Cardioprotective Effect of κ_1-Opioid Receptor Activation and Role of cAMP in Its Realization

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The cardioprotective and antiarrhythmic effects of a selective κ_1-opioid receptor agonist U-50,488 were studied during experimental 45-min total ischemia and 30-min reperfusion of isolated rat heart. The opioid had no effect on the incidence and type of reperfusion arrhythmias. U-50,488 in a concentration of 0.1 μM inhibited reperfusion-induced release of creatine phosphokinase and decreased cAMP concentration in the myocardium by 2 times. These parameters remained unchanged after treatment with U-50,488 in a concentration of 1 μM. The cardioprotective effect of U-50,488 was probably associated with a decrease in cAMP concentration in heart cells. U-50,488 in a concentration of 1 μM produced no cardioprotective effect, which can be explained by its interaction with an unknown non-opioid receptor in cardiomyocytes.

Key Words: *isolated perfused heart; κ_1-opioid receptors; ischemia-reperfusion; cAMP*

Activation of cardiac δ-opioid receptors (OR) improves the resistance of the isolated heart to arrhythmogenic factors and has a cardioprotective effect under conditions of myocardial ischemia and reperfusion [1]. However, the myocardium contains primarily k-OR. The role of these receptors in the regulation of heart resistance to ischemia-reperfusion is poorly understood [13]. There are contradictory data on the k-opioidergic regulation of the myocardial resistance to hypoxia and reoxygenation [2,4,8,11]. It is probably related to the existence of 2 subtypes of κ-OR (κ_1 and κ_2 receptors) on the sarcolemma of cardiomyocytes (CMC) [12]. For example, treatment with various k_1-OR agonists, including U-50,488, ICI 204,448, and BRL 52537, produces a cardioprotective effect [8,9]. By contrast, κ_2-OR agonist bremazocine increases the severity of ischemic and reperfusion damage to the heart [3]. The intracellular mechanisms of these effects are poorly studied. Published data show that activation of κ-OR is followed by inhibition of adenylate cyclase and decrease in cAMP formation in intact CMC [10]. Some authors report that the cardioprotective effect of opioids under conditions of ischemia—reperfusion is associated with a decrease in cAMP concentration in the myocardium [3]. We hypothesized that cAMP plays an important role in the cardioprotective effect of κ_1-OR agonists on the model of total ischemia and reperfusion of the isolated heart.

Here we studied the role of κ_1-OR in the regulation of isolated heart resistance to ischemia and reperfusion. The role of cAMP in cardioprotective activity of κ_1-OR agonist U-50,488 was evaluated *in vitro*.

MATERIALS AND METHODS

Experiments were performed on isolated hearts of male Wistar rats weighing 250-300 g. After thoracotomy the heart was rapidly removed and placed in a bath with cold Krebs—Henseleit solution (4°C). After termination of spontaneous contractions, the

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heart was put in a thermostabilized humid chamber. A cannula was introduced into the ascending aortic arch. Retrograde Langendorff perfusion of the heart with Krebs—Henseleit solution was performed at a constant pressure of 52 mm Hg. Krebs—Henseleit solution was saturated with carbogen (37°C, pH 7.4) and contained 120 mM NaCl, 4.8 mM KCl, 2.0 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 20 mM $NaHCO_3$, and 10 mM glucose (ICN Biomedicals). Total normothermic ischemia of the myocardium was induced by termination of perfusate supply for 45 min. Observations continued over 30-min reperfusion. ECG was continuously recorded in the first 10 min of reperfusion. The incidence of ventricular arrhythmias (extrasystoles, tachycardia, and fibrillation) was estimated.

