Activation of k_1-Opioid Receptor as a Method for Prevention of Ischemic and Reperfusion Arrhythmias: Role of Protein Kinase C and K_{ATP} Channels

A. Yu. Lishmanov, L. N. Maslov, T. V. Lasukova, D. Crawford*, and T. M. Wong**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 2, pp. 145-148, February, 2007
Original article submitted May 23, 2006

Intravenous pretreatment with κ-opioid receptor antagonist (-)-U-50,488 (1 mg/kg) improved heart resistance to the arrhythmogenic effect of coronary occlusion and reperfusion. Selective κ_1-opioid receptor antagonist norbinaltorphimine and nonselective blocker of peripheral opioid receptors methylnaloxone abolished this antiarrhythmic effect. Preliminary blockade of protein kinase C with chelerythrine or inhibition of ATP-dependent K^+ channels (K_{ATP} channels) with glybenclamide abolished the antiarrhythmic effect of κ-opioid receptor activation. Selective inhibitor of sarcolemmal K_{ATP} channels did not modulate the κ-opioid receptor-mediated increase in cardiac electrical stability. Our results suggest that protein kinase C and mitochondrial K_{ATP} channels play an important role in the antiarrhythmic effect associated with activation of peripheral κ-opioid receptors.

Key Words: *opioid receptors; heart; arrhythmias; ischemia; reperfusion*

Antiarrhythmic drugs used in clinical practice do not necessarily abolish or prevent arrhythmias. Moreover, they sometimes provoke arrhythmias. Much attention is paid to the development of new antiarrhythmic drugs, whose mechanism of action differs from that of class I-IV medicinal preparations. κ-Opioid receptor (OR) agonists hold much promise in this respect. Previous studies showed that κ-OR agonists increase cardiac resistance to the arrhythmogenic effect of ischemia/reperfusion [1,11]. These compounds produce no arrhythmogenic or proarrhythmic effect *in vivo* [1,2,9,11,12]. However, antiarrhythmic activity of κ-OR agonists is poorly understood. For example, localization of κ-OR mediating the antiarrhythmic effect remains unknown. Published data show that the antiarrhythmic effect is observed not only during intravenous injection, but also after intracerebral administration of opioids (*i.e.*, via central κ-OR) [9]. Little is known about the mechanisms of the κ-opioidergic increase in myocardial resistance to arrhythmogenic factors. Protein kinase C (PKC) and ATP-dependent K^+ channels (K_{ATP} channels) play a key role in the cardioprotective effect of opioids [3,6]. It was hypothesized that the antiarrhythmic effect of opioids is realized via the interaction of these intracellular signaling systems.

This work was designed to study the role of peripheral κ-OR in the regulation of cardiac electrical stability during coronary occlusion and reperfusion. We evaluated the role of PKC and K_{ATP} channels in the κ-opioidergic increase in myocardial resistance to the arrhythmogenic effect of ischemia/reperfusion.

Institute of Cardiology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences; *Tocris Cookson, Bristol; **Hong Kong University. ***Address for correspondence:*** maslov@cardio.tsu.ru. L. N. Maslov

MATERIALS AND METHODS

Experiments were performed on ketamine-narcotized (50 mg/kg intraperitoneally) male Wistar rats weighing 180-200 g. Acute 10-min myocardial ischemia was induced by ligation of the left coronary artery, while 10-min reperfusion was simulated by removal of the ligature [6]. Artificial ventilation with room air was performed using a RO-2 device. ECG in standard lead I was recorded during ischemia and reperfusion using an UBF4-03 biopotential amplifier and Pentium computer (original software). The percent of animals without arrhythmias, number of rats with multiple ventricular extrasystoles, and episodes of ventricular tachycardia and ventricular fibrillation were determined.

