BIOPHYSICS AND BIOCHEMISTRY

Cannabinoid Receptor Antagonists SR141716 and SR144528 Exhibit Properties of Partial Agonists in Experiments on Isolated Perfused Rat Heart

A. V. Krylatov*, L. N. Maslov*,, O. V. Lasukova**, and R. G. Pertwee*****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 5, pp. 512-516, May, 2005
Original article submitted July 12, 2004

We studied the effect of selective cannabinoid receptor ligands on contractility of isolated Langendorff-perfused rat heart. It was found that 10-min perfusion of rat heart with a solution containing selective agonist of CB1 and CB2 receptors HU-210 (10 nM) decreased left ventricular developed pressure and maximum rates of contraction and relaxation. However, HU-210 had no effect on heart rate and end-diastolic pressure. Treatment with selective CB1 receptor antagonist SR141716 (1 μM) and selective CB2 receptor antagonist SR144528 (1 μM) decreased left ventricular developed pressure and maximum rates of contraction and relaxation, but had no effect on heart rate and end-diastolic pressure. Ten-minute perfusion of rat heart with a solution containing selective agonist of CB1 and CB2 receptors HU-210 (10 nM) decreased cAMP concentration in the heart. CB receptor antagonists had little effect on cAMP concentration in the heart. The negative inotropic effect of HU-210 and CB receptor antagonists is probably mediated by activation of CB1 receptors. It can be hypothesized that the decrease in heart cAMP concentration is related to stimulation of CB2 receptors. Our results suggest that selective CB receptor antagonists SR141716 and SR144528 in a final concentration of 1 μM exhibit properties of partial CB receptor agonists.

Key Words: *cannabinoid receptors; isolated rat heart; cAMP; HU-210; SR141716; SR144528*

Cannabinoid (CB) receptors belong to the superfamily of G protein-coupled receptors (GPCR) [7,12]. An important characteristic of GPCR is their ability to spontaneous activation in the absence of agonists [4, 13]. P. L. Prather descried 4 conformations of GPCR: R (inactive), R*1 (active), R*2 (active), and R*3 (active) [13]. "Reverse agonists" have high affinity for R conformation of GPCR. Spontaneously activated receptors exist in R*1 conformation. R*2 conformation results from the interaction of GPCR with an agonist. R*1 and R*2 conformations are induced by different G proteins. Signal effects of R*3 conformation are mediated by β-arrestin and do not depend on G proteins. An equilibrium between these conformations can be shifted, which depends on the presence of GPCR ligands. For example, reverse agonists and agonists shifts the equilibrium toward R and R*2, respectively. P. L. Prather reported that neutral antagonists interact with different conformations of GPCR [13]. It was assumed that neutral antagonists do not modulate cell function, but abolish the effect of agonists. The

*Tomsk State Pedagogical University; **Institute of Cardiology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences; ***Aberdeen University, Foresthill, Aberdeen, United Kingdom. ***Address for correspondence:*** maslov@cardio.tsu.ru. L. N. Maslov

0007-4888/05/1395-0558

interaction between reverse agonists produces an opposite effect (as compared to the action of agonists) [4,13]. Published data show that cannabinoids activate G_i and G_o proteins coupled to CB receptors and decrease adenylate cyclase activity [7,12]. In this case, reverse agonists should inhibit G_i and G_o proteins, but activate adenylate cyclase [7,12].

Selective CB1 receptor antagonist SR141716 and CB2 receptor antagonist SR144528 interact with cloned CB1 and CB2 receptors and exhibit properties of reverse agonists [2,3]. It remains unclear whether SR141716 and SR144528 *in vitro* exhibit properties of reverse agonists in relation to native CB receptors. Our experiments were performed on isolated perfused heart. Previous studies showed that cardiac cells express CB receptors, and cannabinoids produce a negative inotropic effect [1,14]. We hypothesized that if SR141716 and SR144528 interact with native CB receptors as reverse agonists, addition of these substances to the perfusate should produce a positive inotropic effect. Published data show that activation of CB receptors is followed by inhibition of adenylate cyclase [7,12]. Thus, SR141716 and SR144528 should produce an opposite effect (*i.e.*, activation of cAMP synthesis).

Here we studied whether SR141716 and SR144528 exhibit properties of reverse agonists in isolated perfused rat heart.

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, 2.0 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 20.0 mM $NaHCO_3$, and 10.0 mM D-glucose (ICN Biomedicals).

The catheter with a fluid-filled latex balloon 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. Pumping function of the heart under isovolumic conditions was evaluated by heart rate (HR, bpm), left ventricular developed pressure (mm Hg), and maximum rates of contraction and relaxation (mm Hg/sec). Left ventricular developed pressure 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 (HR, 236±13 bpm; left ventricular developed pressure, 89.6±7.9 mm Hg/sec; maximum rate of contraction, 35.5±2.4 mm Hg; maximum rate of relaxation, 23.9±2.15 mm Hg/sec).

