GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Activation of Type II Cannabinoid Receptors Improves Myocardial Tolerance to Arrhythmogenic Effects of Coronary Occlusion and Reperfusion

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 6, pp. 617-620, June, 2001
Original article submitted January 31, 2001

Preliminary intravenous injection of cannabinoid receptor agonist HU-210 (0.05 mg/kg) reduced the incidence of ventricular arrhythmias during 10-min coronary occlusion and 10-min reperfusion in chloralose-anesthetized rats. Preliminary injection of type I cannabinoid receptor antagonist SR 141716A (3 mg/kg) had no effect on the antiarrhythmic effect of HU-210, while type II cannabinoid receptor antagonist SR 144528 (1 mg/kg) completely abolished the effect of HU-210. Preconditioning with glibenclamide (0.3 mg/kg), an inhibitor of ATP-dependent K^+-channels, did not affect the antiarrhythmic activity of HU-210. These findings suggest that antiarrhythmic effect of HU-210 is mediated through activation of type II cannabinoid receptors rather than activation of K^+-channels.

Key Words: *arrhythmias*; *cannabinoid receptors*; *K^+ channels*

Agonists of cannabinoid (CB) receptors produce inotropic and chronotropic effects *in vivo* [13]. In addition, CB_1-receptor agonist Δ^9-tetrahydrocannabinol potentates the arrhythmogenic effect of epinephrine [6]. However, this substance stimulates central CB_1-receptors and inhibits peripheral CB_2-receptors [1], and therefore it remains unclear whether its proarrhythmogenic effect is mediated through activation of CB_1- or inhibition of CB_2-receptors. Moreover, it is not clear whether cannabinoids modulate myocardial resistance to ischemia-reperfusion-induced arrhythmias.

The effects of cannabinoids on the cardiovascular system [13], their antinociceptive activity [10], and modulatory effect on the intracellular signaling pathways [10] are similar to those of opioids [5]. Moreover, the analgesic effect of CB agonists is related to mobilization of endogenous opioids and activation of opioid receptors [14]. It can be hypothesized that the cardiotropic effects of cannabinoids are mediated via activation of ATP-dependent K^+-channels (K_{ATP}) [12].

Our aim was to study the effect of activation of CB_1- and CB_2-receptors on myocardial resistance to arrhythmias provoked by coronary occlusion and reperfusion and the role of K_{ATP}-channels in this phenomenon.

MATERIALS AND METHODS

Experiments were performed on Wistar rats weighing 250-300 g, narcotized with α-chloralose (100 mg/kg, intraperitoneally) and ketamine (5 mg/kg, intravenously) and artificially ventilated with room air using a

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0007-4888/01/1316-0523$25.00

RO-2 apparatus. Acute ischemia and reperfusion were modeled by occlusion of the left coronary artery [13]. ECG was recorded in standard lead I, amplified using an UBF4-03 amplifier, and analyzed using an original software. The episodes of ventricular extrasystoles, ventricular tachycardia (VT), and ventricular fibrillations (VF) were counted throughout the periods of ischemia and reperfusion.

The following substances were used: HU-210, a potent agonist for CB_1- and CB_2-receptors synthesized by Prof. R. Mechoulam [10]; selective CB_1-receptor antagonist SR141716A ([N-piperidine-1-yl]-5-(4-chlorophenyl)-1-(1,2-dichlorphenyl)-4-methyl-1H-pyrazole-3-carboxamide HCl) [8]; selective CB_2-receptor antagonist SR144528 ([N-(1S)-endo-1,3,3-trimethyl bicyclo [2.2.1] hepta-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide) [2]. CB-receptor antagonists were synthesized by Dr. V. Parker from the Research Triangle Institute, USA. K_{ATP}-channel blocker glibenclamide (ICN Biomedicals) was injected in a dose of 0.3 mg/kg 45 min before occlusion [12]. All substances, except glibenclamide, were diluted *ex tempore* in a Cremophore EL: ethanol:0.9% NaCl mixture (1:1:18). Glibenclamide was dissolved in 45% 2-hydroxypropyl-β-cyclodextrin water solution (RBI). HU-210 (50 μg/kg) was administered 15 min before coronary occlusion. SR141716A (3 mg/kg) and SR144528 (1 mg/kg) were injected 10 min before HU-210 administration or 25 min before coronary occlusion. The doses of CB-receptor ligands, solvents, and pharmacological schedules were chosen on the basis of published data on their cardiovascular and antinociceptive activities [2,8,9,14]. Control animals received solvents before occlusion.

