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Original article

Cannabinoid CB_1 and CB_2 receptors antagonists AM251 and AM630 differentially modulate the chronotropic and inotropic effects of isoprenaline in isolated rat atria

Jolanta Weresa, Anna Pędzińska-Betiuk, Rafał Kossakowski, Barbara Malinowska*

Department of Experimental Physiology and Pathophysiology, Medical University of Bialystok, Białystok, Poland

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ABSTRACT

Background: Drugs targeting CB_1 and CB_2 receptors have been suggested to possess therapeutic benefit in cardiovascular disorders associated with elevated sympathetic tone. Limited data suggest cannabinoid ligands interact with postsynaptic β-adrenoceptors. The aim of this study was to examine the effects of CB_1 and CB_2 antagonists, AM251 and AM630, respectively, at functional cardiac β-adrenoceptors.
Methods: Experiments were carried out in isolated spontaneously beating right atria and paced left atria where inotropic and chronotropic increases were induced by isoprenaline and selective agonists of β_1 and β_2-adrenergic receptors.
Results: We found four different effects of AM251 and AM630 on the cardiostimulatory action of isoprenaline: (1) both CB receptor antagonists 1 μM enhanced the isoprenaline-induced increase in atrial rate, and AM630 1 μM enhanced the inotropic effect of isoprenaline; (2) AM251 1 μM decreased the efficacy of the inotropic effect of isoprenaline; (3) AM251 0.1 and 3 μM and AM630 3 μM reduced the isoprenaline-induced increases in atrial rate; (4) AM630 0.1 and 3 μM enhanced the inotropic effect of isoprenaline, which was not changed by the same concentrations of AM251.
Conclusions: Our results show that the CB_1 and CB_2 receptor antagonists AM251 and AM630 have bidirectional effects on the cardiostimulatory action of isoprenaline, most likely related to an interaction with β_1-adrenoceptors. Provided that the results translate to human heart, caution should be taken when using CB_1 and CB_2 receptor antagonists, as an enhanced sympathetic tone accompanies many cardiovascular disorders.

Introduction

Cannabinoids (CB) exhibit complex cardiovascular effects. In contrast to the large body of information on cannabinoid vascular effects, surprisingly few studies have focused on cardiac effects. The most well-known acute and chronic physiological changes produced by cannabis preparations in humans are tachycardia [1,2] and symptomatic sinus bradycardia and ventricular asystole [3], respectively. In rats, bradycardia is elicited by acute injection of the major psychoactive component of the cannabis plant, Δ^9-tetrahydrocannabinol (Δ^9-THC), the endocannabinoid anandamide, its stable analogue methanandamide, or other synthetic cannabinoids [1,2].

Cannabinoids are mainly known to decrease cardiac contractility *via* CB_1 receptors. The above effect is probably responsible in hypertensive rats for the decrease in blood pressure induced by anandamide and two of its degradation inhibitors, URB597 [4] and AM3506 [5]. The CB_1-receptor-dependent negative inotropic effects of anandamide, methanandamide, and the synthetic cannabinoid CB receptor agonists HU-210 or CP55940 have been determined in isolated human atrial muscle [6] and in rat heart [7] and atria [8,9]. A CB_2 receptor-mediated positive inotropic effect of anandamide was identified in rat atria in the presence of the CB_1 receptor antagonist AM251 [8]. Under physiological conditions none of the CB receptor antagonists modified cardiovascular parameters [1,2]. Unexpectedly, two pairs of antagonists, AM251 (CB_1) and AM630 (CB_2) in rat ventricular myocytes [10], and SR141716 (CB_1) and SR144528 (CB_2) in isolated heart [7], consistently led to negative inotropic effects.

Moreover, presynaptic CB_1 receptors on sympathetic nerve endings innervating the heart inhibit neurogenic tachycardia and/

* Corresponding author.
E-mail address: bmalin@umb.edu.pl (B. Malinowska).

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or noradrenaline release in human atrial appendages [11] and pithed rats [12]. In rat atria, anandamide [13] or anandamide and CP55940 [14], respectively, decreased or failed to modify the CB_1 receptor-mediated neurogenic tachycardia.

An interaction of cannabinoid ligands with postsynaptic β-adrenoceptors has also been described. The CB receptor agonists WIN55212-2 and HU-210 inhibited cAMP production and/or positive ino- and chronotropic effects elicited by the non-selective β-adrenoceptor agonist isoprenaline in rat cultured neonatal cardiomyocytes [15] and Langendorff perfused heart [16]. AM251 restored the blunted response to isoprenaline of rat ventricular papillary muscle isolated from bile duct-ligated rats [17] and attenuated endotoxin/lipopolysaccharide-induced tachycardia in rats [18]. Acute and chronic inhibition of anandamide degradation reduced the occurrence of isoprenaline-induced ventricular tachyarrhythmia in rats [19] and differentially modified the ino- and chronotropic effects of isoprenaline in atria and hearts isolated from hypertensive and normotensive rats [9].

