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Chronic cannabidiol treatment reduces the carbachol-induced coronary constriction and left ventricular cardiomyocyte width of the isolated hypertensive rat heart

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ABSTRACT

Cannabidiol (CBD) is suggested to possess cardioprotective properties. We examined the influence of chronic (10 mg/kg once daily for 2 weeks) CBD administration on heart structure (e.g. cardiomyocyte width) and function (e.g. stimulatory and inhibitory responses induced by β-adrenoceptor (isoprenaline) and muscarinic receptor (carbachol) activation, respectively). Experiments were performed on hearts and/or left atria isolated from spontaneously (SHR) and deoxycorticosterone (DOCA-salt) hypertensive rats; Wistar-Kyoto (WKY) and sham-operated rats (SHAM) served as the respective normotensive controls. CBD diminished the width of cardiomyocytes in left ventricle and reduced the carbachol-induced vasoconstriction of coronary arteries both in DOCA-salt and SHR. However, it failed to affect left ventricular hypertrophy and even aggravated the impaired positive and negative lusitropic effects elicited by isoprenaline and carbachol, respectively. In *normotensive* hearts CBD led to untoward structural and functional effects, which occurred only in WKY or SHAM or, like the decrease in β_1-adrenoceptor density, in either control strain. In conclusion, due to its modest beneficial effect in hypertension and its adverse effects in normotensive hearts, caution should be taken when using CBD as a drug in therapy.

1. Introduction

Cannabidiol (CBD) is a non-psychoactive constituent of marijuana and possesses a complex mechanism of action including anti-apoptotic, anti-oxidant, and anti-inflammatory properties (reviewed in: Pertwee et al. 2010; Pisanti et al. 2017; Pacher et al. 2020). CBD is indicated for the treatment of rare types of epilepsy (Arzimanoglou et al. 2020) and, in combination with Δ^9-tetrahydrocannabinol, for the therapy of multiple sclerosis-associated spasticity (Comi et al. 2020). Moreover, CBD has been postulated to have a potential therapeutic action in anxiety disorders, schizophrenia, depression, Alzheimer's and Parkinson's disease, chronic pain, cancer, inflammatory and autoimmune diseases and diabetic complications (Pisanti et al. 2017; Millar et al. 2019; Cassano et al. 2020; Pauli et al. 2020).

Recently, it has been suggested that CBD might be a promising candidate as a an antihypertensive and cardioprotective drug as well (Stanley et al. 2013; Sultan et al. 2017; Shayesteh et al. 2019; Pacher et al. 2020). Our group was the first to study whether a blood pressure-lowering effect occurs in deoxycorticosterone-salt (DOCA-salt) and spontaneously hypertensive rats (SHR); sham-operated (SHAM) and Wistar-Kyoto rats (WKY) served as the respective normotensive controls. In the first study, acute intraperitoneal (i.p.) administration at a dose of 10 mg/kg failed to modify cardiovascular parameters in SHR (Kossakowski et al. 2019). The lack of effect on blood pressure might be related to the fact that, as we could show in more reductionistic experiments of this study, CBD possesses both stimulatory and inhibitory effects on the cardiovascular system. Thus, its administration to urethane-anaesthetized SHR (and WKY) induced a short-lasting bradycardia and

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hypotension (the so-called Bezold-Jarisch reflex) whereas its administration to pithed rats disclosed a peripheral sympathomimetic effect (Kossakowski et al. 2019).

In our second study, systemic blood pressure was not affected by chronic administration (daily injections of 10 mg/kg for 14 days) to SHR and DOCA-salt (Remiszewski et al. 2020). Additional experiments within that study revealed that CBD (given for 2 weeks) possesses antioxidant effects in the heart and plasma of both hypertensive rat strains. The possibility that levels of endocannabinoids or their degrading enzymes are affected had to be considered since CBD to some extent influences the endocannabinoid system. Our experiments revealed that endocannabinoid levels in both hypertensive models are influenced in opposite direction. Unexpectedly, CBD increased some pro-oxidative parameters in the WKY and SHAM controls (Remiszewski et al. 2020). In contrast to the lack of blood pressure changes in the two models of systemic hypertension, we have found recently that chronic administration of the same dose of CBD for 21 days was sufficient to reduce monocrotaline-induced pulmonary hypertension in rats; e.g. it reduced the elevated right ventricular systolic pressure by almost 80% and restored blood oxygen saturation to the control value (Sadowska et al. 2020).