The data were statistically analyzed by χ^2 test.

RESULTS

A 10-min coronary occlusion induced ventricular arrhythmias (VA) in 85% rats of the control group (Table 1). Malignant arrhythmias (VT and VF) were observed in 59% rats. Reperfusion provoked life-threatening ventricular arrhythmias (VT and VF) in 89% control rats.

The nonselective agonist of CB_1- and CB_2-receptors HU-210 improved myocardial tolerance to arrhythmogenic effects of ischemia and reperfusion (Table 1): malignant arrhythmias developed in only 1 of 14 rats and in 86% rats no arrhythmias occurred after coronary occlusion. During reperfusion, VT developed in only 1 of 14 rats, while 10 rats were resistant to arrhythmogenic effect of reperfusion. As cannabinoid was effective in a very low dose (50 μg/kg), the observed antiarrhythmic effect is most likely receptor-mediated.

The effects of SR141716A and SR144528 on the incidence of arrhythmias induced by coronary occlusion and reperfusion were insignificant (Table 1), hence endogenous CB-receptor agonists do not play a role in arrhythmia genesis during 10-min myocardial ischemia or reperfusion. It was found that selective CB_2-receptor blocker SR144528 abolished the antiarrhythmic effect of HU-210, while CB_1-receptor inhibitor SR141716A was ineffective. This suggests that the antiarrhythmic effect of HU-210 is mediated via CB_2-receptors.

Since psychotropic side effects of cannabinoids are associated with activation of type I receptors, agonists of type II CB-receptors are perspective agents for developing novel antiarrhythmic drugs. For instance, cannabinoid HU-210 possesses high antiarrhythmic activity and produces a pronounced effect in a dose of

TABLE 1. Effects of Cannabinoid Receptor Ligands on Incidence (Number of Animals) of Ischemia and Reperfusion Arrhythmias

Period of observation	Control (*n*=27)	HU-210, 50 μg/kg (*n*=14)	SR141716A, 3 mg/kg		SR144528, 1 mg/kg		Glibenclamide, 0.3 mg/kg+HU-210 (*n*=14)
			without HU-210 (*n*=14)	+HU-210 (*n*=14)	without HU-210 (*n*=14)	+HU-210 (*n*=14)	
10-min ischemia							
without VA (%)	4 (15)	12* (86)	2 (14)	12* (86)	2 (14)	3 (21)	13* (93)
MVA (%)	21 (78)	1* (7)	12.(86)	2* (14)	11 (79)	11 (79)	1* (7)
VT (%)	14 (52)	1* (7)	8 (57)	2** (14)	7 (50)	8 (57)	1** (7)
VF (%)	2 (7)	0	0	0	0	0	0
10-min reperfusion							
without VA (%)	4 (11)	10* (64)	2 (14)	12*(86)	2 (14)	2 (14)	11* (79)
MVA (%)	24 (89)	4* (36)	12 (86)	2* (14)	11 (79)	12 (86)	2* (14)
VT (%)	21 (78)	1* (7)	11 (79)	2* (14)	10 (71)	10 (71)	2 (14)
VF (%)	3 (11)	0	1 (7)	1 (7)	2 (14)	2 (14)	0

Note: *p<0.001, **p<0.01 compared to the control.