The following pharmacological agents were used: selective κ_1-OR agonist *trans*(-)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide hydrochloride ((-)-U-50,488, 1 mg/kg, Tocris Cookson) [5]; κ-OR antagonist norbinaltorphimine hydrochloride (NBT, 9 mg/kg, Research Triangle Institute) [5]; nonselective OR antagonist methylnaloxone not crossing the blood-brain barrier (3 mg/kg, Sigma) [13]; blocker of sarcolemmal and mitochondrial K_{ATP} channels glybenclamide (0.3 mg/kg) [6]; blocker of sarcolemmal K_{ATP} channels 1-[[5-(5-chloro-*o*-anisamido)ethyl]-2-methoxyphenyl]sulfonyl]-3-methylthiourea sodium (HMR 1098, 3 mg/kg, Sanofi-Aventis) [6]; and PKC inhibitor chelerythrine (5 mg/kg, Sigma, LCLabs Company) [8]. (-)-U-50,488 was infused intravenously 15 min before ischemia modeling. NBT was administered 1.5 h before coronary artery ligation. Glybenclamide was injected intravenously 45 min before the start of the study. Chelerythrine was administered 20 min before coronary occlusion. Methylnaloxone and HMR 1098 were administered 25 min before coronary occlusion. The doses and schemes of treatment with these preparations were selected from published data on the cardiotropic effects of pharmacological agents [1,3,5,6,8]. OR ligands, HMR 1098, and chelerythrine were dissolved in 0.9% NaCl. Chelerythrine was dissolved in NaCl heated to 55°C. Glybenclamide (3 mg) was dissolved in 0.1 ml dimethylsulfoxide. The solution (10 μl) was added to 1 ml 20% hydroxypropyl-β-cyclodextrin (Tocris Cookson). Hydroxypropyl-β-cyclodextrin (200 mg) was dissolved in 1 ml H_2O.

Control animals with coronary occlusion and reperfusion did not receive the test preparations. Statistical treatment of experimental data involved χ^2 test.

RESULTS

Acute coronary occlusion was accompanied by the development of ventricular arrhythmias in 96% animals. Severe arrhythmias (ventricular tachycardia and ventricular fibrillation) were found in 83% animals. Reperfusion induced various types of ventricular arrhythmias in all experimental animals. Multiple extrasystoles, ventricular tachycardia, and episodes of ventricular fibrillation were revealed in 74, 87, and 17% animals, respectively.

Intravenous pretreatment of rats with (-)-U-50,488 was accompanied by a significant decrease in the incidence of ventricular extrasystoles and tachycardia during acute ischemia (Fig. 1, *a*). Besides this, 47% rats receiving a κ-OR agonist became resistant to the arrhythmogenic effect reperfusion. The incidence of ventricular extrasystoles

