Role of Cannabinoid Receptors in the Regulation of Cardiac Contractility during Ischemia/Reperfusion

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 11, pp. 500-504, November, 2006
Original article submitted March 7, 2006

We studied the effect of selective ligands of cannabinoid (CB) receptors on contractility of isolated Langendorff-perfused rat heart under conditions of 45-min total ischemia and 30-min reperfusion. Perfusion with a solution containing selective CB receptor agonist HU-210 for 10 min before ischemia increased the severity of reperfusion contractile dysfunction. This drug decreased left ventricular developed pressure and maximum rates of contraction and relaxation, but had no effect on heart rate and end-diastolic pressure. The negative inotropic effect of the drug was transitory and disappeared after 5-min reperfusion. Pretreatment with selective CB1 receptor antagonist SR141716A and selective CB2 receptor antagonist SR144528 had no effect on heart rate and myocardial contractility during reperfusion. Our results indicate that stimulation of CB receptors can increase the degree of reperfusion-induced cardiac contractile dysfunction. However, endogenous cannabinoids are not involved in the development of myocardial contractile dysfunction during ischemia/reperfusion of the isolated heart.

Key Words: *cannabinoid receptors; cardiac contractility; ischemia; reperfusion; isolated heart*

Ischemia and reperfusion are followed by myocardial contractile dysfunction [2], which often abolishes the positive effect of thrombolytic therapy in patients with acute myocardial infarction and complicates the postoperation period in cardiosurgical patients [1]. There are no drugs for the effective treatment or prevention of this disorder. It is probably due to poor knowledge of the pathogenesis of reperfusion stunning of the heart. The role of endogenous cannabinoids in the pathogenesis of myocardial contractile dysfunction remains unknown. Previous studies showed that cannabinoid (CB) receptor agonists produce a negative inotropic effect *in vitro* [3,6] and *in vivo* [11] and contribute to the decrease in myocardial contractility during reperfusion.

CB receptors belong to the family of transmembrane $G_{i/o}$ protein-coupled receptors. Activation of these receptors promotes the decrease in adenylate cyclase activity [8]. Systemic administration of cannabinoids produces hypotensive and negative chronotropic effects [11]. However, there is no general agreement about the role of these compounds in the regulation of cardiac inotropic function during ischemia/reperfusion.

Here we studied the role of CB receptors in the regulation of myocardial contractility during ischemia/reperfusion.

MATERIALS AND METHODS

Experiments were performed on isolated hearts from male Wistar rats weighing 250-300 g. After thora-

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0007-4888/06/1425-0557

cotomy the heart was rapidly removed and placed in a bath with cold Krebs-Henseleit solution (4°C). Isotonic solution was delivered through a cannula inserted into the ascending aortic arch. Retrograde perfusion of the heart with Krebs—Henseleit solution was performed by the method of Langendorff at a constant pressure of 55 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, 20.0 mM $NaHCO_3$, and 10 mM D-glucose (ICN Biomedicals, Costa Mesa). Krebs—Henseleit solution and reagents were prepared using distilled water obtained on a Simplicity device (Millipore).

A catheter with a latex balloon was introduced into the left ventricle to study cardiac contractile activity. The balloon was filled with water. Ventricular diastolic pressure was set at 10-15 mm Hg. Pump function of the heart was determined by heart rate (HR, bpm), left ventricular developed pressure (LVDP, mm Hg), and maximum rates of contraction and relaxation (mm Hg/sec). LVDP was calculated as the difference between systolic and end-diastolic pressure (mm Hg). CB receptor blockade was induced by treatment with a selective CB1 receptor antagonist SR141716A (N-[piperidin-1-yl]-5-(4-chlorophenyl)-1-[2,4-dichlorophenyl]-4-methyl-1H-pyrazole-3-carboxamid hydrochloride) [9] and selective CB2 receptor antagonist SR144528 (N-(1,3,3-trimethylbicyclo(2.2.1)heptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methyl-benzyl)-pyrazole-3-carboxamide) [3]. These compounds were dissolved in dimethylsulfoxide (DMSO) and after a stabilization period were added to the perfusion solution. The final concentration of the test compounds was ≤1 μM. A special series was conducted with DMSO in a final concentration 0.01%. DMSO in the specified concentration has no effect on cardiac contractility. Cardiac contractility was recorded after 10-min perfusion of the heart with Krebs—Henseleit solution containing one of the antagonists. CB receptor antagonists were synthesized at the Research Triangle Institute (Research Triangle Park). Selective CB receptor agonist HU-210 ((6aR)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo [b,d]pyran-9-methanol, Tocric Cookson) in a final concentration of 1 μM was added to the perfusate for CB receptor activation [10]. HU-210 was dissolved in DMSO immediately before the experiment. After 20-min adaptation, the hearts were perfused with Krebs—Henseleit solution containing (10 min) or not containing HU-210 (10 min). This procedure was followed by 45-min total normothermic ischemia and 30-min perfusion.

