Negative Chronotropic Effect of Cannabinoids and Their Water-Soluble Emulsion Is Related to Activation of Cardiac CB1 Receptors

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Intravenous injection of cannabinoids dissolved in cremophore EL:ethanol:NaCl mixture and water-soluble emulsion of the same cannabinoids caused identical negative chronotropic effects in chloralose-narcotized rats. Selective CB1 and CB2 receptor antagonist HU-210 also induced a negative chronotropic effect in rats, while pre-injection of CB1 receptor antagonist SR 141716A completely abolished this effect of HU-210. Selective CB2 receptor antagonist SR 144528 had no effect on HU-210-induced bradycardia. Preinjection of ganglioblocker hexamethonium also did not abolish the negative chronotropic effect of HU-210 and ACPA. Perfusion of isolated rat heart with Krebs—Henseleit solution containing HU-210 in a final concentration of 100 nM reduced heart rate. It was shown that the negative chronotropic effect of cannabinoids is mediated through activation of cardiac CB1 receptors.

Key Words: *cannabinoids; heart rhythm*

Cannabinoid (CB) receptors belong to the G-protein-conjugated receptor superfamily [5,6]. Two receptor types are distinguished: CB1 and CB2. Activation of cloned CB receptors leads to adenylate cyclase inhibition [4]. Endogenous agonists of CB receptors are identified: arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol. CB1 receptors were detected in the myocardium [3], but their role in the regulation of cardiac function remains not quite clear. It was found that selective CB agonist HU-210, injected intravenously, caused long-lasting bradycardia in rats [9]. It remained unclear whether this effect was related to activation of CB receptors and where CB receptors regulating heart rate (HR) are located in the body.

We studied the receptor nature of the negative chronotropic effect (CTE) of cannabinoids and localization of CB receptors regulating heart rhythm.

MATERIALS AND METHODS

Experiments were carried out *in vivo* on Wistar rats (200-250 g) narcotized with α-chloralose (50 mg/kg intraperitoneally) and *in vitro* on isolated perfused hearts of these animals.

ECG in thoracic lead II was recorded for 15 min after injection of CB agonists and for 25 min after intravenous injection of CB antagonists using an UBF4-03 biopotential amplifier and original applied software for ECG recording and processing [5,6]. HR was evaluated using ECG. The animals were intravenously injected with boluses: selective

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CB1 and CB2 receptors agonist HU-210 ((6a*R*)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[b,d]pyran-9-methanol) in a dose of 0.1 mg/kg (n=8); selective CB1 agonist ACPA (arachidonylcyclopropylamide) in a dose of 0.125 mg/kg (n=7); endogenous cannabinoid anandamide in a dose of 2.5 mg/kg (n=7); enzyme-resistant synthetic anandamide analog, a selective CB1 receptor agonist methanandamide ((*R*)-*N*-(2-hydroxy-1-methylethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide) in a dose of 2.5 mg/kg (n=7) (all agonist manufactured by Tocris Cookson Ltd). The following CB receptor antagonists were used: selective CB1 antagonist SR 141716A (N-[piperidin-1-yl]-5-(4-chlorophenyl)-1-[2,4-dichlorophenyl]-4-methyl-1H-pyrazole-3-carboxamide hydrochloride) in a dose of 1 mg/kg (n=13) and selective CB2 antagonist SR 144528 (N-(1,3,3-trimethylbicyclo(2,2,1)heptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methyl-benzyl)pyrazole-3-carboxamide) in a dose of 1 mg/kg (n=13), which were also injected intravenously 10 min before injection of cannabinoids. CB receptor antagonists were synthesized at Research Triangle Institute, USA. Peripheral autonomic ganglion blocker hexamethonium (Sigma) was injected in a dose of 10 mg/kg intravenously 10 min before HU-210 (n=15) and ACPA (n=11).