Drugs targeting CB_1 [e.g. 20] and CB_2 [e.g. 21] receptors have been suggested as potential remedies against cardiovascular disorders associated with elevated sympathetic tone, such as hypertension or heart failure [22]. The CB_1 receptor antagonists modify hemodynamics and cardiac contractility functions [20]. The cardioprotective influence of CB_2 receptor stimulation is connected primarily with its immunosuppressive properties [21]. The possibility that cannabinoid receptor antagonists interact with β-adrenoceptors, thereby leading to cardiac side effects, must be considered. The aim of the present study was to examine the effect of the CB_1 antagonist AM251 and the CB_2 antagonist AM630 on the chronotropic and inotropic effects of isoprenaline in isolated rat atria. In order to determine the precise mechanism of the interaction, we also replaced (1) AM251 and AM630 by the non-selective CB receptor agonist CP55940 [23] and cannabidiol (a cannabis plant-derived constituent with a low affinity at both CB receptors [23] that has been already approved for the treatment of spasticity in multiple sclerosis or intractable epilepsies [24] and (2) isoprenaline by the phosphodiesterase inhibitor IBMX, by agonists of β_1- (xamoterol) and β_2-adrenoceptors (fenoterol) and by an agonist of the low-affinity state of the β_1-adrenoceptor (CGP12177 [25]).

Materials and methods

Animals

Experiments were conducted in accordance with the European Directive 2010/63/EU and with the approval of the local Animal Ethics Committee in Białystok (Poland). They were performed on male Wistar rats (320–400 g), housed at a constant temperature (21–22 °C) with a 12-h light/dark cycle and ad libitum access to standard chow and water.

Preparation of isolated atria

Rats were anaesthetized by intraperitoneal injection of pentobarbital sodium 300 μmol/kg. Hearts were removed, and the right and left atria were dissected and suspended in an organ bath containing 10 ml Krebs solution (for details see Ref. [9]). Each preparation was stretched to approximately 5 mN of force and allowed to equilibrate for 60 min. Right atria worked spontaneously. Left atria were continuously stimulated by electrical field, applied using a bipolar platinum electrode with square waves (just over threshold, 5 ms duration, 2 Hz). Force and frequency (HR) of contractions were recorded using an isometric force transducer (PIM 100RE, BIO-SYS-TECH, Białystok, Poland).

Experimental protocols

Atria were pretreated for 30 min with AM251, AM630, CP55940 or cannabidiol, for 60 min with the β_1-adrenoceptor antagonist CGP20712A or for 90 min with the β_2-adrenoceptor antagonist ICI118551. Concentration-response curves (CRCs) were constructed by cumulative addition of each agonist (for each preparation only one CRC was determined): isoprenaline, IBMX, xamoterol, fenoterol, or CGP12177. CGP12177 was examined in the presence of propranolol 200 nM, a concentration that does not modify the cardiostimulant effects of CGP12177 [27]. All antagonists, CP55940 or cannabidiol were present during the construction of the CRSs.

Drugs used

(-)-Isoprenaline (±)-bitartrate salt, (±)-CGP12177 [(±)-4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropyl]-1,3-dihydro-2H-benzimidazole-2-one hydrochloride], (±)-CGP20712A [(±)-2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluoromethyl)-1H-imidazol-2-yl]phenoxy]-propyl]amino]ethoxy]-benzamide methanesulfonate], 3-isobutyl-1-methylxanthine (IBMX), CP55940 [(-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol], (S)-(−)-propranolol hydrochloride (Sigma-Aldrich, Steinheim, Germany); ICI118551 [erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochlo-ride], xamoterol hemifumarate, AM630 [6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)methanone], AM251 [N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide], (-)-cannabidiol (Tocris Bioscience, Bristol, UK); fenoterol hydrobromide (MP Biomedicals, Solon, OH, USA) and pentobarbital sodium (Biowet, Puławy, Poland) were used. Stock solutions of isoprenaline, IBMX, CGP12177, CGP20712A, ICI118551, fenoterol, xamoterol, and propranolol were prepared using distilled water. AM630, AM251, CP55940, and cannnabidiol were dissolved in dimethyl sulphoxide (DMSO, Sigma-Aldrich, Steinheim, Germany). The final concentration of DMSO in the organ bath was 0.1% v/v, which enhanced basal HR by about 5% and diminished basal force by about 10%. Further dilutions were made with Krebs solution.

Data analysis

Results are given as the mean ± SEM (n = number of animals). Positive chronotropic effects are shown as changes from baseline values. Positive inotropic effects are shown as a percentage of the maximum responses to isoprenaline, fenoterol or IBMX. To determine the maximal effects (E_{max}) and the potency of agonists, the pEC_{50} values (the negative logarithm of the concentration causing a half-maximum effect) were determined from the individual CRCs. Statistical analysis was performed using Graph Pad Prism version 5.0 (La Jolla, CA, USA). To compare the mean values of several compounds with the same control, one-way analysis of variance (ANOVA) followed by the Dunnett test was used. The effects of particular antagonists on basal values were estimated with Student's t-test for paired data. Differences were considered significant when $p < 0.05$.