The isolated rat hearts perfused with Krebs—Henseleit solution containing DMSO in a final concentration ≤0.01% served as the control. The scheme of treatment was similar to that in experiments with HU-210.

The results were analyzed by Student's *t* test and Mann—Whitney test.

RESULTS

Coronary reperfusion after 45-min total ischemia was followed by a decrease in cardiac contractility in control animals. During the reperfusion period, HR decreased by 1.5-2.0 times compared to that observed before total ischemia (223±10 bpm; Fig. 1, *a*). Bradycardia persisted until the end of reoxygenation. LVDP of the isolated heart was 80.4±4.2 mm Hg before ischemia and decreased to 30% of the preischemic level after 5-min reperfusion (Fig. 1, *b*). By the end of reperfusion, LVDP slightly increased and corresponded to 50% of the basal level (Fig. 1, *b*).

After total ischemia, end-diastolic pressure increased by 2 times compared to the basal level. End-diastolic pressure increased after the start of reperfusion and remained high over the reperfusion period (Fig. 1, *c*). The maximum rate of contraction in the beginning of reperfusion was 3.5-fold lower compared to the preischemic level (31.4±2.2 mm Hg/sec). After 30-min reperfusion, the maximum rate of contraction corresponded to 50% of the basal level (Fig. 1, *d*). The maximum rate of relaxation underwent similar changes (Fig. 1, *e*).

HU-210 in a dose of 1 μM had no effect on HR of the isolated heart during postischemic reperfusion (Fig. 1, *a*). HR in these rats did not differ from that in control animals (Fig. 1, *a*). Activation of CB receptors was not followed by significant changes in end-diastolic pressure during the reperfusion period (Fig. 1, *c*). However, preactivation of CB receptors was accompanied by a short-term decrease in contractility of the isolated heart during reperfusion followed by its recovery to the control level (Fig. 1, *b*). LVDP after 5-min reperfusion was 1.5-fold below the control. By the 15th and 30th minutes, the force of isolated heart contraction increased to the control level (Fig. 1, *b*). After CB receptor activation, the contraction rate returned to normal more slowly than in the control. The rate of contraction after 5-min reperfusion was 2-fold lower compared to the control. By the end of the study, the contraction rate increased to the control level (Fig. 1, *d*). Changes in the relaxation rate were similar to those in the contraction rate (Fig. 1, *e*). After 5-min reperfusion, the relaxation rate was 1.5-fold lower than in

