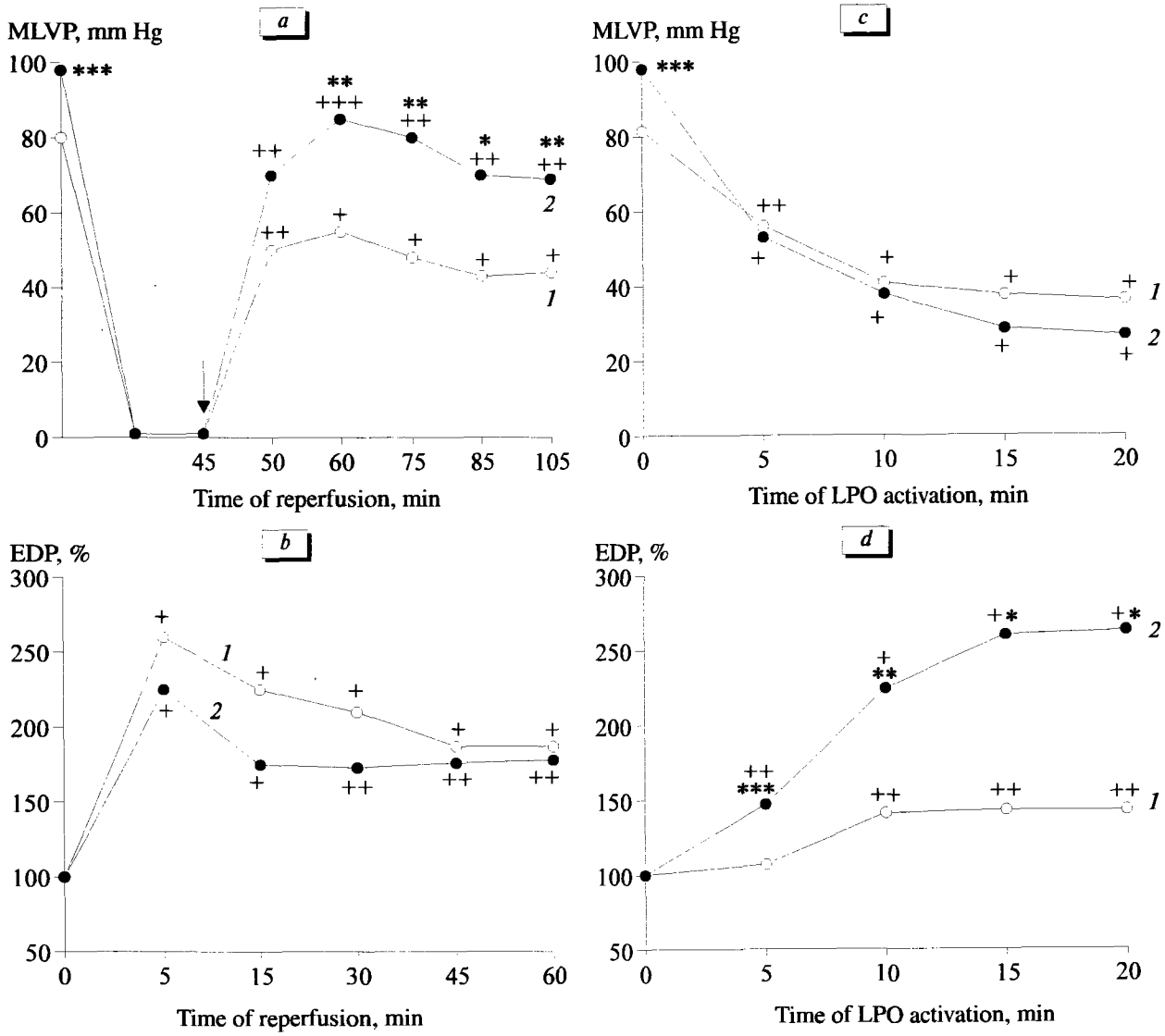

Fig. 1. Effects of DAMGO on contractility of isolated heart during ischemia—reperfusion (*a*, *b*) and oxidative stress (*c*, *d*). MLVP: maximum left ventricular pressure; EDP: end-diastolic pressure. Arrow: start of reperfusion without (control, *1*) and with DAMGO (*2*). Here and in Fig. 3: $^{*}p<0.001$, $^{**}p<0.01$, and $^{***}p<0.05$ compared to the control; $^{+}p<0.001$, $^{++}p<0.01$, and $^{+++}p<0.05$ compared to the initial level.

