Activation of Cannabinoid Receptors Decreases the Area of Ischemic Myocardial Necrosis

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We studied the possibility of decreasing the area of ischemic necrosis during myocardial infarction with HU-210, a selective cannabinoid receptor agonist. Activation of cannabinoid receptors with HU-210 had practically no effect on collateral blood flow in the myocardium, but considerably decreased the area of necrosis. There results indicate that cannabinoid receptor agonist HU-210 possesses cardioprotective activity and delays the formation of necrotic zones during coronary occlusion and reperfusion.

Key Words: *cannabinoid receptors; myocardial infarction*

Endogenous cannabinoids (CB) are a new class of endogenous biologically active substances possessing cardiovascular activity [5,6]. These CB receptor agonists affect the autonomic nervous system [7,13], which probably contributes to their influence on myocardial activity. However, the presence of specific CB receptors in the myocardium [6] suggests that these substances can directly modulate cardiac activity. CB receptors are coupled via G proteins with intracellular signaling systems [4,10]. CB inhibits cAMP synthesis in the myocardium [8]; interestingly, similar effect is produced by propranolol that decreases the area of myocardial infarction. Our previous studies showed that the CB receptor agonist HU-210 (50 μg/kg, intravenously) possesses pronounced antiarrhythmic properties during epinephrine-induced arrhythmias, which indicates that antiadrenergic activity of this CB is high and comparable with that of propranolol [2]. We hypothesized that CB receptor agonists have cardioprotective activity.

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Here we studied whether the activation of CB receptors can decrease the area of myocardial ischemic necrosis.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 250-300 g. Myocardial infarction was induced by 45-min ischemia and 120-min reperfusion under barbamyl anesthesia (60 mg/kg intravenously at 30-min intervals) [11]. Artificial ventilation with atmospheric air was performed using a RO-2 device. After 120-min reperfusion the coronary artery was ligated. Staining agent Patent violent blue (Sigma) was injected intravenously to visualize nonischemic regions and zone of hypoperfusion (risk zone). The heart was dissected, ventricles were separated from atria, and 5-6 cross-sections (2 mm) were prepared. The risk zone was separated from nonischemic regions and incubated with 0.1% nitroblue tetrazolium (Sigma) in phosphate buffer (pH 7.4) at 37°C for 15 min. The zone of myocardial infarction was separated from the risk zone (hypoperfusion) using a binocular lens and weighted [11].

The 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]py-

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ran-9-methanol, was synthesized at the Tocris Cookson Company [10]. The preparation was dissolved *ex tempore* in a mixture of Cremophore EL (Sigma), 96% ethanol, and 0.9% NaCl (ratio 1:1:18) and administered in a dose of 50 μg/kg 15 min before coronary artery ligation. The scheme of treatment and doses of the CB receptor agonist and solvent were selected taking into account published data on antiarrhythmic activity of preparations [2]. Control animals were intravenously injected with the solvent before coronary occlusion.

The results were analyzed by Student's *t* test.

RESULTS

In control rats with myocardial infarction the zone of hypoperfusion constituted about 50% of left ventricle weight. This region was not oxygenated during coronary occlusion (Table 1). In animals receiving HU-210, the hypoperfused area did not differ from the control, which indicates that activation of CB receptors had no effect on collateral blood flow in the myocardium. However, the absolute and relative weights of the necrotic zone in rats injected with HU-210 were lower than in control animals (Table 1).

Our results show that the selective CB receptor agonist HU-210 produced a cardioprotective effect. In our experiments HU-210 was injected in low doses (50 μg/kg), which suggests that cardioprotective activity of this preparation is receptor-dependent and not associated with nonspecific membrane-stabilizing effects. HU-210 is the agonist of CB1 and CB2 receptors [9]. It remains unclear, which type of receptors mediates the effect of this preparation. CB1 receptors are localized in the central nervous system. CB2 receptors are present in peripheral organ and tissues, including the myocardium [6]. Our previous studies showed that peripheral, but not central administration of this cannabinoid produces an antiarrhythmic effect during epinephrine-induced arrhythmias [2]. Antiarrhythmic activity of HU-210 during coronary occlusion and reperfusion is related to activation of peripheral CB2 receptors [1]. Vagotomy and chemical sympathectomy do not abolish the cardiovascular effect of this preparation [14]. These data suggest that the protective effect of HU-210 is not realized via the autonomic nervous system. Cardioprotective activity of the preparation is probably associated with activation of peripheral CB2 receptors. The type of receptors and mechanisms underlying cardioprotective activity of HU-210 require further investigations.

TABLE 1. Effect of Pretreatment with HU-210 (50 μg/kg) on the Area of Myocardial Infarction in Rats (*M*±*m*)

Parameter	Control	HU-210
Weight of the left ventricle, g	0.556±0.021	0.503±0.017
Weight of perfusion zone, g	0.290±0.016	0.236±0.011
Weight of hypoperfused zone, g	0.265±0.009	0.244±0.008
Weight of necrotic zone, g	0.141±0.007	0.111±0.006*
Necrotic zone/hypoperfusion zone, %	53.21±2.16	45.49±2.76**

Note. *$p<0.01$ and **$p<0.05$ compared to the control.

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REFERENCES

1. D. S. Ugdyzhekova, N. A. Bernatskaya, A. V. Krylatov, *et al*., *Byull. Eksp. Biol. Med*., **131**, No. 6, 617-620 (2001).
2. D. S. Ugdyzhekova, L. A. Maimeskulova, Yu. G. Davydova, *et al*., *Ibid*., **130**, No. 11, 552-554 (2001).
3. W. S. Aronow and J. N. Cassidy, *N. Engl. J. Med*., **291**, No. 2, 65-67 (1974).
4. M. Bouaboula, N. Desnoyer, P. Carayon, *et al*., *Mol. Pharmacol*., **55**, 473-480 (1999).
5. C. C. Felder and M. Glass, *Ann. Rev. Pharmacol. Toxicol*., **38**, 179-200 (1998).
6. S. Galiegue, S. Mary, J. Marchand, *et al*., *Eur. J. Biochem*., **232**, 54-61 (1995).
7. E. J. Ishac, L. Jiang, K. D. Lake, *et al*., *Br. J. Pharmacol*., **118**, 2023-2028 (1996).
8. D. M. F. Li and C. K. M. Ng, *Clin. Exp. Pharmacol. Physiol*., **11**, No. 1, 81-85 (1984).
9. R. Mechoulam, J. J. Feigenbaum, N. Lander, *et al., Experientia*, **44**, 762-764 (1988).
10. R. G. Pertwee, *Curr. Med. Chem*., **6**, 635-664 (1999).
11. J. E. J. Schultz, A. K. Hsu, H. Nagase, and G. J. Gross, *Am. J. Physiol*., **274**, H909-H914 (1998).
12. K. Varga, K. D. Lake, D. Huangfu, *et al*., *Hypertension*, **28**, 682-686 (1996).
13. R. R. Volmer, I. Cavero, R. J. Ertel, *et al*., *J. Pharm. Pharmacol*., **26**, No. 3, 186-194 (1974).
14. S. P. Welch, *J. Pharmacol. Exp. Ther*., **265**, No. 2, 633-640 (1993).