Selective κ_1-OR agonist U-50,488 (trans(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl] benzeneacetamide hydrochloride, The Upjohn Company; kindly provided by Dr. P. F. Von Voigtlander) [6] was used for receptor activation. The heart was adapted to normoxic perfusion (20 min), perfused with Krebs—Henseleit solution containing U-50,488 (10 min), and washed to remove this ligand (10 min). U-50,488 was dissolved in physiological saline immediately before the start of the study. The final concentrations of U-50,488 were 0.1 and 1 μM. The isolated hearts exposed to 40-min adaptation, 45-min total ischemia, and 30-min reperfusion served as the control. U-50,488 concentration of 0.1 μM was selected taking into account published data on inhibition of adenylate cyclase in isolated CMC in the presence of this compound [10] and on its affinity for κ_1-OR in CMC (K_i=32 nM) [5]. U-50,488 concentration of 1 μM was selected taking into account that this κ_1-OR agonist *in vitro* has an antiarrhythmic effect during experimental norepinephrine-induced arrhythmias [11].

The severity of damage to CMC was estimated by creatine phosphokinase (CPK) activity in the perfusate. CPK activity was measured using CK-Nac Biocon Diagnostik enzyme kits (Vohl/Marienhagen) and calculated per 1 g heart tissue over 30-min reperfusion.

After reperfusion, the isolated hearts were rapidly frozen and stored in liquid nitrogen. cAMP was extracted from the heart tissue with ethyl alcohol [7]. cAMP concentration was measured using RIA AMPc/cAMP radioimmune kits (Immunotech). Radioactivity in samples was measured on a Gamm-12 γ-counter.

The results were analyzed by Mann—Whitney test and χ^2 test. The linear correlation coefficient was calculated.

RESULTS

In the control group, reperfusion of the isolated heart after total ischemia was accompanied by arrhythmia and damage to CMC membrane. This conclusion was derived from a 5-fold increase in cAMP concentration in the outflow perfusate (Table 1, Fig. 1). Arrhythmia was observed practically in all hearts over the first 10 min after coronary reperfusion (Table 1). Ventricular extrasystoles, tachycardia, and fibrillation were revealed in 92, 57, and 42% hearts, respectively. Heart rhythm disturbances were reversible and resulted in the recovery of normal sinus rhythm or development of ventricular extrasystoles.

Prestimulation of κ_1-OR after addition of U-50,488 in final concentrations of 0.1 and 1 μM to the perfusate had no effect on the type and incidence of reperfusion arrhythmias. The κ_1-OR agonist at the micromolar concentration tended to decrease the incidence of ventricular fibrillation (Table 1).

Hence, activation of cardiac κ_1-OR has no effect on the resistance of the isolated heart to the arrhythmogenic effect of ischemia-reperfusion. *In vivo* experiments showed that κ_1-OR agonist U-50,488 prevents arrhythmias induced by coronary occlusion and reperfusion [2]. The antiarrhythmic effect of U-50,488 probably manifested only in the whole organism. It can be hypothesized that κ_1-OR on the sarcolemma of CMC are not involved in myocardial resistance to the arrhythmogenic effect of reperfusion. The *in vivo* antiarrhythmic effect of this κ_1-OR agonist is related to κ-OR-mediated

TABLE 1. Effect of U-50,488 Addition to the Perfusate before Ischemia on Reperfusion Arrhythmias (*n*=14)

Group	Multiple ventricular extrasystoles		Ventricular fibrillation		Ventricular tachycardia	
Control	13	92	8	57	6	42
U-50,488, 0.1 μM	12	85	9	64	5	35
U-50,488, 1 μM	9	64	7	50	4	28