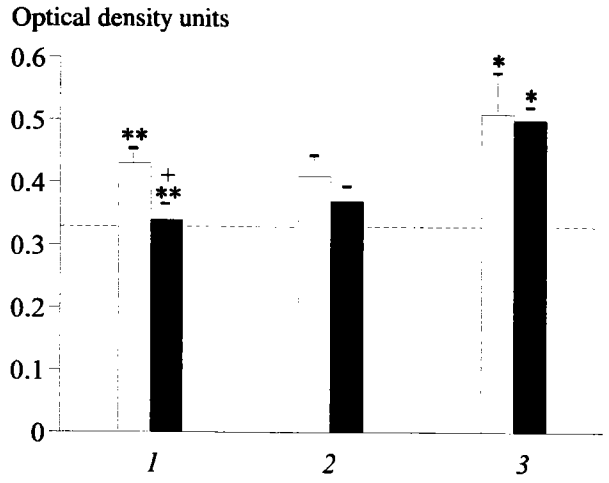

Fig. 2. Effects of DAMGO on the content of conjugated dienes in rat myocardium: ischemia+reperfusion, 5 min (*1*), ischemia+reperfusion, 60 min (*2*), oxidative stress, 20 min (*3*). $^{*}p<0.001$ and $^{**}p<0.01$ compared to normoxic control (dotted line), $^{+}p<0.05$ compared to the control. Here and in Fig. 3: light bars: without DAMGO (control); dark bars: with DAMGO.

perfusion (Fig. 1, *b*). These results agree with published data that low energy formation in cardiomyocytes caused by ischemia impairs pump functions of the heart [1,5].

Five minutes after the start of reperfusion, the content of CD in the myocardium increased by 30% compared to normoxic control (Fig. 2). The content of LPO products decreased to the control level by the end of reoxygenation. Similar dynamics of CD accumulation in the myocardium was reported previously [1,5]. Creatine phosphokinase activity by the 5th and 60th min of reperfusion 5-fold surpassed the initial level (Fig. 3).

Stimulation of μ-OR was accompanied by the rise of MLVP before total ischemia and during reperfusion. The initial contraction force after DAMGO administration was 23% higher than in normoxic control. By the 60th min of reoxygenation, this parameter was 45% higher than in reperfusion control (Fig. 1, *a*). It can be assumed that this effect is due to the systolic rise of $[Ca^{2+}]_i$ in cardiomyocyte sarcolemma. T. M. Wong *et al.* [11] showed that activation of μ-OR in isolated cardiomyocytes promoted the increase in $[Ca^{2+}]_i$ due to its mobilization from the sarcoplasmic reticulum. At the same time, in our experiments activation of μ-OR had no effect on the severity of contracture (Fig. 1, *b*).

DAMGO-produced activation of μ-OR led to a significant decrease in creatine phosphokinase activity on the 5th and 60th min of reperfusion compared to that in ischemic and reperfusion controls (Fig. 3). These results are consistent with published data that μ-agonists display *in vivo* cardioprotective effects during myocardial reperfusion [15]. The membrane-protective effect was probably due to inhibition of LPO in cardiomyocyte membranes, because CD content in the myocardium decreased in parallel with the reduction of creatine phosphokinase activity in the perfusate (Fig. 2).

After 20-min perfusion with the Fe^{2+}-ascorbate medium, MLVP decreased 2.7-fold (Fig. 1, *c*) and EDP increased 2-fold (Fig. 1, *d*) indicating the development of contracture. Initiation of free radical processes in the myocardium by the Fe^{2+}-ascorbate mixture considerably increased (by 25%) the content of CD compared to normoxic control (Fig. 2). A 5-fold rise of creatine phosphokinase activity in the effluent 20 min after LPO activation confirmed free radical-induced damages to cardiomyocyte membranes (Fig. 3).

Activation of μ-OR before 20-min perfusion of the isolated heart with Fe^{2+}-ascorbate mixture pro-