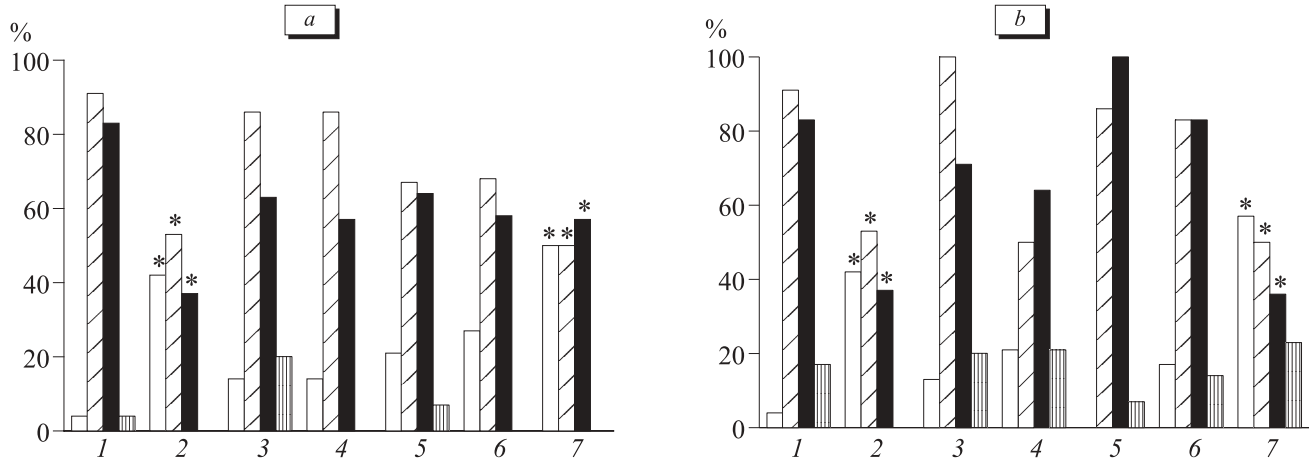

Fig. 1. Effect of κ-OR activation on the incidence of ventricular arrhythmias during 10-min ischemia (*a*) and 10-min reperfusion (*b*). Light bars: without ventricular arrhythmias; dark bars: multiple ventricular extrasystoles; shaded bars: ventricular tachycardia; vertical shading: ventricular fibrillation. Control (*1*), (-)-U-50,488 (*2*), NBT+(-)-U-50,488 (*3*), methylnaloxone+(-)-U-50,488 (*4*), chelerythrine+(-)-U-50,488 (*5*), glybenclamide+(-)-U-50,488 (*6*), and NMR 1098+(-)-U-50,488 (*7*). *$p<0.01$ compared to the control.

decreased by 34%, while ventricular fibrillation was not observed in these animals (Fig. 1, *b*). For evaluation of the type of receptors mediating the antiarrhythmic effect of (-)-U-50,488, we administered this ligand after blockade of κ-OR with NBT. NBT completely abolished the antiarrhythmic effect of (-)-U-50,488 during acute ischemia and reperfusion (Fig. 1). Hence, the antiarrhythmic effect was determined by activation of κ-OR. These findings are of considerable importance, since the type of receptors mediating the influence of (-)-U-50,488 remained unknown. Moreover, several authors believed that the antiarrhythmic effect of κ-OR agonists is associated with nonspecific blockade of Na^+ or K^+ channels [12]. NBT did not modulate the incidence and type of arrhythmias during coronary occlusion and reperfusion. Therefore, endogenous agonists of κ-OR synthesized in the myocardium do not play a role in myocardial resistance to the arrhythmogenic effect of ischemia and reperfusion.

Experiments with methylnaloxone were conducted to evaluate the localization of κ-OR determining myocardial resistance to the arrhythmogenic effect of coronary occlusion/reperfusion [13]. The percentage of animals with ischemic and reperfusion ventricular arrhythmias receiving (-)-U-50,488 after inactivation of peripheral OR did not differ from the control (Fig. 1). These data indicate that κ-OR localized outside the central nervous system play a role in the improvement of heart resistance to the arrhythmogenic effect of acute ischemia and reperfusion.

The mechanisms underlying the effects of peripheral κ-OR activation were estimated. Published data suggest that the protective effect of κ-OR agonist is realized via PKC [6]. Moreover, it cannot be excluded that the κ-opioidergic increase in electrical stability of the myocardium is mediated by K_{ATP} channels [3,6].

For evaluation of the role of PKC, (-)-U-50,488 was infused intravenously after chelerythrine blockade [8]. Chelerythrine did not modulate the incidence and type of arrhythmias during coronary occlusion and reperfusion. However, chelerythrine abolished the antiarrhythmic effect of κ-OR agonist. Hence, the increase in myocardial resistance to the arrhythmogenic effect of acute ischemia/reperfusion observed during activation of κ_1-OR is mediated by PKC.

The type of arrhythmias in rats with ischemia/reperfusion remained unchanged after inactivation of K_{ATP} channels with glybenclamide. (-)-U-50,488 produced no antiarrhythmic effect under these conditions.

A relationship was found between the antiarrhythmic effect of κ-OR stimulation, PKC, and K_{ATP} channels. (-)-U-50,488 was administered after blockade of sarcolemmal K_{ATP} channels with selective blocker HMR 1098 to evaluate the type of K_{ATP} channels mediating antiarrhythmic activity of (-)-U-50,488. HMR 1098 did not modulate the incidence of ventricular arrhythmias during ischemia and reperfusion (Fig. 1). The antiarrhythmic effect of κ-OR agonist (-)-U-50,488 was preserved after blockade of sarcolemmal K_{ATP} channels with HMR 1098. The incidence of acute ischemia-induced ventricular extrasystoles and episodes of ventricular fibrillation significantly decreased after combined administration of HMR 1098 and (-)-U-50,488 (Fig. 1, *a*). The incidence of ventricular extrasystoles decreased by 41%, while ventricular fibrillation was not observed. (-)-U-50,488 produced a similar antiarrhythmic effect during reperfusion (Fig. 1, *b*).