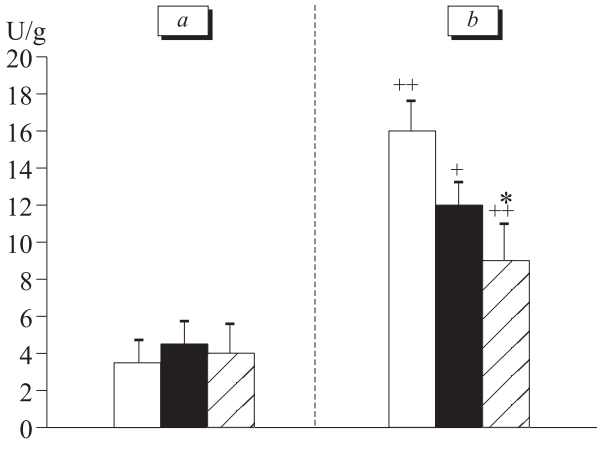

Fig. 1. CPK activity in the perfusate before ischemia (*a*) and during reperfusion (*b*) after prestimulation with U-50,488. Light bars: control; dark bars: 1 μM U-50,488; and shaded bars: 0.1 μM U-50,488. *$p<0.05$ compared to the control; $^{+}p<0.05$ and $^{++}p<0.01$ compared to the basal level.

changes in the function of the autonomic nervous system, which plays an important role in the regulation of cardiac electrical stability.

Perfusion of the isolated heart with a solution of U-50,488 did not modulate the preischemic level of CPK activity (Fig. 1). Stimulation of κ_1-OR with U-50,488 in a concentration of 0.1 μM was followed by a significant decrease in the number of CMC dying after ischemia and reperfusion. CPK activity in the outflow perfusate decreased by 45% compared to the control. Increasing the concentration of U-50,488 to 1 μM did not potentiate, but even abolished the cardioprotective effect (Fig. 1).

The protective effect of U-50,488 is probably associated with activation of cardiac κ_1-OR. The agonist has high affinity for these receptors [5]. Disappearance of cytoprotective activity with increasing U-50,488 concentration probably resulted from activation of non-opioid receptors. Experiments on isolated heart showed that δ-OR agonist DADLE has the cardioprotective effect. This effect disappeared with increasing peptide concentration in the perfusate. The cardioprotective effect of δ-OR agonist was accompanied by a decrease in cAMP concentration in the isolated heart [3]. Published data show that activation of κ-OR is followed by the inhibition of adenylate cyclase in isolated CMC [10]. The κ-OR-mediated decrease in myocardial cAMP concentration probably contributes to the cardioprotective effect of U-50,488.

The postreperfusion level of cAMP after treatment with U-50,488 in a concentration of 0.1 μM was far below the control (12.0±0.9 and 23.1±1.1 nmol/g, respectively, $p<0.05$). Little changes in cAMP concentration were revealed after addition of 1 μM κ_1-OR agonist (18.2±2.1 nmol/g). Therefore, the cardioprotective effect of U-50,488 in a concentration of 0.1 μM was accompanied by a decrease in cAMP concentration. The coefficient of correlation between myocardial cAMP concentration and CPK activity in the perfusate was 0.89 ($p<0.01$). Hence, the cardioprotective effect of U-50,488 can be related to a decrease in cAMP concentration in CMC. cAMP increases pump function of the myocardium. The decrease in cAMP concentration inhibits contractile function of the heart and decreases myocardial demands for O_2. The decrease in myocardial demands for O_2 under ischemic conditions probably contributes to the increase in CMC tolerance to the pathogenic effect of ischemia—reperfusion. These changes were observed in our experiments.

Our results suggest that the κ-opioidergic decrease in myocardial cAMP concentration results from the decrease in adenylate cyclase activity. This conclusion is derived from the results of studies with isolated CMC [10]. It cannot be excluded that U-50,488 activates phosphodiesterase due to the increase in cAMP degradation. However, there are no data to support this assumption. The decrease in myocardial cAMP concentration after addition of U-50,488 to the perfusate is most likely related to inhibition of adenylate cyclase after activation of κ_1-OR.

We conclude that activation of cardiac κ_1-OR has no effect on the incidence of reperfusion arrhythmias. Pretreatment with a κ_1-OR agonist U-50,488 has a cardioprotective effect associated with activation of cardiac κ_1-OR. The cardioprotective effect of U-50,488 correlates with a decrease in cAMP concentration in the myocardium.

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