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Selective Cannabinoid Receptor Agonist HU-210 Decreases Pump Function of Isolated Perfused Heart: Role of cAMP and cGMP

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The rate and strength of heart contractions decreased after 10-min perfusion of rat myocardium with Krebs—Henseleit solution containing a selective cannabinoid receptor agonist HU-210 in a final concentration of 10 nM. HU-210 completely blocked the positive inotropic and chronotropic effect of β-adrenoceptor agonist isoproterenol, decreased the basal level of cAMP, and abolished the isoproterenol-induced increase in myocardial cAMP concentration. cGMP concentration remained unchanged under these conditions. The decrease in myocardial cAMP concentration after activation of cannabinoid receptors did not correlate with changes in the strength and rate of heart contractions. Our results suggest that the negative inotropic and chronotropic effects of HU-210 are not associated with decreased cAMP concentration in the myocardium.

Key Words: *cannabinoid receptors; isolated heart; cAMP; cGMP*

Cannabinoid receptors (CR) with unknown function were recently found in mammalian organs and tissues [4,8]. Two subtypes of central and peripheral CR were identified [8]. Central CR are present in the bone marrow, brain, and some visceral organs. Peripheral CR were revealed only in peripheral organs and tissues [8,9]. mRNA encoding central CR was identified in cardiomyocytes, which serves as indirect evidence for the presence of CR in the myocardium [9]. The role of CR in the regulation of cardiac activity is poorly understood. Previous studies showed that systemic administration of cannabinoids leads to hypotension and bradycardia [1,11]. It remains unclear whether the cardiovascular effect is related to the direct influence of cannabinoids on the heart or they are mediated via the autonomic nervous system and circulating humoral factors. The role of intracellular signal systems (cAMP and cGMP) in the inotropic effect associated with stimulation of CR is unknown. These intracellular messengers regulate the cardiac rhythm and contractility of cardiomyocytes. CR are coupled with adenylate cyclase through G proteins. Stimulation of CR reduces adenylate cyclase activity, which is followed by a decrease in intracellular cAMP concentration [2,3]. It is unknown whether activation of CR can modulate cAMP synthesis in cardiomyocytes. Previous experiments were performed on neuronal cells [3] and cloned CR [2].

Here we studied the effect of a selective CR agonist HU-210 on contractile activity and concentrations of cAMP and cGMP in isolated perfused heart.

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MATERIALS AND METHODS

Experiments were performed on isolated hearts from male Wistar rats. After thoracotomy the hearts were rapidly removed and placed in a bath with cold Krebs—Henseleit solution (4°C). Isotonic solution was delivered through a cannula inserted into the ascending aortic arch. Retrograde perfusion of the heart with Krebs—Henseleit solution was performed by the method of Langendorff at a constant pressure of 55 mm Hg. Krebs-Henseleit solution was saturated with carbogen (37°C, pH 7.4) and contained 120 mM NaCl, 4.8 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 20 mM $NaHCO_3$, and 10 mM D-glucose (ICN Biomedicals).

A catheter attached to a latex balloon filled with water was introduced into the left ventricle to study contractile activity of the heart. Ventricular diastolic pressure was set at a level of 10-15 mm Hg. Pump function of the heart under isovolumic conditions was determined by heart rate (HR), left ventricular developed pressure (LVDP), and maximum rates of contraction and relaxation. LVDP was calculated as the difference between systolic and diastolic pressures. Experiments were performed on isolated hearts with initial contractility corresponding to the standard value estimated in our laboratory.

Selective CR agonist HU-210 ((6aR)-trans-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol, Tocris Cookson [6]) in a final concentration of 10 nM (3.7 μg/liter) was added to the perfusate for stimulation of CR. The dose of HU-210 was selected taking into account the ability of this preparation in concentrations of 1-10 nM to decrease adenylate cyclase activity in neuroblastoma cells [7]. HU-210 was dissolved in dimethylsulfoxide (DMSO) and added to Krebs—Henseleit solution. DMSO concentration in the perfusate did not exceed 0.01 mg/liter. Our previous studies showed that DMSO in the specified concentration had no effect on heart contractility. Contractile function of the isolated heart was stabilized after 20-min adaptation. The myocardium was perfused with Krebs—Henseleit solution and HU-210 for 10 min and immediately frozen in liquid nitrogen to measure the concentrations of cAMP and cGMP. β-Adrenoceptor agonist isoproterenol (IP) in a final concentration of 100 nM was used to evaluate whether activation of CR would modulate the basal and pharmacologically altered level of cAMP. Preliminary experiments showed that IP in a concentration of 100 nM increases myocardial cAMP concentration and produces a positive inotropic effect. After 20-min stabilization period, the hearts were consecutively perfused with solutions containing HU-210 (10 min) and IP (10 min). The effect of IP on contractility of the hearts not pretreated with HU-210 was studied in a special series. IP was added to Krebs—Henseleit solution after 30-min adaptation, and perfusion continued for 10 min.

The hearts were rapidly frozen in liquid nitrogen. cAMP and cGMP were extracted from heart tissue with ethyl alcohol [5]. The concentrations of cAMP and cGMP in samples were measured using standard commercial radioimmune RIA AMPc/cAMP and RIA cGMP kits (Immunotech), respectively. Radioactivity in samples was estimated on a Gamma-12 counter.

Isolated rat hearts perfused with Krebs—Henseleit solution containing DMSO in a final concentration of 0.01% served as the control.