Ligands of CB receptors were *ex tempore* dissolved in the cremophore EL (Sigma):ethanol:0.9% NaCl mixture (1:1:18). Water soluble emulsions containing ACPA, anandamide, and methanandamide (Tocris Cookson Ltd.) were used in some experimental series. Hexamethonium was dissolved in 0.9% NaCl. The choice of the doses and concentrations was based on published data [3,5,6,8,9] and our findings [1].

For *in vitro* experiments the heart was rapidly removed after thoracotomy and placed into cold (4°C) Krebs—Henseleit solution (KHS; ICN Biomedicals) until cessation of spontaneous beats and then was placed into a Langendorff device and retrograde open-contour Langendorff perfusion of the heart was started. Contractile function was recorded during perfusion at a constant pressure of 52 mm Hg with carbogen-saturated KHS (37°C, pH 7.4), containing (in mM): 120.0 NaCl, 4.8 KCl, 2.0 CaCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 20.0 $NaHCO_3$, and 10.0 glucose. Cardiac CB receptors were stimulated by 10-min perfusion of the heart with KHS containing HU-210 in a concentration of 100 nM (38 μg/kg) [2,4]. Heart rate was determined by ECG data directly after 20 min stabilization period, after 10-min perfusion with HU-210, and 10 min after perfusion without the preparation (washout). The preparation was dissolved in DMSO (10 μg/liter; Sigma) directly before the experiment. We previously found that DMSO in a concentration of 10 μg/liter did not modify cardiac rhythm and myocardial contractility.

All studies were carried out with the blind method. The results were statistically processed using Student's t test and Wilcoxon—Mann—Whitney test.

RESULTS

Treatment with HU-210 caused stable bradycardia in narcotized rats. As early as 5 min after injection, HR decreased by 28% in comparison with the initial values (Fig. 1, *a*). Similar effect of HU-210 was detected previously [9]. The solvent did not change HR. ACPA also caused bradycardia (-8% on mi-

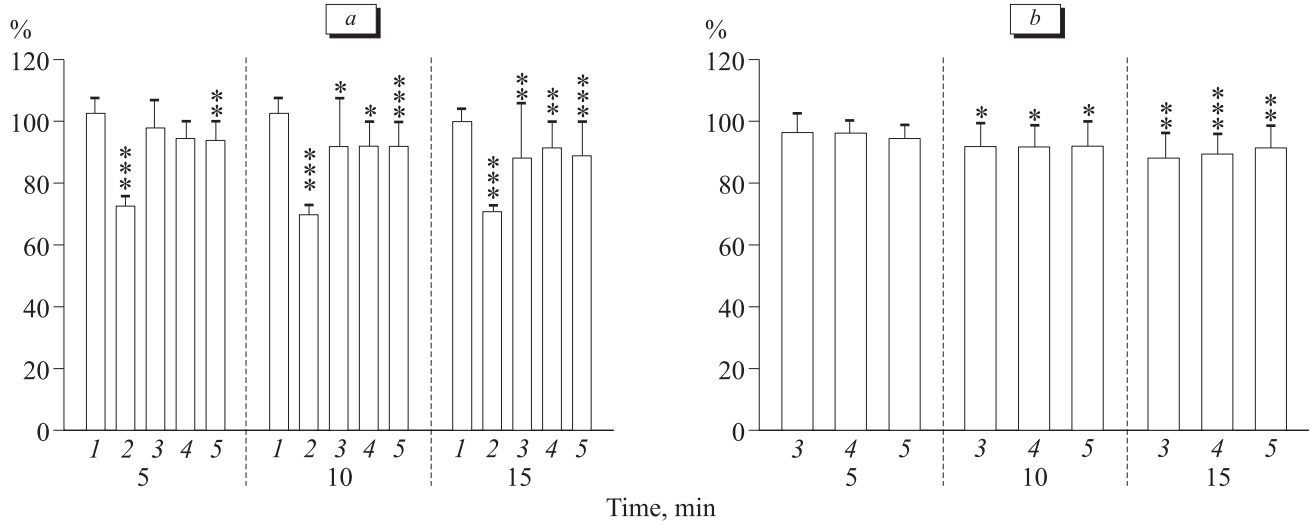

Fig. 1. Heart rate after intravenous injections of fat-soluble (*a*) and water-soluble (*b*) forms of CB receptor agonists. *1*) solvent; *2*) HU-210; *3*) ACPA; *4*) anandamide; *5*) methanandamide. *p<0.01, **p<0.001, ***p<0.0001 compared to initial HR (100%).

