Endogenous Cannabinoids Improve Myocardial Resistance to Arrhythmogenic Effects of Coronary Occlusion and Reperfusion: a Possible Mechanism

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Stimulation of cannabinoid receptors with endogenous cannabinoid anandamide and its enzyme-resistant analogue R-(+)-methanandamide improved cardiac resistance to arrhythmias induced by coronary occlusion and reperfusion. This antiarrhythmic effect was not associated with activation of NO synthase, since pretreatment with N^G-nitro-L-arginine methyl ester had no effect on the incidence of ischemia/reperfusion-induced arrhythmias. Blockade of ATP-dependent K^+ channels with glybenclamide did not abolish the antiarrhythmic effect of R-(+)-methanandamide. Antiarrhythmic activity of endogenous cannabinoids is probably associated with their direct effects on the myocardium.

Key Words: *cannabinoid receptors; arrhythmias; endogenous cannabinoids*

Coronary occlusion and reperfusion produce a pronounced arrhythmogenic effect. The therapy and prevention of ischemic and reperfusion arrhythmias are an urgent medical problem [2]. Arrhythmias provoked by these pathological conditions are modulated by various neurotransmitter systems possessing cardiovascular activity [6]. It can be hypothesized that endogenous cannabinoids produce similar effects, because these bioactive substances induce a wide spectrum of cardiovascular responses [3]. Experiments on isolated heart showed that cannabinoids inhibit the synthesis of cAMP [10], which acts as the endogenous arrhythmogenic factor [6]. Endogenous cannabinoid anandamide inactivates L-type Ca^{2+} channels [9], which improves myocardial resistance to coronary occlusion and reperfusion [6]. We previously demonstrated antiarrhythmic activity of a synthetic cannabinoid receptors agonist [1]. In light of this the hypothesis on the involvement of endogenous cannabinoids in the regulation of myocardial resistance to acute ischemia and reperfusion is quite reasonable.

Published data suggest that anandamide stimulates secretion of NO, a compound possessing antiarrhythmic activity [11], in the endothelium of myocardial vessels [7]. Cannabinoid and opiate receptors belong to the $G_{i/o}$-coupled receptor family [7]. The antiarrhythmic effect of opiate receptor agonists is associated with activation of ATP-dependent K^+ channels (K_{ATP} channels) [8]. We hypothesized that the antiarrhythmic effect of cannabinoids is related to activation of K_{ATP} channels or intensification of NO synthesis.

Here we studied the effects of endogenous cannabinoid anandamide and its enzyme-resistant ana-

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logue R-(+)-methanandamide on myocardial resistance to arrhythmias induced by coronary occlusion and reperfusion and evaluated the contribution of NO and ATP-dependent K^+ channels in the realization of this effect.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-250 g narcotized with chloralose and ketamine (100 and 5 mg/kg intramuscularly, respectively). Acute myocardial ischemia was produced by ligation of the left coronary artery for 10 min, after that coronary blood flow was restored (10-min reperfusion) [12]. Artificial ventilation was performed using a RO-2 device. ECG (lead II) was monitored and recorded during 10-min coronary occlusion and 10-min reperfusion. We estimated the percent of animals without arrhythmias (no ventricular arrhythmias, NVA) and evaluated the incidence of multiple ventricular extrasystoles (MVE) and episodes of ventricular tachycardia (VT) and ventricular fibrillation. ECG was recorded and analyzed using an UBF4-03 biological potential amplifier equipped with special software.

The endogenous agonist of cannabinoid receptors anandamide (arachidonylethanolamide) [4] and its enzyme-resistant analogue R-(+)-methanandamide (R-(+)-arachidonyl-1'-hydroxy-2'-propylamide) [4] were used in doses of 10 and 5 mg/kg, respectively. Anandamide and R-(+)-methanandamide were synthesized at the Laboratory headed by A. Makriyannis. Blockade of K_{ATP} channels was induced by intravenous injection of 0.3 mg/kg glybenclamide [12]. Nonselective NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 50 mg/kg) was used to block NO synthesis [5]. Glybenclamide, L-NAME, chloralose, and Cremaphore EL were purchased from Sigma, and ketamine from Moscow Plant for Endocrine Preparations. Cannabinoid receptor agonists were dissolved *ex tempore* in an ethanol:Cremaphore EL:0.9% NaCl mixture (ratio 1:1:18). Anandamide and R-(+)-methanandamide were injected intravenously 10 min before coronary occlusion. Glybenclamide was administered 45 min before ischemia. L-NAME was injected into the femoral artery 15 min before administration of endogenous cannabinoids. The doses of preparations, methods for dissolution, and route of treatment were selected taking into account published data on *in vivo* antinociceptive effects of anandamide and R-(+)-methanandamide [4]. Control animals were intravenously injected with ethanol:Cremaphore EL:0.9% NaCl (ratio 1:1:18) mixture before coronary occlusion.