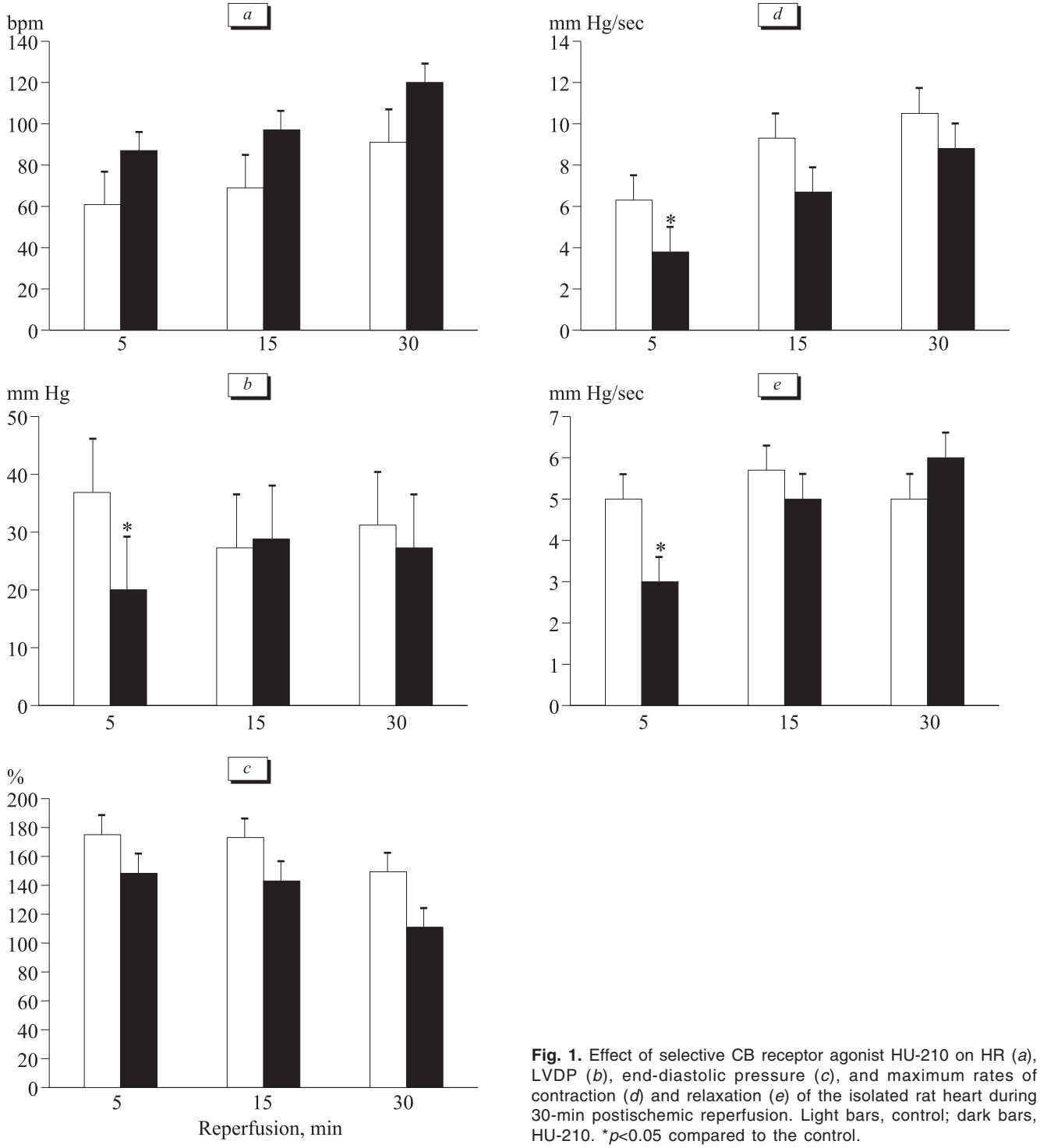

Fig. 1. Effect of selective CB receptor agonist HU-210 on HR (*a*), LVDP (*b*), end-diastolic pressure (*c*), and maximum rates of contraction (*d*) and relaxation (*e*) of the isolated rat heart during 30-min postischemic reperfusion. Light bars, control; dark bars, HU-210. *$p<0.05$ compared to the control.

the control. However, the relaxation rate increased to the control level by the 15th and 30th minutes of study (Fig. 1, *e*).

These data show that stimulation of myocardial CB receptors is accompanied by the decrease in LVDP and rates of left ventricle contraction and relaxation during the reperfusion period. It can be hypothesized that activation of CB receptors aggravates cardiac contractile dysfunction during reperfusion. The negative inotropic effect was transitory, since the test parameters returned to normal after 15-min reoxygenation. This transitory inotropic effect of HU-210 is probably associated with dissociation of the HU-210-CB receptor complex or desensitization of CB receptors. The negative inotropic of HU-210 was observed under