Selective CB receptor agonist HU-210 in a final concentration of 10 nM was added to the perfusate. The dose of HU-210 was selected taking into account that this preparation in a concentration of 1-10 nM decreases adenylate cyclase activity in neuroblastoma cells (S. Mukhopadhyay *et al*. [11]). Selective CB receptor 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 [10]) 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 has no effect on heart contractility. Contractile function of isolated heart was stabilized after 20-min adaptation. The myocardium was perfused with HU-210-containing solution for 10 min.

SR141716A (N-[piperidine-1-yl]-1-[1,2-dichlorophenyl]-4-methyl-1H-pyrazole-3-carboxamide HCl) [8] and SR144528 (N-(1S)-endo-1,3,3-trimethylbicyclo[2.2.1]hepta-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide) [3] served as selective antagonists of CB1 and CB2 receptors, respectively. These preparations were dissolved in DMSO and added to the perfusate after stabilization (1 μM). Contractile activity was recorded after 10-min perfusion of the heart with Krebs—Henseleit solution containing one of these antagonists. The concentration of antagonists was selected taking into account the results of previous studies [5,6]. CB receptor antagonists were synthesized at the Research Triangle Institute (Research Triangle Park).

The hearts were rapidly frozen in liquid nitrogen. cAMP was extracted from the heart tissue with ethyl alcohol [9]. The concentration of cAMP in samples was measured using standard commercial radioimmune RIA AMPc/cAMP kit (Immunotech, Beckman Coulter Company). 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% (solvent) instead of HU-210 served as the control. The results were analyzed by Student's *t* test and correlation analysis.

RESULTS

Ten-minute perfusion of the isolated heart with Krebs—Henseleit solution containing 10 nM HU-210 decreased left ventricular developed pressure and maximum rates of contraction and relaxation by 24, 33, and 29%,

respectively, compared to the baseline level (Fig. 1). HU-210 had no effect on the end-diastolic pressure and HR. The baseline left ventricular developed pressure, maximum rate of contraction, maximum rate of relaxation, HR, and end-diastolic pressure were 80 mm Hg, 37.3 mm Hg/sec, 22.0 mm Hg/sec, 200 bpm, and 10 mm Hg, respectively. DMSO had no effect on HR, end-diastolic pressure, and contractility of the isolated heart. Our results agree with published data that CB *in vitro* produce a negative inotropic effect on the isolated perfused rat heart [5] and isolated human cardiac trabeculae [1]. A negative inotropic effect of HU-210 is probably associated with activation of CB receptors in the heart. It should be emphasized that the negative inotropic effect is observed after treatment with low dose of HU-210 (10 nM), which acts as a strong selective agonist of CB receptors [7,12]. Our findings exclude the possibility that HU-210 produces a nonspecific effect on the myocardium.

Radioimmune assay showed that 10-min perfusion of the isolated heart with a solution containing 10 nM HU-210 decreases cAMP concentration in the myocardium by 26% compared to the control (Fig. 2). cAMP concentration in heart samples perfused with Krebs—Henseleit solution not containing CB receptor ligands was 10.8 nmol/g. Therefore, activation of CB receptors in the myocardium decreased cAMP concentration. Tissue cAMP concentration depends on catalytic activities of adenylate cyclase (synthesis of cAMP) and phosphodiesterase (conversion of cAMP into AMP). We found no published data on the effect of CB on phosphodiesterase activity. It can be hypothesized that the decrease in cAMP concentration in the heart tissue results from inhibition of adenylate cyclase by CB (similarly to other organs, tissues, and cells) [7,12]. Myocardial cAMP plays an important role in the regulation of Ca^{2+} transport in cardiomyocytes and pumping function of the heart. We assumed that the negative inotropic effect of HU-210 is associated with the decrease in myocardial cAMP concentration. However, no significant correlation was found between the decrease in cAMP concentration