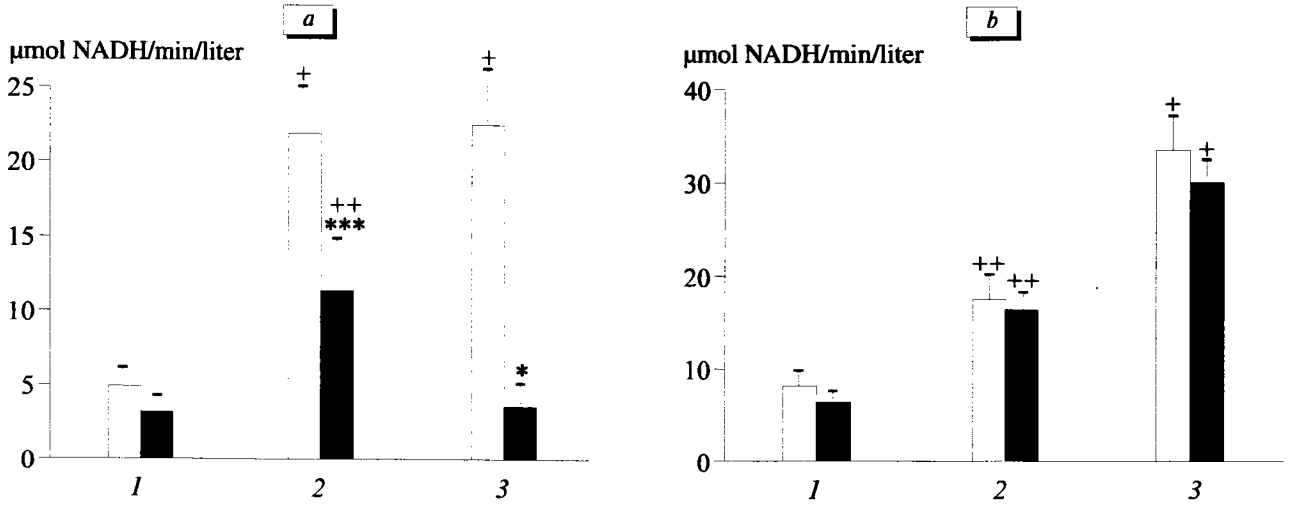

Fig. 3. Creatine phosphokinase activity in the perfusate during ischemia-reperfusion (*a*) and oxidative stress (*b*). *1*) initial level; *2*) 5-min reperfusion (*a*) or LPO activation (*b*); 3) 60-min reperfusion (*a*) or 20-min LPO activation (*b*).

duced no cardioprotective effect. The content of CD in the myocardium of these rats practically did no differ from that in rats with activation of free radical LPO (Fig. 2). Creatine phosphokinase activity in the perfusate after 20 min also significantly surpassed the initial level (Fig. 3). However, under these conditions DAMGO increased the severity of contracture: after 20 min, EDP surpassed the control level by 184% (Fig. 1, *c*). Probably, the effect of DAMGO on $[Ca^{2+}]_i$ became more pronounced under conditions of oxidative stress impairing the function of Ca^{2+} pump [16].

Hence, the data suggest that activation of μ-OR prevents ischemia-reperfusion-induced contractile and metabolic disturbances in the myocardium, but has no effect on free radical-produced injuries. In the latter case, DAMGO produced no cardioprotective effects probably due to the absence of antioxidant properties. Therefore, the protective effect of μ-OR stimulation during ischemia-reperfusion is only partially due to LPO inhibition, but the major role in the realization of this action is played by other mechanisms.

It is known that activation of $[Ca^{2+}]_i$-independent phospholipase A_2 is an important factor contributing to cardiomyocyte damages during ischemia-reperfusion [1,14]. Previous studies showed that inhibition of this enzyme attenuates ischemic damages to cardiomyocytes. Furthermore, cardioprotective effect of ATP-dependent K^+-channel activators inversely depends on phospholipase A_2 activity [10,14]. Stimulation of cardiac μ-OR is accompanied by activation of ATP-dependent K^+-channels [15]. The data suggest that protective effects of DAMGO are realized via activation of these channels and inhibition of $[Ca^{2+}]_i$-independent phospholipase A_2.

Cardioprotective properties of DAMGO can also be related to the following fact. Total ischemia is accompanied by accumulation of lactate and cardiomyocyte acidosis promoting activation of phospholipase A_2 with pH optimum 4.0-6.5 [1,5]. It can be assumed that μ-OR activation during ischemia-reperfusion leads to alkalization of the cardiomyocyte myoplasm, since opioids are known to normalize the content of lactate in the ischemic myocardium [2]. DAMGO probably inhibits hydrolysis of phospholipids and activation of LPO, which are known to play a role in the pathogenesis of cell damages [1,5].

Hence, stimulation of μ-OR prevents ischemia-reperfusion disturbances in myocardial contractility and protects cardiomyocyte membranes during reoxygenation. These effects probably result from activation of ATP-dependent K^+-channels and inhibition of phospholipase A_2. At the same time, inefficiency of this μ-OR agonist under conditions of oxidative stress indicates that cardioprotective action of DAMGO during reperfusion is not directly related to LPO inhibition.

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