Endogenous Cannabinoid Anandamide Increases Heart Resistance to Arrhythmogenic Effects of Epinephrine: Role of CB_1 and CB_2 Receptors

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Intravenous injection of 10 mg/kg anandamide reduces the incidence and duration of epinephrine-induced arrhythmias in rats. SR141716A and SR144528, antagonists of cannabinoid receptor I and II did not abolish the antiarrhythmic effect of anandamide. These data suggest that the antiarrhythmic effect of anandamide is nonspecific or mediated via unknown cannabinoid receptors, but not associated with activation of cannabinoid receptors I and II.

Key Words: *cannabinoid receptors; epinephrine; arrhythmias*

Recent studies revealed cannabinoid receptors (CB-R) and their endogenous agonists, including arachidonylethanolamide (anandamide) [4]. Type I CB-R (CB_1-R) are localized in the central and peripheral nervous systems [5]. Peripheral organs and tissues contain type II CB-R (CB_2-R) [5]. Endogenous cannabinoids possess pronounced cardiovascular activity [10,11,15]. CB-R modulate activity of intracellular signaling systems via G proteins [2]. In cardiomyocytes cannabinoids decrease activity of adenylate cyclase coupled with β-adrenoceptors [7] and inhibit cAMP synthesis thus playing an important role in the pathogenesis of arrhythmias [8]. These data suggest that cannabinoids counteract the arrhythmogenic effects of epinephrine. However, the role of endogenous cannabinoids in the pathogenesis of arrhythmias remains unclear. Here we studied the role of the endogenous cannabinoid system in the regulation of heart resistance to arrhythmogenic factors. We evaluated the effect of anandamide on heart resistance to epinephrine-induced arrhythmia.

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MATERIALS AND METHODS

Experiments were performed on Wistar rats weighing 150-200 g. Arrhythmias were induced by intravenous injection of 120 μg/kg epinephrine under ether anesthesia. Electrocardiogram (ECG) was recorded (lead I) for 5 min after epinephrine administration, and the incidence of ventricular extrasystoles (VE), ventricular tachycardia (VT), and ventricular fibrillation (VF) was estimated using a UBF4-03 amplifier, personal computer, and original computer software. Six-sixteen extrasystoles over 5-min ECG recording were considered as single VE.

We used the endogenous CB-R agonist anandamide (arachidonylethanolamide) [4], selective CB_1-R antagonist SR141716A (N-[piperidine-1-yl]-5-[4-chlorphenyl]-1-[1,2-dichlorphenyl]-4-methyl-1H-pyrazol-3-carboxamide HCl) [9], and selective CB_2-R antagonist SR144528(N-(1S)-endo-1,3,3-trimethylbicyclo [2.2.1]hepta-2-yl]-5-(4-chlor-3-methylphenyl)-1-(4-methylbenzyl)-pyrazol-3-carboxamide) [2].

Arachidonic acid (NitroMed) was used as a reference substance. CB-R ligands were synthesized by Dr. V. Parker (Research Triangle Institute, USA). The preparations were dissolved in a Cremophore EL: ethanol:physiological saline mixture (1:1:18) immediately

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before intravenous injection. Anandamide and arachidonic acid were injected 10 min before epinephrine administration. SR141716A and SR144528 were injected 10 min before anandamide administration. Control animals were intravenously injected with the solvent before epinephrine administration. The results were analyzed by χ^2 and Student's *t* tests.

RESULTS

Intravenous injection of 120 μg/kg epinephrine produced VE, VT, and VF in 86, 46, and 15% rats, respectively (Fig. 1). The solvent for CB-R ligands did not affect the type and incidence of epinephrine-induced arrhythmias (Fig. 1). Therefore, the animals injected with the solvent before epinephrine administration served as the control.

Anandamide increased the number of rats resistant to arrhythmogenic effects of epinephrine and decreased the number of animals with multiple VE by 50 and 40%, respectively, compared to the control. Therefore, anandamide possesses pronounced antiarrhythmic activity.