Results

General

Almost all basal values were comparable (HR in spontaneously beating right atria and force in stimulated left atria), immediately before the construction of CRCs for particular ligands (Tables 1 and 2). The following exceptions were noted (compared to

Table 1
Effects of different agonists and antagonists on the concentration-response curves of isoprenaline in isolated atria.

Drug under study or its solvent	Pharmacological activity	Concentration (μM)	Right atrium				Left atrium			
			n	Basal HR (beats/min)	pEC_{50}	E_{max} (beats/min)	n	Basal force (mN)	pEC_{50}	E_{max} (%)
control		–	19	352 ± 7	8.4 ± 0.1	117 ± 6	21	2.2 ± 0.1	7.7 ± 0.1	97 ± 1
AM251	(-) CB_1	0.1	5	360 ± 18	8.4 ± 0.2	90 ± 15*	4	2.1 ± 0.1	8.4 ± 0.2***	97 ± 14
		1	7	358 ± 7	8.8 ± 0.1*	132 ± 13	6	2.2 ± 0.1	7.7 ± 0.2	46 ± 6***
		3	6	353 ± 10	8.6 ± 0.1	77 ± 6**	5	2.1 ± 0.1	8.0 ± 0.2	107 ± 13
AM630	(-) CB_2	0.1	5	374 ± 5	8.8 ± 0.2	115 ± 15	4	2.2 ± 0.1	7.9 ± 0.1	169 ± 26***
		1	7	347 ± 6	9.1 ± 0.1***	155 ± 10**	5	2.3 ± 0.1	9.6 ± 0.1***	90 ± 4
		3	6	364 ± 19	8.5 ± 0.3	67 ± 15***	5	2.2 ± 0.1	8.4 ± 0.1*	148 ± 16***
control		–	7	339 ± 7	8.4 ± 0.1	129 ± 6	9	2.3 ± 0.1	7.6 ± 0.1	98 ± 1
cannabidiol[1]	(+) CB_1/CB_2	1	4	298 ± 12	8.6 ± 0.1	122 ± 7	6	2.3 ± 0.2	8.5 ± 0.2**	79 ± 15
CP55940	(+) CB_1/CB_2	1	6	315 ± 17	8.9 ± 0.1**	125 ± 14	5	2.3 ± 0.1	8.5 ± 0.2**	130 ± 27
control		–	5	331 ± 13	8.6 ± 0.1	149 ± 14	5	2.2 ± 0.2	8.3 ± 0.1	97 ± 2
ICI118551	(-) β_2-AR	0.05	5	317 ± 15	8.8 ± 0.1	136 ± 11	5	1.6 ± 0.1	8.2 ± 0.2	81 ± 13
CGP20712A	(-) β_1-AR	0.3	5	244 ± 9***	6.7 ± 0.1***	132 ± 24	4	1.6 ± 0.4	6.7 ± 0.2***	55 ± 10*

Basal parameters are recorded immediately before the first concentration of isoprenaline. [1]very low affinity; E_{max} - maximal changes from baseline (right atria) and percentages of the maximum response (left atria) to isoprenaline; (+) stimulation; (-) inhibition; AR – adrenoceptor; CB – cannabinoid. Data are presented as means ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ *vs.* the corresponding control.

Table 2
Effects of CB_1 and CB_2 receptor antagonists AM251 and AM630 on the concentration-response curves of xamoterol, CGP12177, fenoterol, and IBMX in isolated atria.

Antagonist or its solvent	Concentration (μM)	Agonist	Pharmacological activity	Right atrium				Left atrium			
				n	Basal HR (beats/min)	pEC_{50}	E_{max} (beats/min)	n	Basal force (mN)	pEC_{50}	E_{max} (%)
control	–	xamoterol	(+) β_1-AR	4	300 ± 26	7.9 ± 0.3	71 ± 12				
AM251	1.0			5	287 ± 14	7.8 ± 0.2	80 ± 9				
AM630	1.0			5	309 ± 28	7.8 ± 0.4	85 ± 21				
control	–	CGP12177 (in the	(+) low-affinity state of β_1-AR	5	308 ± 12	6.9 ± 0.2	76 ± 11				
AM251	1.0	presence		3	308 ± 52	6.8 ± 0.2	90 ± 19				
AM630	1.0	of propranolol)		3	331 ± 6	7.2 ± 0.2	69 ± 14				
control	–	fenoterol	(+) β_2-AR	5	330 ± 22	6.9 ± 0.1	137 ± 13	3	2.2 ± 0.1	5.9 ± 0.1	100
AM251	1.0			4	286 ± 12	7.2 ± 0.1	141 ± 20	4	2.4 ± 0.1	6.4 ± 0.2	91 ± 14
AM630	1.0			6	331 ± 21	6.7 ± 0.1	106 ± 16	5	2.1 ± 0.2	6.4 ± 0.2	77 ± 17
control	–	IBMX	(-) PDE	4	315 ± 13	5.3 ± 0.1	201 ± 16	5	2.4 ± 0.1	5.0 ± 0.1	100
AM251	1.0			5	305 ± 12	5.6 ± 0.1	158 ± 15	4	2.4 ± 0.3	4.9 ± 0.1	101 ± 16
AM630	1.0			4	327 ± 17	5.4 ± 0.1	160 ± 20	4	2.5 ± 0.3	5.0 ± 0.2	96 ± 20

Basal parameters were recorded immediately before the first dose of each particular agonist. E_{max} - maximal changes from baseline (right atria) and % of basal values or percentages of the maximum response (left atria) to IBMX or to fenoterol. (+) stimulation; (-) inhibition; AR – adrenoceptor; CB – cannabinoid; PDE- phosphodiesterase. Data are presented as means ± SEM.

respective controls; Table 1): (1) CGP20712A diminished basal HR by approximately 25%; (2) ICI118551 and CGP20712A tended to diminish basal force by approximately 25%.