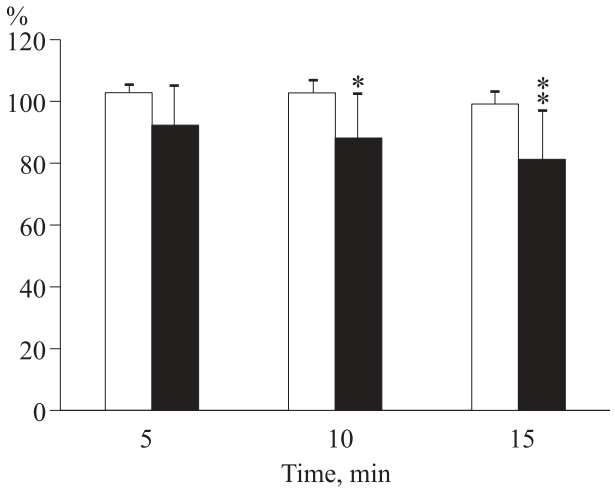

Fig. 2. Effect of pre-injection of SR 141716A (light bars) and SR 144528 agonists (dark bars) and HU-210 on the rat HR. Here and in Fig. 3: *p<0.05, **p<0.01 compared to initial HR (100%).

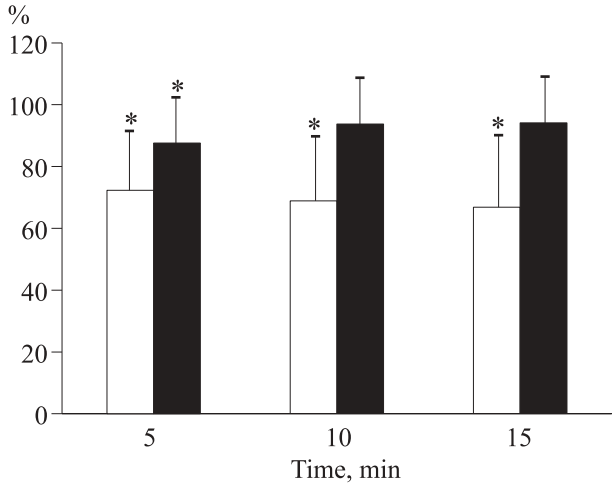

Fig. 3. Effect of pre-injection of ganglioblocker hexamethonium and HU-210 (light bars) and ACPA (dark bars) on the rat HR.

nute 15 after injection), but this effect was less pronounced compared to that of HU-210. Water-soluble ACPA emulsion produced a more potent effect than fat-soluble preparation, but weaker than HU-210 (Fig. 1, *b*). Anandamide in the fat-soluble form caused a significant reduction of HR as early as 5 min after injection (-7%), and after 10 min HR was by 9% below the initial value (Fig. 1). Water-soluble emulsion of anandamide reduced HR on minute 10 after injection (-9%). Injection of methanandamide (2.5 mg/kg) in the solvent caused bradycardia (-8%) after 10 min. Water-soluble emulsion of methanandamide produced similar effect (Fig. 1, *b*): HR on minutes 10 and 15 of observation decreased by 8 and 9%, respectively.

At first glance, anandamide and methanandamide exhibited a more pronounced negative CTE than ACPA. But the effect of ACPA was in fact more potent than of these cannabinoids, because anandamide and methanandamide caused bradycardia after injection in a dose of 2.5 mg/kg, while ACPA reduced HR in a dose of 0.125 mg/kg.