The results were analyzed by χ^2 test.

RESULTS

Anandamide markedly decreased the incidence of MVE and VT compared to the control (Table 1). The number of rats without cardiac arrhythmias increased from 18 to 79%. In animals receiving anandamide, reperfusion caused less severe heart rhythm disturbances compared to controls (Table 1). The number of animals with normal heart rhythm increased by 4 times compared to the control. Therefore, endogenous cannabinoid anandamide improves cardiac resistance to the arrhythmogenic effect of coronary occlusion and reperfusion.

TABLE 1. Effect of Intravenous Administration of Cannabinoid Receptor Agonists on the Incidence of Ischemic and Reperfusion Arrhythmias in Rats

Period	Control (n=17)	Anandamide (n=14)	Anandamide+ L-NAME (n=13)	R-(+)-methanandamide		
				without blockers (n=14)	+L-NAME (n=13)	+glybenclamide (n=15)
Ischemia, 10 min						
NVA	3 (18)	11 (79)*	10 (77)**	11 (79)*	10 (71)**	12 (80)*
MVE	13 (76)	3 (21)**	2 (15)**	3 (21)**	4 (29)**	3 (20)*
VT	8 (47)	1 (7)***	0 (0)***	2 (14)	3 (21)	2 (13)***
Reperfusion, 10 min						
NVA	3 (18)	10 (71)**	11 (85)*	11 (79)*	11 (79)*	8 (53)***
MVE	14 (82)	4 (29)**	2 (15)*	1 (7)*	3 (21)*	7 (47)***
VT	10 (59)	3 (21)***	0 (0)**	1 (7)**	2 (14)***	4 (27)

Note. *p<0.001, **p<0.01, and ***p<0.025 compared to the control. In parentheses: percent of the total number of animals in each group.

Pretreatment with R-(+)-methanandamide increased the number of rats without ventricular arrhythmias and decreased the incidence of MVE (similarly to anandamide, Table 1). During myocardial reperfusion the number of animals without cardiac arrhythmias increased by 61%. Under these conditions the incidence of MVE and VT decreased to 7%; episodes of ventricular fibrillation were not observed. These results indicate that R-(+)-methanandamide possesses antiarrhythmic activity (similarly to anandamide). It should be emphasized that the dose of R-(+)-methanandamide was 2 times lower than the concentration of anandamide. Therefore, R-(+)-methanandamide is 2-fold more effective than anandamide. Since R-(+)-methanandamide is more resistant to enzymes than anandamide, antiarrhythmic activity is produced by endogenous cannabinoids rather than their metabolites.

Our previous studies showed that cannabinoids induces NO synthesis in the endothelium of coronary arteries [7]. NO improves myocardial resistance to the arrhythmogenic effect of ischemia and reperfusion [11]. We hypothesized that antiarrhythmic activity of endogenous cannabinoids is related to activation of NO synthase. However, pretreatment with the NO synthase blocker L-NAME did not modulate the antiarrhythmic effect of anandamide and R-(+)-methanandamide (Table 1). Therefore, the cannabinoid-induced increase in cardiac resistance to arrhythmias accompanying coronary occlusion and reperfusion was not related to NO synthase activation.

Preliminary blockade of K_{ATP} channels with glybenclamide did not abolish the protective effect of R-(+)-methanandamide (Table 1). Since glybenclamide has no effect on ischemic and reperfusion arrhythmias (data not shown), it can be hypothesized that the methanandamide-induced increase in cardiac resistance to coronary occlusion and reperfusion is not associated with activation of K_{ATP} channels.

Experiments on isolated heart showed that cannabinoids inhibit the synthesis of cAMP [10], which acts as the endogenous arrhythmogenic factor [6]. The data suggest that the antiarrhythmic effect of endogenous cannabinoids is related to inhibition of intracellular cAMP production. Moreover, the blockade of norepinephrine release from adrenergic cardiac nerves during activation of presynaptic cannabinoid receptors with anandamide [13] also contributes to the improvement of cardiac resistance to the arrhythmogenic effect of ischemia and reperfusion.

Thus, endogenous cannabinoids increase cardiac resistance to the arrhythmogenic effect of coronary occlusion and reperfusion. Antiarrhythmic activity of endogenous cannabinoids is not associated with activation of NO synthase or stimulation of ATP-dependent K^+ channels.

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