Influence of cannabinoid ligands on isoprenaline-induced cardiostimulatory effects

Isoprenaline (0.001 nM – 30 μM) caused concentration-dependent increases in HR and force, respectively, in right and left atria (Fig. 1). The positive chronotropic effect of isoprenaline was enhanced slightly (only for its lower concentrations) by AM251 1 μM (Fig. 1A) and strongly (E_{max} increased approximately 30%) by AM630 1 μM (Fig. 1B). In contrast, the effect was reduced by AM251 3 μM and AM630 3 μM (E_{max} reduced approximately 35% and 40%, respectively). The lowest concentrations (0.1 μM) of AM251 (but not AM630) reduced the increase in HR (E_{max} reduced approximately 30%) induced by higher concentrations of isoprenaline. The positive inotropic effect of isoprenaline was modified by only one concentration of AM251 1 μM (E_{max}, reduced approximately 50%, Fig. 1C). AM630 1 μM shifted isoprenaline's CRC to the left and did not modify its E_{max} value. In contrast, AM630 0.1 and 3 μM enhanced the positive inotropic effect of isoprenaline (E_{max} increased approximately 70% and 50%, respectively) (Fig. 1D). The potency of isoprenaline's chrono- and inotropic effects was enhanced by both CB receptor antagonists at 1 μM with the exception of the inotropic effect of isoprenaline in the presence of AM251. Additionally, the potency of isoprenaline's inotropic effect was increased by AM251 0.1 μM and AM630 3 μM (Table 1). In further studies, we examined the effect of a single concentration of both CB receptor antagonists, 1 μM, which effectively modified all cardiostimulatory effects of isoprenaline.

The positive chronotropic effect of isoprenaline was unmodified by cannabidiol 1 μM, while CP55940 1 μM increased its potency but not efficacy (Fig. 2A). CP55940 and cannabidiol increased potency of the positive inotropic effect of isoprenaline but did not affect its E_{max} (Fig. 2; Table 1).

Influence of β-adrenoceptor antagonists on isoprenaline-induced cardiostimulatory effects

CGP20712A 300 nM strongly shifted the CRCs of isoprenaline-induced chrono- and inotropic effects to the right and decreased