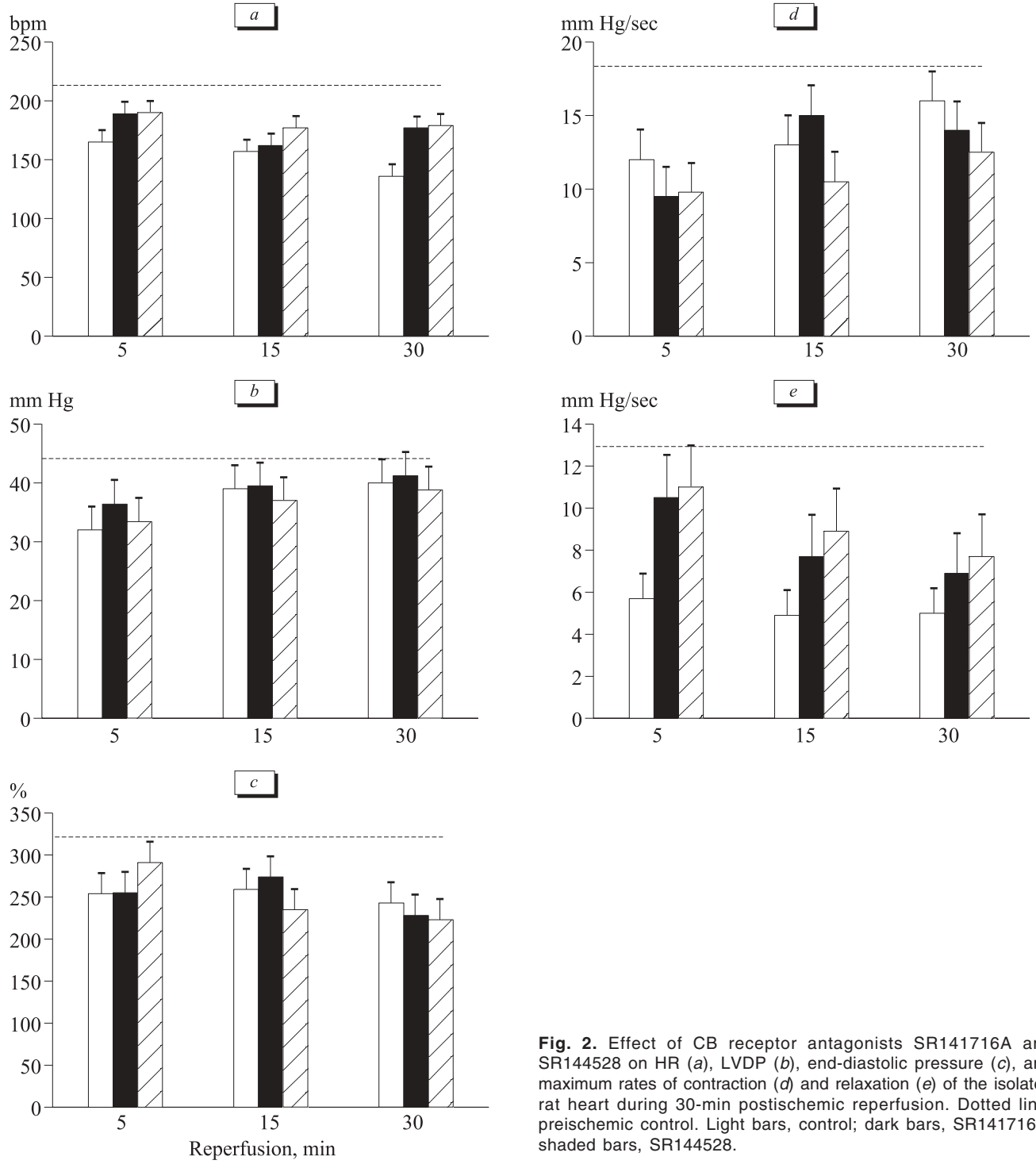

Fig. 2. Effect of CB receptor antagonists SR141716A and SR144528 on HR (*a*), LVDP (*b*), end-diastolic pressure (*c*), and maximum rates of contraction (*d*) and relaxation (*e*) of the isolated rat heart during 30-min postischemic reperfusion. Dotted line, preischemic control. Light bars, control; dark bars, SR141716A; shaded bars, SR144528.

normoxic conditions, but became less significant by the 15th minute of reperfusion. These results are consistent with published data that CB receptor activation leads to a decrease in the force and rate of contraction of intact isolated rat heart [6]. Our study indicates that the negative inotropic effect of cannabinoids persists during coronary reperfusion.

The inotropic effects of CB receptor agonist are realized via cAMP. This conclusion is derived from published data that stimulation of CB receptors results in dose-dependent inhibition of intracellular cAMP synthesis [8]. Moreover, synthetic enzyme-resistant cAMP analogue 8-bromo-cAMP increases the force of contraction and accelerates contraction and relaxation of the isolated papillary muscle from

guinea pigs. These data suggest that the decrease in the force of contraction and rates of cardiac contraction and relaxation under conditions of CB receptor stimulation results from inhibition of cAMP synthesis. This hypothesis requires further investigations.

HR, the force of isolated heart contraction, end-diastolic pressure, and postischemic rates of contraction and relaxation after CB1 receptor blockade with SR141716 (1 μM) did not differ from the control (Fig. 2). It can be hypothesized that endogenous agonists of CB1 receptors are not involved in contractile dysfunction of the isolated heart during reperfusion.

CB2 receptor blockade after 10-min perfusion of the isolated heart with SR144528-containing solution was followed by minor changes in HR, force of cardiac contractions, LVDP, end-diastolic pressure, and rates of contraction and relaxation during the reperfusion period (Fig. 2). Hence, *in vitro* pump dysfunction of the heart during reperfusion is not related to CB2 receptor activation with endogenous cannabinoids.

We conclude that CB receptor stimulation *in vitro* aggravates the impairment of cardiac contractility during ischemia/reperfusion. Endogenous cannabinoids are not involved in the development of contractile dysfunction of the isolated myocardium during reperfusion.

This work was supported by the Russian Foundation for Basic Research. We are grateful to Dr. J. Kevin Gormley (NIDA) for CB receptor antagonists. We thank Yu. B. Lishmanov (Corresponding Member of the Russian Academy of Medical Sciences) for helpful remarks.

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