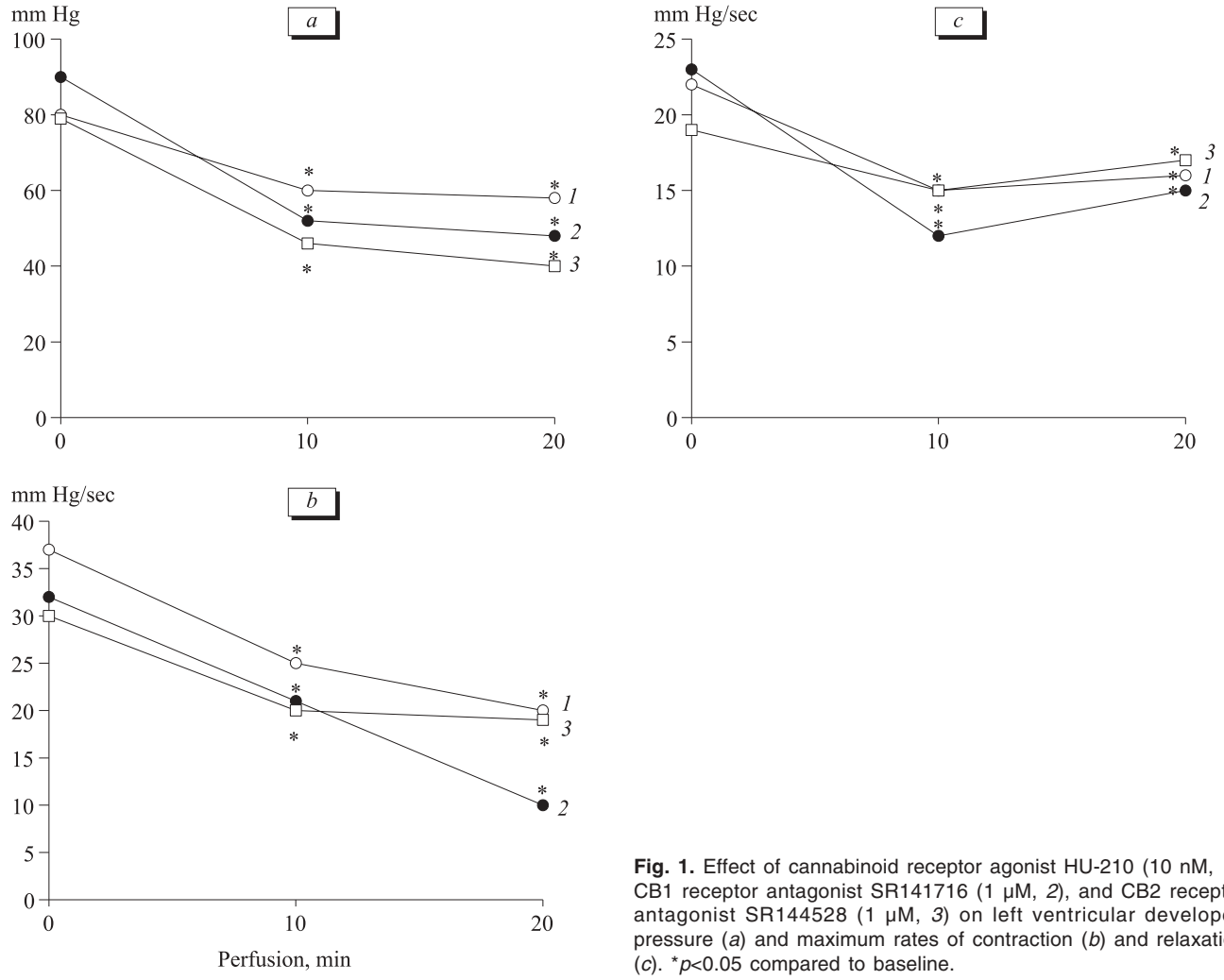

Fig. 1. Effect of cannabinoid receptor agonist HU-210 (10 nM, *1*), CB1 receptor antagonist SR141716 (1 μM, *2*), and CB2 receptor antagonist SR144528 (1 μM, *3*) on left ventricular developed pressure (*a*) and maximum rates of contraction (*b*) and relaxation (*c*). **p*<0.05 compared to baseline.

and reduction of the pumping function (left ventricular developed pressure and maximum rates of contraction and relaxation). Hence, the negative inotropic effect of HU-210 is not related to the decrease in myocardial cAMP concentration, but can be realized via other signal system differing from cAMP (*e.g.*, mitogen-activated protein kinase, whose activity increases after occupation of CB receptors by agonists) [3].

Ten-minute perfusion of the isolated heart with Krebs—Henseleit solution containing SR141716A decreased left ventricular developed pressure and maximum rates of contraction and relaxation by 43, 35, and 48%, respectively, compared to the baseline level (Fig. 1). End-diastolic pressure and HR remained unchanged under these conditions. Administration of CB2 receptor antagonist SR144528 into the perfusate decreased left ventricular developed pressure and maximum rates of contraction and relaxation by 47, 34, and 33%, respectively. SR144528 had no effect on end-diastolic pressure and HR. These data show that CB receptor antagonists (1 μM) and HU-210 (10 nM) produce similar effects on the myocardium.

It can be hypothesized that CB receptor antagonists exhibit properties of partial CB receptor agonists and decrease cAMP concentration in the myocardium (similarly to HU-210). However, the decrease in myocardial cAMP concentration under the influence of CB receptor antagonists was statistically insignificant (Fig. 2). SR141716A insignificantly increased cAMP concentration in the myocardium.