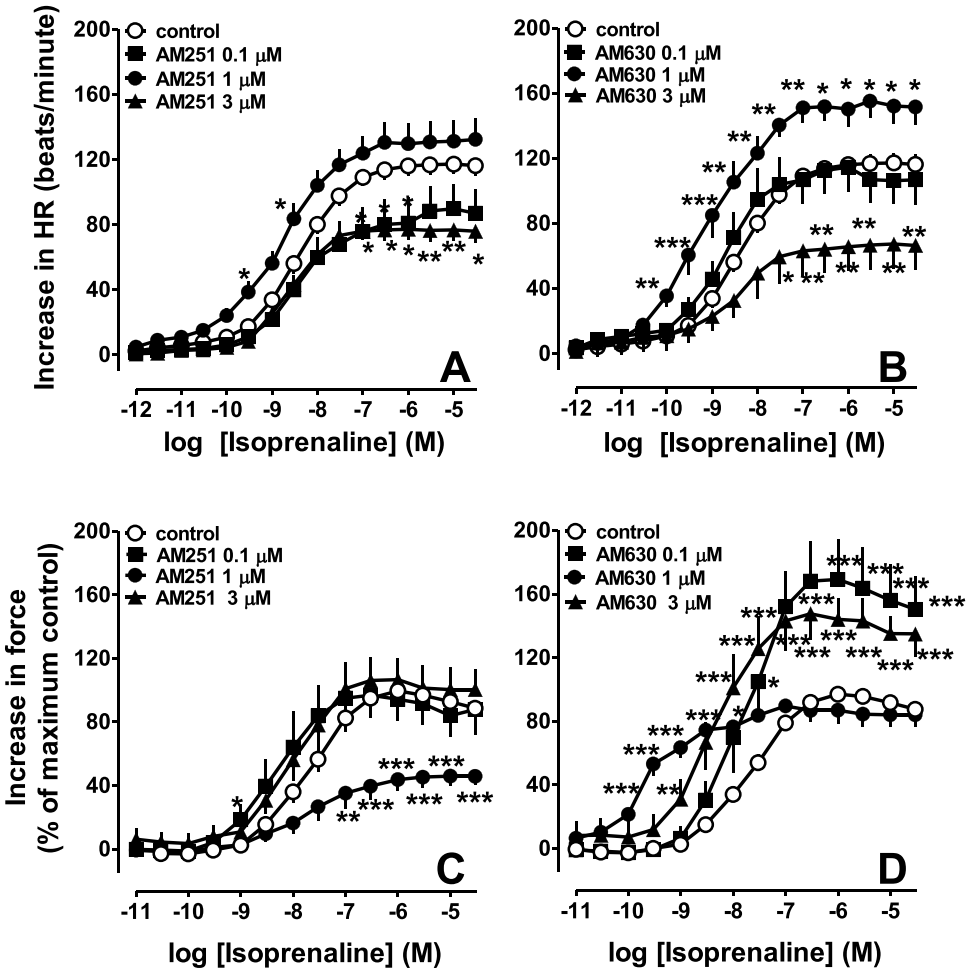

Fig. 1. Influence of AM251 and AM630 or their vehicle (control) on the positive chronotropic (A, B) and inotropic (C, D) effects of isoprenaline. Data are expressed as changes from baseline values (right atria) and as percentages of the maximum response (left atria) to isoprenaline (control) (Table 1). Data are presented as means ± SEM of 4–21 experiments; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to respective values in control groups.

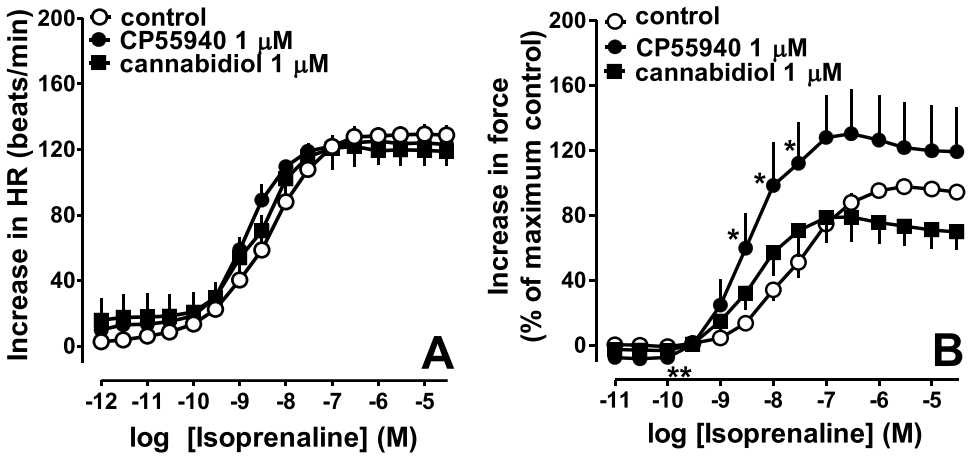

Fig. 2. Influence of CP55940 and cannabidiol or their vehicle (control) on the positive chronotropic (A) and inotropic (B) effects of isoprenaline. Data are expressed as changes from baseline values (right atria) and as percentages of the maximum response (left atria) to isoprenaline (control) (Table 1). Data are presented as means ± SEM of 4–9 experiments; $^{*}p < 0.05$ compared to respective values in control groups.

agonist potency (Fig. 3, Table 1). ICI118551 50 nM did not affect the chrono- and inotropic effects of isoprenaline.

Influence of cannabinoid antagonists on the cardiostimulatory effects induced by β-adrenoceptor agonists and a phosphodiesterase inhibitor

Xamoterol (1 nM – 30 μM), fenoterol (0.1 nM – 30 μM), CGP12177 (1 nM – 30 μM), IBMX (1 nM – 100 μM), caused concentration-dependent increases in HR of right atria maximally by approximately 70 (Fig. 4A), 140 (Fig. 4B), 75 (Fig. 4C), and 200 (determined for the highest concentration; Fig. 5A) beats/min, respectively. Only the positive chronotropic effect induced by the lower concentrations of fenoterol was reduced by AM630 but not by AM251 (1 μM each).

Fenoterol (10 nM–30 μM; Fig. 4D) and IBMX (1 nM – 100 μM; Fig. 5B) caused a concentration-dependent increase in left atrial contractile force. Neither AM251 nor AM630 (1 μM each) changed the inotropic atrial response for IBMX and fenoterol (for the respective pEC_{50} and E_{max} values, see Table 2).

Discussion

Our paper aimed to examine the influence of cannabinoid CB_1 (AM251) and CB_2 (AM630) receptor antagonists on cardiac functions mediated by β-adrenoceptors. These receptors are present in rat atria [9,25,26] and we used a simple model of isolated rat right atria, to estimate changes in spontaneously beating rate, and paced left atria, where contractility is independent of contraction frequency. We can exclude the involvement of cardiac presynaptic CB_1 receptors on sympathetic nerve endings, which modulate heart function via inhibition of noradrenaline release [11–14]. Thus, atria were paced with punctate electrodes and the voltage was just above threshold to avoid endogenous catecholamines release. Moreover, it was previously shown that the contractile effects of CB_1 and CB_2 agonists were independent of endogenous adrenergic mechanisms since they were not modified by atropine, propranolol, or chemical sympathectomy with 6-hydroxydopamine [27], and CP55940 did not affect the electrically stimulated rat atrial rate [14].