Glybenclamide, which blocks both pools of K_{ATP} channels, abolished the antiarrhythmic effect of (-)-U-50,488. A blocker of sarcolemmal K_{ATP} channels did not modulate this effect. Therefore, mitochondrial K_{ATP} channels play a key role in a κ-opioidergic improvement of myocardial resistance to the arrhythmogenic effect of coronary occlusion and reperfusion. The contribution of these channels into the regulation of electrical stability is poorly known. Previous studies showed that activation of K_{ATP} channels contributes to a decrease in the ischemic and reperfusion overload of cardiomyocytes with Ca^{2+} [4]. Accumulation of this cation in cardiomyocyte cytoplasm during ischemia represents an important pathogenetic stage of arrhythmogenesis, which is realized via the mechanism of trigger automatism [10]. Activation of mitochondrial K_{ATP} channels delays the development of energy deficiency in cardiomyocytes during ischemia [7] and, therefore, contributes to normal function of ion pumps (*e.g.*, sarcoplasmic reticulum Ca^{2+}-ATPase). These changes prevent calcium overload of cells, which serves as a trigger mechanism for ventricular arrhythmias during myocardial ischemia and reperfusion.

Our results indicate that activation of peripheral κ-OR is accompanied by an increase in electrical stability of the myocardium during acute ischemia/reperfusion. PKC and mitochondrial K_{ATP} channels play a key role in this antiarrhythmic effect.

This work was supported by the Russian Foundation for Basic Research.

We are grateful to Dr. Heinz Goegelein, Dr. Juergen Puenter (Sanofi-Aventis), and Dr. Kevin J. Gormley (NIDA, Bethesda, USA) for κ-OR ligands.

REFERENCES

1. L. N. Maslov, Yu. B. Lishmanov, N. V. Solenkova, *et al*., *Eksper. Klin. Farmakol*., **68**, No. 1, 25-29 (2005).
2. S. D. Mikhailova, T. V. Vasil'eva, T. M. Semushkina, and G. I. Storazhikov, *Byull. Eksp. Biol. Med*., **129**, No. 6, 34-36 (2000).
3. N. V. Solenkova, A. V. Krylatov, and V. Yu. Serebrov, *Ibid*., Suppl. 1, 23-25 (2001).
4. R. W. Behling and H. J. Malone, *J. Mol. Cell. Cardiol*., **27**, No. 9, 1809-1817 (1995).
5. B. N. Dhawan, F. Cesselin, R. Raghubir, *et al*., *Pharmacol. Rev*., **48**, No. 4, 567-592 (1996).
6. R. M. Fryer, Y. Wang, A. K. Hsu, and G. J. Gross, *Am. J. Physiol. Heart Circ. Physiol.*, **280**, No. 3, H1346-H1353 (2001).
7. G. J. Gross, *Basic Res. Cardiol*., **95**, 280-284 (2000).
8. A. B. Hassouna, B. M. Matata, and M. Galinanes, *Am. J. Physiol. Cell. Physiol.*, **287**, No. 5, C1418-C1425 (2004).
9. Yu. B. Lishmanov, L. N. Maslov, and D. S. Ugdyzhekova, *Clin. Exp. Pharmacol. Physiol.*, **26**, 1325-1335 (1999).
10. W. F. Lubbe, T. Podzuweit, and L. H. Opie, *J. Am. Coll. Cardiol*., **19**, No. 7, 1622-1633 (1992).
11. M. K. Pugsley, W. P. Penz, M. J. Walker, and T. M. Wong, *Eur. J. Pharmacol*., **212**, No. 1, 15-19 (1992).
12. M. K. Pugsley, D. A. Saint, and M. J. Walker, *Ibid*., **261**, No. 3, 303-309 (1994).
13. J. Russell, P. Bass, L. I. Goldberg, and H. Merz, *Ibid*., **78**, No. 3, 255-261 (1982).
14. M. L. Zhuo, Y. Huang, D. P. Liu, and C. C. Liang, *Int. J. Biochem. Cell. Biol.*, **37**, No. 4, 751-764 (2005).