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

RESULTS

Activation of CR with HU-210 decreased in the rate and strength of isolated heart contractions by 30% compared to the basal level. These changes were accompanied by a 1.5-fold decrease in the rate of contraction and relaxation (Table 1), which is consistent with published data [10]. It should be emphasized that previous experiments were performed with low-affinity CR agonists Δ^8- and Δ^9-tetrahydrocannabinol in high concentration (0.3-31.0 mg/liter), which produces a nonspecific effect on the myocardium [10]. Treatment with high-affinity CR agonist HU-210 in a concentration of 0.0037 mg/liter reduces the probability of nonspecific effects. We believe that the negative inotropic effect of HU-210 results from activation of cardiac CR.

The increase in myocardial cAMP concentration stimulates pump function of heart and elevates HR. By contrast, stimulation of cGMP synthesis decreases the strength of heart contractions and produces bradycardia. CR are coupled with adenylate cyclase through G proteins. Activation of CR dose-dependently inhibits cAMP synthesis in cells [2,3]. We hypothesized that the negative inotropic effect of HU-210 results from the decrease in cardiomyocyte cAMP concentration or increase in myocardial cGMP content. Myocardial cAMP concentration decreased by 29%, while cGMP content remained practically unchanged after 10-min stimulation of CR with HU-210 (Table 1). These data suggest that cGMP does not play a role in the negative inotropic effect of HU-210. The reduction of myocardial contractility after activation of CR was probably associated with the decrease in cAMP concentration. Correlation analysis was performed to confirm this hypothesis. However, no correlations were revealed between HR, LVDP, maximum rates of contraction and relaxation, and cAMP concentration. The

TABLE 1. Effect of Individual or Combined Treatment with HU-210 and IP on Contractility of Isolated Heart and Concentrations of cAMP and cGMP in Myocardial Tissue (*M*±*m*)

Parameter		Control	HU-210	IP	HU-210+IP
LVDP, mm Hg	adaptation	75±9	80.0±5.9	76±13	77.0±7.4
	perfusion	72±8	61.0±6.8*+	116±10*+	51±8+
HR, bpm	adaptation	232±20	200±14	261±29	201±12
	perfusion	222±31	179±6*	358±30*	229±31
Maximum rate of contraction,	adaptation	29.4±4.0	37.3±5.0	27.9±3.0	34.8±3.5
mm Hg/sec	perfusion	30±4	25±4+	53.0±9.4*+	28.0±2.8
Maximum rate of relaxation,	adaptation	22±4	22.0±1.9	21.0±3.4	18±6
mm Hg/sec	perfusion	21.0±4.1	15.6±2.0*	27.0±5.3	16.0±1.6
cAMP concentration, nmol/g		6.83±0.41	5.10±0.68*	8.90±0.47*	5.40±0.74
cGMP concentration, nmol/g		0.210±0.025	0.244±0.050	0.300±0.037	0.26±0.03

Note. $p<0.05$: *compared to the control; +compared to adaptation.

coefficient of linear correlation did not exceed 0.4 ($p>0.05$). Our results indicate that the decrease in cardiac contractility after activation of CR does not depend on changes in intracellular cAMP concentration. The effect of CR agonist can be realized via other signal systems with the involvement of inositol triphosphate and diacylglycerol as the key secondary messengers.

We assumed that the decrease in basal cAMP level in the myocardium produced by perfusion with HU-210 results from inactivation of adenylate cyclase. Studies with neuronal cells [3] and cloned CR [2] showed that activation of CR coupled with adenylate cyclase through $G_{i/o}$ proteins is followed by inhibition of cAMP synthesis. Should this be the case then activation of CR would abolish the increase in cAMP production in response to stimulation of β-adrenoceptors coupled with adenylate cyclase through G_s proteins. We studied the effect of CR activation on an IP-induced increase in myocardial cAMP concentration. IP is a standard β-adrenoceptor agonist and is often used to stimulate adenylate cyclase in cardiomyocytes. Perfusion of the isolated heart with the solution containing IP significantly increased in HR and strength and rate of heart contractions (Table 1). IP increased cAMP concentration by 35%. The correlation analysis showed that the positive inotropic effect of IP is associated with stimulation of cAMP synthesis. cAMP concentration linearly correlated with HR (0.74) and LVDP (0.77, $p<0.05$).

IP had no positive inotropic effect ubder conditions of CP activation. After consecutive perfusion with the solution containing HU-210 and IP, contractility of the myocardium did not differ from the control (Table 1). Under these conditions myocardial cAMP concentration increased less significantly compared to experiments with IP. Therefore, CR agonist HU-210 abolished the IP-induced increase in myocardial contractility and cAMP concentration.

Our results suggest that changes in contractility after activation of CR are not related to variations in cardiomyocyte cAMP concentration. Activation of cardiac CR can be accompanied by inhibition of cAMP synthesis in cardiomyocytes. It cannot be excluded that HU-210 activates phosphodiesterase and stimulates cleavage of cAMP. However, there are no data that stimulation of CR promotes the increase in phosphodiesterase activity. Inactivation of adenylate cyclase after activation of CR is the most probable mechanism for the decrease in cAMP concentration produced by HU-210.

Our study showed that selective CR agonist HU-210 has a direct inotropic and chronotropic effect on the myocardium. Activation of myocardial CR is accompanied by a decrease in cAMP concentration, but has no effect on cGMP level. Changes in contractility of the isolated heart in response to stimulation of CR do not correlate with the decrease in myocardial cAMP concentration.

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