As expected [28], the nonselective β-adrenoceptor agonist isoprenaline concentration-dependently increased atrial rate and contractility. We identified four different bidirectional effects of AM251 and AM630 on the cardiostimulatory action of isoprenaline. Firstly, both CB receptor antagonists, at 1 μM, enhanced the isoprenaline-induced increase in atrial rate (affinity and, for AM630, also efficacy). Moreover, the same concentration of AM630 enhanced the affinity (but not efficacy) of the inotropic effect of isoprenaline. Secondly, AM251 1 μM decreased the efficacy (but not affinity) of isoprenaline's inotropic effect. Thirdly, AM251 0.1 and 3 μM and AM630 3 μM reduced the isoprenaline-induced increases in HR. Fourthly, the inotropic effect of isoprenaline was enhanced by AM630 0.1 and 3 μM, but was not changed by the same concentrations of AM251. Additionally, CP55940 1 μM, which activates CB_1 and CB_2 receptors (K_i values of 0.5–5.0 and 0.69–2.8 nM, respectively), and cannabidiol, which possesses low CB_1 and CB_2 receptor affinities [23], enhanced the potency of isoprenaline's inotropic effects, though cannabidiol to a lesser degree. CP55940 slightly increased the potency of isoprenaline's chronotropic action. Differential effects have been noted for AM251, e.g. in rat cultured spinal cord neurons [29], and for AM630, where in CHO cells transfected with CB_1 and CB_2 receptors AM630 respectively decreased and increased cAMP production [30]. Chronic inhibition of anandamide degradation enhanced the isoprenaline-induced inotropic effect in left atria of Wistar Kyoto rats (WKY) but reduced its chrono- and inotropic actions in isolated heart of WKY and Wistar rats [9].

In order to determine the type of β-adrenoceptors modulated by the CB receptor antagonists (1 μM), we used selective β_1- and β_2-adrenoceptor antagonists, respectively, CGP20712A and ICI118551. The positive chrono- and inotropic effects of isoprenaline were antagonized by the β_1- but hardly affected by the β_2-adrenoceptor antagonist. This is in contrast to the inhibition of the inotropic effect of isoprenaline by ICI118551 in human cardiac tissues [31] but in concordance with its lack of effect in rat left atria [32]. CGP20712A decreased HR and atrial force, suggesting activation of β_1-adrenoceptors by endogenous catecholamines.

Since isoprenaline acts *via* β_1-adrenoceptors under the conditions of our study, the interaction of the two CB antagonists with the β_1-adrenoceptor agonist xamoterol [33] was examined. CGP12177, which is an agonist at the low-affinity site of the β_1-adrenoceptor, was also evaluated but in the presence of the non-selective β-adrenoceptor antagonist propranolol, in order to block classical β_1- and β_2-adrenoceptors [25]. Both CB antagonists' lack of effect may be related to the partial β_1-adrenoceptor agonists xamoterol [34] and CGP12177 eliciting lower acceleration in atrial rates compared to isoprenaline (e.g., approximately 40% lower E_{max}) [25].

The mechanism(s) underlying the effects of CB antagonists on the isoprenaline-induced cardiostimulation are unclear. The possibility that endogenously formed cannabinoids come into

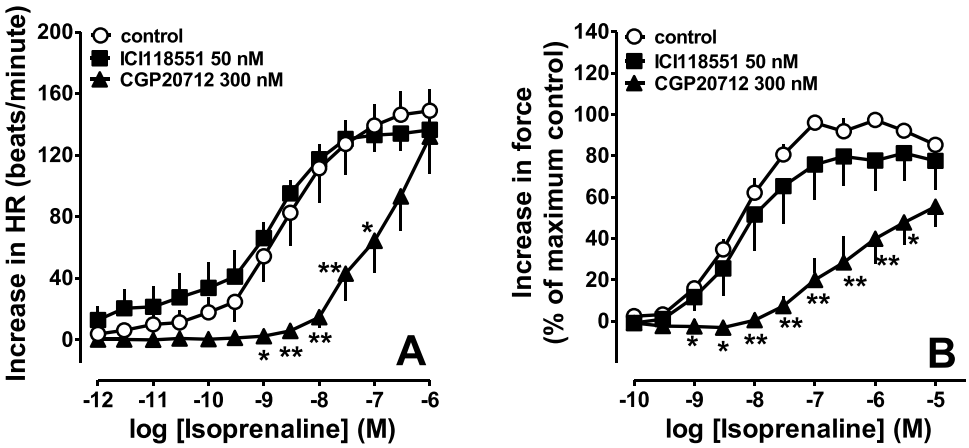

Fig. 3. Influence of CGP20712A and ICI118551 or their vehicle (control) on the positive chronotropic (A) and inotropic (B) effects of isoprenaline. Data are expressed as changes from baseline values (right atria) and as percentages of the maximum response (left atria) to isoprenaline (control) (Table 1). Data are presented as means ± SEM of 4–5 experiments; $^{*}p < 0.05$; $^{**}p < 0.01$ compared to respective values in control groups.