0.05 mg/kg, while the effective doses of conventional antiarrythmics verapamil and propranolol for preventing the occlusion-reperfusion-induced arrhythmias are 1-2 mg/kg [3].

Taking into account the fact that cannabinoids and opioids produce [10] similar effects on the second messenger system [5], we hypothesized that the antiarrhythmic effect of HU-210 can also be mediated via activation of K_{ATP}-channels. However, K_{ATP}-channel blocker glibenclamide did not eliminate the effect of HU-210 in our experiments. Hence, the antiarrhythmic activity of this cannabinoid are not related to changes in K^+ efflux in cardiomyocytes. It seems unlikely that the autonomic nervous system is involved in the protective action of HU-210, because vagotomy or sympathectomy did not abolish its cardiovascular effects [14]. Mobilization of catecholamines from sympathetic nerve endings in the myocardium [4,7] during ischemia and reperfusion and subsequent activation of adenylate cyclase and accumulation of cyclic AMP, a potent endogenous arrhythmogenic factor, play an important role in the pathogenesis of arrhythmia [4]. Since CB_2-receptor antagonists inhibit adenylate cyclase [10], the antiarrhythmic effect of HU-210 can result from inhibition of cAMP synthesis in cardiomyocytes, although this hypothesis requires experimental validation.

An electrophysiological mechanism underlying the cardioprotective effect of HU-210 should me mentioned. VF and VT during 10-min coronary occlusion and subsequent reperfusion are mediated by the re-entry mechanism [3,7,11]. It can be hypothesized that HU-210 prevents re-entrant arrhythmias, but this assumption should be studied in detail.

Thus, activation of peripheral CB_2-receptors improves myocardial tolerance to arrhythmias induced by coronary occlusion and reperfusion, and this effect is not related to K_{ATP}-channel activation.

We thank Dr. Kevin Gormley (NIDA, USA) for the kind donations of CB-receptor antagonists and Corresponding Member of RAMS Yu. B. Lishmanov for valuable discussion of the results. This work was supported by Wellcome Trust grant No. 047980 (to Prof. R. Pertwee).

REFERENCES

1. M. Bayewitch, M.-H. Rhee, T. Avidor-Reiss, *et al*., *J. Biol. Chem*., **271**, No. 17, 9902-9905 (1996).
2. M. Bouaboula, N. Desnoyer, P. Carayon, *et al*., *Mol. Pharmacol*., **55**, 473-480 (1999).
3. J. H. Botting, M. J. Curtis, and M. J. A. Walker, *Mol. Aspects Med.*, **8**, No. 4, 307-422 (1985).
4. M. J. Curtis, M. K. Pugsley, and M. J. A. Walker, *Cardiovasc. Res.*, **27**, No. 5, 703-719 (1993).
5. B. N. Dhawan, F. Cesselin, R. Raghubir, *et al*., *Pharmacol. Rev.*, **48**, No. 4, 567-592 (1996).
6. U. Estrada, D. A. Brase, B. R. Martin, W. L. Dewey, *Life Sci.*, **41**, 79-97 (1987).
7. M. J. Janse and A. L. Wit, *Physiol. Rev.*, **69**, No. 4, 1049-1169 (1989).
8. R. S. Mansbach, C. C. Rovertti, E. N. Winston, and J. A. Lowe, *Psychopharmacology*, **124**, 315-322 (1996).
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. D. C. Russel, J. S. Lawrie, R. A. Riemersma, and M. F. Oliver, *Am. J. Cardiol.*, **53**, 307-312 (1984).
12. J. E. J. Schultz, A. K. Hsu, H. Nagase, and G. J. Gross, *Am. J. Physiol.*, **274**, H909-H914 (1998).
13. H. Vidrio, M. A. Sanchez-Salvatori, and M. Medina, *J. Cardiovasc. Pharmacol.*, **28**, 332-336 (1996).
14. S. P. Welch, *J. Pharmacol. Exp. Ther.*, **265**, No. 2, 633-640 (1993).