Hence, the negative CTE of the test cannabinoids decreases in the following order: HU-210> ACPA>methandamide=anandamide. By affinity for CB1 receptors the cannabinoids could be ranked as follows: HU-210>ACPA>methandamide>anandamide [6]. Hence, the negative CTE of cannabinoids is most likely a result of CB1 receptor activation. Further experiments confirmed this hypothesis. HU-210 did not modify cardiac rhythm under conditions of CB1 receptor blockade with selective CB1 antagonist SR 141716A (Fig. 2). On the other hand, negative CTE of HU-210 was retained after selective CB2 receptor inhibition with CB2 antagonist SR 144528 (Fig. 2). CB receptor inhibitors did not modify HR.

The mechanism of negative CTE of cannabinoid can be attributed to the fact that occupation of presynaptic CB receptors limits norepinephrine release from adrenergic terminals in the myocardium and, hence, reduces HR [7]. However, it was shown that the negative CTE of HU-210 was retained under conditions of vagotomy, while chemical sympathectomy even potentiated HU-210-induced bradycardia [9]. These facts do not confirm the key role of the autonomic nervous system in the realization of the negative CTE of cannabinoid.

For blockade of peripheral sympathetic and parasympathetic ganglia we used hexamethonium, a nicotinic cholinoreceptor blocker disrupting sym-

TABLE 1. Heart Rate (bpm) of Isolated Rat Heart during Perfusion with KHS and DMSO (Control) and Treatment with HU-210 ($M\pm m$)

Period of study	Control (n=20)	HU-210 (n=17)
20-min adaptation	205.0±15.8	210.7±11.2
10-min perfusion with preparation	189.0±15.5	163.0±12.9*
10-min perfusion without preparation	175.0±16.1	146.7±15.7*

Note. *p<0.05 compared to initial value.

pathetic transmission [8]. Hexamethonium did not abolish the negative CTE of cannabinoids (Fig. 3), while injected alone it reduced HR by 7% only on minute 10 after injection; heart rhythm returned to normal after 15 min. Since in combined use of the ganglioblocker and cannabinoids the ECG was recorded on minute 15 after injection of nicotinic cholinoreceptor blocker, the CTE induced by hexamethonium could not influence the final result. Hence, the negative CTE of HU-210 and ACPA did not depend on the autonomic nervous system.

In order to clear out whether this effect is a result of direct influence of cannabinoid on the heart or it is associated with alteration of blood concentrations of humoral factors (for example, epinephrine), which could modulate the heart rhythm, we carried out experiments on isolated perfused heart. Isolated heart perfused with oxygenated KHS (not blood) is an ideal model for the study of the direct effects of various drugs on the heart. In experiments on isolated heart we used HU-210 in a final concentration of 100 nM (38 μg/liter), comparable to HU-210 dose of 100 μg/kg *in vivo* [4]. After 10-min perfusion of the isolated heart with KHS containing HU-210, HR decreased by 23% in comparison with the initial values (Table 1). Bradycardia progressed 10 min after washout from the cannabinoid, HR was below the initial values by 31%.

Hence, the negative CTE of cannabinoids does not depend on the type of the solvent. The negative CTE of HU-210 is related to activation of CB1 receptors in the heart. The negative CTE of ACPA and methanandamide directly depends on their affinity for CB1 receptors [6]. Anandamide is characterized by 4-fold lower affinity for CB1 than methanandamide [6]. Moreover, anandamide rapidly undergoes enzymatic hydrolysis in the blood and tissues [5]. Arachidonoylethanolamide and methanandamide produce virtually identical negative CTE. The formation of negative CTE in response to anandamide injection seems to be regulated not only by CB1 receptors, but also by vanilloid receptors and the so-called anandamide receptors, for which arachidonoylethanolamide exhibits very high affinity [5].

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