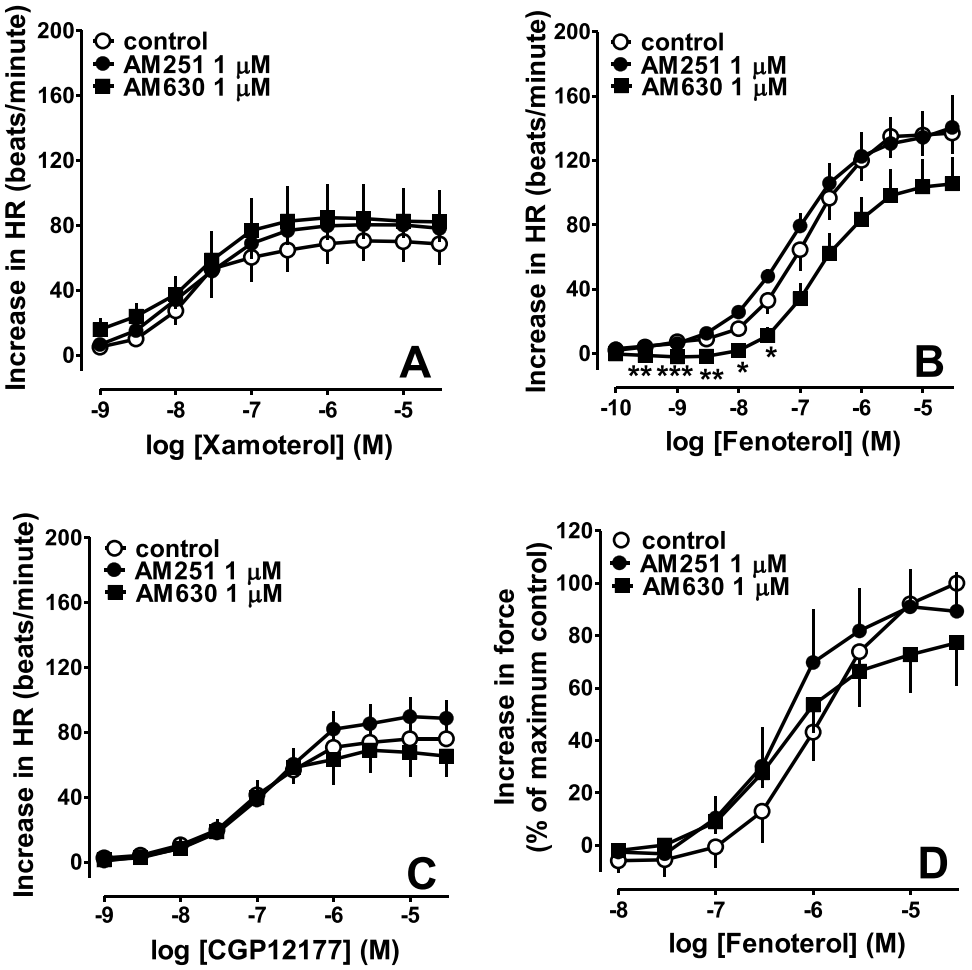

Fig. 4. Influence of AM251 and AM630 or their vehicle (control) on the positive chronotropic effects of xamoterol (A), fenoterol (B) and CGP12177 (C) and on the positive inotropic effects of fenoterol (D). Data are expressed as changes from baseline values (right atria) and as percentages of the maximum response (left atria) to fenoterol (control) (Table 2). Data are presented as means ± SEM of 3–6 experiments; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to respective values in control groups.

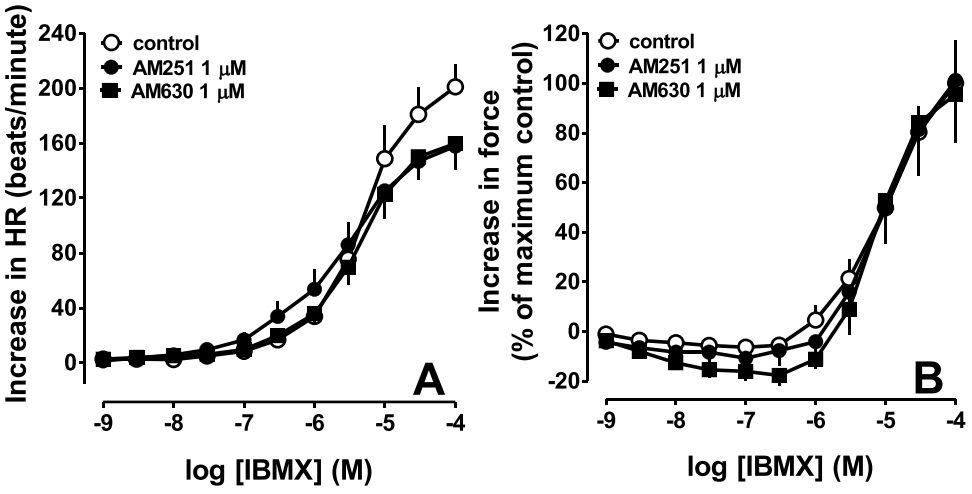

Fig. 5. Influence of AM251 and AM630 or their vehicle (control) on the positive chronotropic (A) and inotropic (B) effects of IBMX. Data are expressed as changes from baseline values (right atria) and as percentages of the maximum response (left atria) to IBMX (control) (Table 2). Data are presented as means ± SEM of 4–5 experiments.