On the one hand, CB receptor agonist HU-210 produces a negative inotropic effect and decreases cAMP concentration. It should be emphasized that no correlation was found between variations in cAMP concentration and heart contractility. On the other hand, CB receptor antagonists produce a negative inotropic effect, but do not modulate cAMP concentration. An attempt was made to study the cause of these conflicting results.

We hypothesized that cardiac cells express CB1 and CB2 receptors, while cardiomyocytes express only CB1 receptors. A negative inotropic effect of CB is associated with activation of CB1 receptors [1,5]. We believe that the negative inotropic effect of HU-210, SR141716A, and SR144528 is related to activation of these receptors. SR141716A and SR144528 exhibit properties of partial CB1 receptor agonists. Inhibition of cAMP synthesis results from occupation of CB2 receptors that are absent in cardiomyocytes and expressed in cardiac cells not involved in the pumping function of the myocardium (fibroblasts, macrophages, endotheliocytes, and vascular smooth muscle cells). Probably, these receptors can be activated only by HU-210. In this case the decrease in cAMP concentration induced by HU-210 does not correlate with changes in the pumping function of the heart (as shown in our experiments). Published data support this hypothesis. For example, HU-210 displays high affinity for CB1 and CB2 receptors [7,12]. Therefore, this agent activates CB1 receptors on cardiomyocytes and occupies CB2 receptors on other cells of the heart. Our hypothesis requires further detailed investigations.

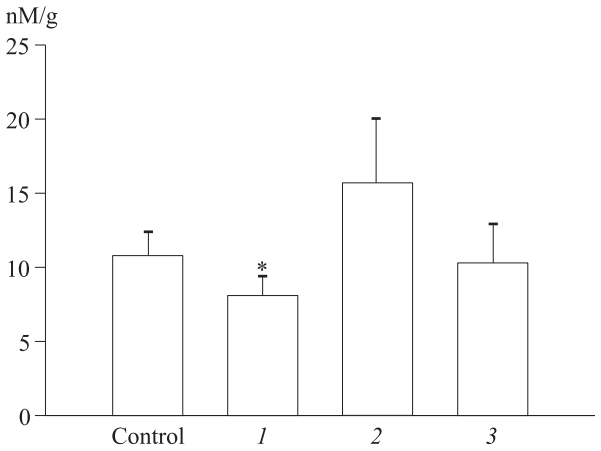

Fig. 2. Effect of cannabinoid receptor agonist HU-210 (10 nM, *1*), CB1 receptor antagonist SR141716 (1 μM, *2*), and CB2 receptor antagonist SR144528 (1 μM, *3*) on cAMP concentration in rat myocardium. *$p<0.05$ compared to normoxic control.

This work was supported by the Russian Ministry of Education and Russian Foundation for Basic Research.

We are grateful to Kevin J. Gormley (NIDA, USA) for ligands of CB receptors.

REFERENCES

1. A. Bonz, M. Laser, S. Kullmer, *et al.*, *J. Cardiovasc. Pharmacol.*, **41**, No. 4, 657-664 (2003).
2. M. Bouaboula, N. Desnoyer, P. Carayon, *et al.*, *Mol. Pharmacol.*, **55**, 473-480 (1999).
3. M. Bouaboula, S. Perrachon, L. Milligan, *et al.*, *J. Biol. Chem.*, **272**, 22,330-22,339 (1997).
4. T. Costa and A. Herz, *Proc. Natl. Acad. Sci. USA*, **86**, 7321-7325 (1989).
5. W. R. Ford, S. A. Honan, R. White, and C. R. Hiley, *Br. J. Pharmacol.*, **135**, No. 5, 1191-1198 (2002).
6. W. S. Ho and C. R. Hiley, *Ibid.*, **139**, No. 3, 585-597 (2003).
7. A. C. Howlett, F. Barth, T. I. Bonner, *et al.*, *Pharmacol. Rev.*, **54**, No. 2, 161-202 (2002).
8. R. S. Mansbach, C. C. Rovertti, E. N. Winston, and J. A. Lowe, *Psychopharmacology*, **124**, 315-322 (1996).
9. L. Maslov and Y. B. Lishmanov, *Clinical Exp. Pharmacol. Physiol.*, **22**, 812-816 (1995).
10. R. Mechoulam, J. J. Feigenbaum, N. Lander, *et al.*, *Experientia*, **44**, 762-764 (1984).
11. S. Mukhopadhyay, J.-Y. Shim, A. A. Assi, *et al.*, *Chem. Physics Lipids*, **121**, 91-109 (2002).
12. R. G. Pertwee, *Current Med. Chem.*, **6**, 635-664 (1999).
13. P. L. Prather, *Sci. STKE*, **215** (2004).
14. J. A. Wagner, K. Hu, J. Karcher, *et al.*, *Br. J. Pharmacol.*, **138**, No. 7, 1251-1258 (2003).