Selective CB_1-R and CB_2-R antagonists were used to evaluate receptor specificity of the anandamide-induced antiarrhythmic effects. SR141716A and SR144528 did not inhibit antiarrhythmic activity of this endogenous cannabinoid and had no effect on epinephrine-induced arrhythmias (Fig. 1). Thus, anandamide increases heart resistance to epinephrine-induced arrhythmias, and this effect is not mediated via central CB_1-R and peripheral CB_2-R. It should be emphasized that apart from CB-R, peripheral vessels contain anandamide receptors, whose activation causes vasorelaxation [15]. Probably, the effects of this endogenous cannabinoid are mediated via anandamide receptors localized in the myocardium. Previous studies showed that CB-R agonists (*e.g.*, anandamide) block adenylate cyclase in rat ventricular myocytes [7] and inhibit forskolin-induced cAMP production [6]. These processes probably underlie the antiarrhythmic effect of anandamide, because intensification of cAMP synthesis is the major factor provoking arrhythmias under conditions of β-adrenoceptor stimulation with epinephrine [8]. cAMP activates sarcolemmal Ca^{2+} channels, induces Ca^{2+} mobilization from the sarcoplasmic reticulum, increases Ca^{2+} concentration in the myoplasm and, therefore, promotes electrical instability of the heart and development of arrhythmias [3,8].

Intravenous injection of anandamide produced bradycardia and led to shortening of the *P—Q* interval (Table 1). This effect was short-lasting, and 10 min after anandamide administration both parameters returned to normal, which was probably related to rapid catabolism of anandamide. Previous studies showed that L-type Ca^{2+} channel blockers caused bradycardia and prolonged the *P—Q* interval. Increasing the vagal tone was accompanied by similar changes [12]. At the same time, vagal stimulation not only decreases heart rate (HR), but also leads to shortening of corrected *Q—T* (*Q—Tc*) intervals [12,13]. In our experiments, the *Q—Tc* interval remained unchanged (Table 1). These data suggest that the antiarrhythmic effect of anandamide is realized via a decrease in adenylate cyclase activity or blockade of Ca^{2+} channels in cardiomyocytes, but is not related to functional changes in the parasympathetic nervous system. However, this assumption requires further investigations. Anandamide is rapidly metabolized [1] and, therefore, the antiarrhythmic effect is probably associated with its metabolites (*e.g.*, arachidonic acid). At the same time, arachidonic acid possesses no antiarrhythmic activity (Fig. 1). Thus, the antiarrhythmic effect of arachidonylethanolamide is probably related to the activation of anandamide receptors or nonspecific influence of this eicosanoid.

Our results indicate that endogenous cannabinoid anandamide increases heart resistance to arrhythmo-

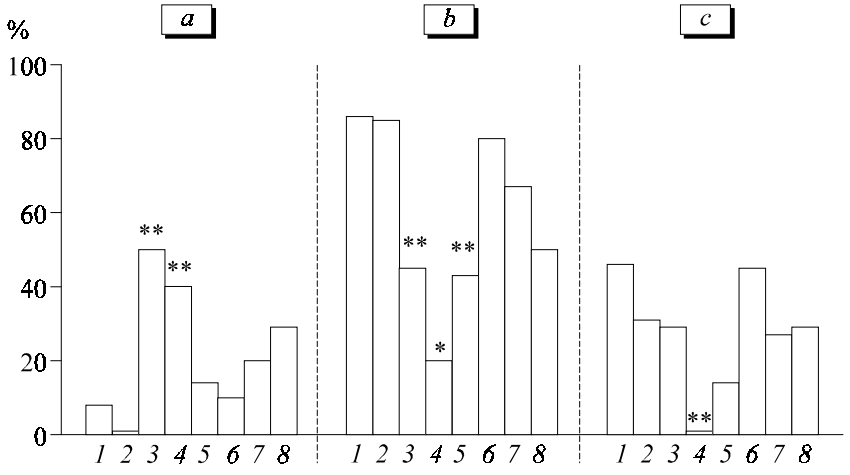

Fig. 1. Effects of anandamide and type I and II cannabinoid receptor blockers on the incidence of epinephrine-induced arrhythmias: number of animals without arrhythmias (*a*) and with ventricular extrasystoles (*b*) and tachycardia (*c*). Epinephrine (*1*), control (solvent, *2*), anandamide (*3*), SR141716A+anandamide (*4*), SR144528+ anadamide (*5*), SR141716A (*6*), SR144528 (*7*), and arachidonic acid (*8*). **p*<0.001 and ***p*<0.01 compared to the control.

TABLE 1. Effect of Anandamide (10 mg/kg Intravenously) on ECG Parameters (*M*±*m*)

Parameter	Before administration	After administration, min	
		5	10
HR	357±7.55	323±6.78*	353±11.17
P—R	47.83±1.96	55.96±2.16**	47.42±2.59
QRS	22.33±0.68	21.75±0.53	20.72±0.77
Q—T	38.5±1.28	37.95±0.53	35.61±1.00
Q—Tc	110.76±1.25	108.73±1.19	107.77±0.95
P—Q	34.90±1.84	42.39±2.06**	37.16±2.75

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

genic effects of epinephrine. This effect is not associated with the activation of CB_1-R and CB_2-R.

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