play has to be considered. In this context, the study by Karpińska et al. [35] in human and rat pulmonary arteries is of interest, in which AM251 although not affecting basal tone enhanced the agonist-induced vasoconstrictor responses probably by blocking the CB_1 receptor-dependent vasodilatory action of endocannabinoids. However, under the conditions of the present study the possible involvement of endocannabinoids in the modulatory effect of CB receptor antagonists on the cardiostimulatory action of

isoprenaline is not plausible. Thus, (1) activation of cardiac CB receptors does not lead to any changes in basal atrial rate and (2) the CB_1 receptor antagonist decreased and the CB_2 receptor antagonist increased the positive inotropic effect of isoprenaline, i.e. both antagonists acted in the same direction as their respective agonists do (which decrease and increase cardiac contractility, respectively [8, the present study].

The opposite influences of CB receptor antagonists did not show a clear concentration dependence, suggesting different mechanisms were involved in these effects. A closer look at the concentration-response curves reveals that positive allosteric modulation may account for, at best, a portion of the effects. The opposing influences of CP55940 and cannabidiol (enhancement; 1 μM each) and AM251 (reduction; 1 μM) on the inotropic effects of isoprenaline implies possible involvement of CB_1 receptors. AM251 is also an agonist of GPR55 receptors [36]. The cardiac function in response to the α_1/β_1-adrenoceptor agonist dobutamine was attenuated in $GPR55^{-/-}$ mice [37], suggesting that this agonist might enhance cardiac function by activation of GPR55 receptors. At the lowest CB receptor antagonist doses (0.1 μM), we noticed clear inhibitory (AM251) and amplifying (AM630) effects on the chrono- and inotropic effect of isoprenaline, respectively. Interestingly, even ultra-low doses of AM251 and Δ^9-THC, respectively, have been demonstrated to enhance the anticonvulsant effects of CB_1 receptor agonist [38] and to act cardioprotectively, i.e. by elevation of fractional shortening [39].

AM251 has also been shown to inhibit the β_2-adrenoceptor-mediated increase in phosphorylated extracellular signal-related kinase in human embryonic kidney cells expressing CB_1/β_2-receptor heterodimers and to potentiate a β_2-adrenoceptor-mediated decrease in primary human trabecular meshwork cells expressing CB_1 receptors and β_2-adrenoceptors [40]. These findings prompted us to examine the effects of fenoterol, a selective β_2-adrenoceptor agonist, on HR and contractile force, even though ICI118551 had not revealed a β_2-adrenoceptor-related component of isoprenaline in our study. However, the positive chrono- and inotropic effects of fenoterol were not altered by AM251, and AM630 only slightly attenuated its positive chronotropic effect.

So far, no interaction between CB_2 receptors or their antagonist, AM630, and β-adrenoceptors has been identified. However, it should be noted that AM630 also acts as a CB_1 and CB_2 receptor inverse agonist [41]. Importantly, in contrast to AM251, all concentrations of AM630 strongly enhanced the inotropic effect of isoprenaline.

Finally, we studied the non-selective phosphodiesterase inhibitor IBMX, which causes an increase in cAMP, the second messenger generated upon β-adrenoceptor stimulation. The positive chrono- and/or inotropic effects elicited by IBMX were not affected by the two antagonists. These data argue against a direct targeting of basal cAMP by the CB antagonists and/or the contribution of a phosphodiesterase-resistant cAMP compartment [42] in their modification of isoprenaline' effects. Similarly, Δ^9-THC [43] and HU210 [44] (but not cannabidiol) did not affect basal adenylate cyclase activity but did increase its activity in rat cardiac ventricular membranes stimulated by isoproterenol via changes in membrane phospholipid order.

In conclusion, our results show that the CB_1 and CB_2 receptor antagonists AM251 and AM630 have bidirectional effects on the cardiostimulatory action of isoprenaline, most likely related to an interaction with β_1-adrenoceptors. In particular, we are the first to show an interaction between the CB_2 receptor antagonist AM630 and β-adrenoceptors, as well as add to currently limited knowledge regarding cannabinoid-mediated chronotropic effects. Even if the effects of both CB antagonists were not observed when a selective β_1-adrenoceptor agonist was used instead of isoprenaline, isoprenaline remains particularly interesting as it shares affinity for more than one adrenoceptor subtype with catecholamines. Provided that the results translate to human heart, caution should be taken when using CB_1 [e.g. 20] and CB_2 [e.g. 21] receptor antagonists, as an enhanced sympathetic tone accompanies many cardiovascular disorders.

Conflict of interests

None declared.

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