With respect to the potential use of CBD as a cardioprotective drug, its effectiveness has been shown in rat ischemic reperfusion (Durst et al. 2007), rat and mouse doxorubicin-induced cardiac injury (Fouad et al. 2013; Hao et al. 2015), mouse autoimmune myocarditis (Lee et al. 2016), mouse diabetic cardiomyopathy (Rajesh et al. 2010) and in the acute cardiac injury induced by ischemia/reperfusion of the rat (Walsh et al. 2010; Gonca and Darıcı, 2015). However, in none of the above publications the effect of chronic CBD administration on cardiac hypertrophy (including hypertensive heart disease) or its influence on the cardiac function modified by β-adrenergic and acetylcholine muscarinic (M) receptors had been studied. A protective effect of CBD against high-glucose-induced arrhythmia and cytotoxicity has also been shown on human cardiac voltage-gated sodium channels in vitro (Fouda et al. 2020).

Although acute or chronic CBD administration failed to influence systemic blood pressure, the drug might have a beneficial effect on hypertensive heart disease, which leads to structural and functional alterations including cardiac fibrosis and remodeling of the atria and ventricles and the arterial system (reviewed in: Nwabuo and Vasan 2020). For example, chronic pirfenidone administration in DOCA-salt hypertension attenuated left ventricular hypertrophy and improved noradrenaline effects in right atria without lowering systolic blood pressure (SBP) (Mirkovic et al. 2002). According to the protective effect of CBD in numerous models of impaired heart function, we hypothesize that it might improve cardiac performance in hypertensive heart disease of DOCA-salt and SHR rats.

To this end, the present investigation was performed using (1) isolated retrograde-perfused Langendorff hearts, (2) isolated left atria, (3) western blotting and (4) histological studies. In detail, we examined the influence of chronic CBD administration on the left ventricular weight and on cardiomyocyte width, on β-adrenoceptor density and on the function of hearts and/or atria under basal conditions and after β-adrenoceptor, muscarinic ACh and cannabinoid receptor activation.

2. Materials and methods

2.1. Animals

All experimental procedures were conducted in agreement with the European Directive (2010/63/EU), Polish legislation and according to the newest ARRIVE guidelines (Percie du Sert et al., 2020). Experimental protocols were approved by the local Animal Ethics Committee in Olsztyn (nr 80/2017). Animals were delivered from the Center for Experimental Medicine of the Medical University of Białystok (Poland). Rats had free access to food and water and were housed in plastic cages in a temperature-controlled room at 22 ± 1 °C under a 12/12 h light/dark cycle.

DOCA-salt hypertension was induced in male Wistar rats (5–6 weeks old; initially weighing 200–300 g) as described previously (Pędzińska-Betiuk et al., 2017; Remiszewski et al. 2020). All animals were anaesthetized with an intraperitoneal (*i.p.*) injection of pentobarbital sodium (70 mg/kg, i.e. ~300 μmol/kg, Biowet, Puławy, Poland) and underwent unilateral nephrectomy. After one week of recovery, rats were divided into two groups: (1) hypertensive animals (DOCA-salt), in which hypertension was induced by subcutaneous injections of 11-deoxycorticosterone acetate (DOCA 25 mg/kg, i.e. ~ 67 μmol/kg; 0.4 ml/kg, Sigma-Aldrich, Munich, Germany) twice weekly for 4 weeks and drinking water was replaced by 1% NaCl solution and (2) normotensive animals (SHAM), which received the vehicle for DOCA (*N*, *N*-dimethylformamide, Sigma-Aldrich, Munich, Germany) twice weekly and drank tap water. *Spontaneously hypertensive rats*. Male 8-9-week-old spontaneously hypertensive rats (SHR) weighing 250-350 g and age-matched normotensive Wistar-Kyoto rats (WKY) weighing 290–380 g were used in experiments.

2.2. Chronic treatment with cannabidiol

(−)-Cannabidiol (CBD; THC Pharm GmbH, Frankfurt, Germany) 10 mg/kg or its vehicle [ethanol, Tween 80 (Sigma-Aldrich, Munich, Germany), 0.9% NaCl - 3:1:16] were injected *i.p.* every 24 h for 14 days to the following 4 groups of rats: hypertensive (1) DOCA-salt; (2) SHR and their normotensive controls: (3) SHAM and (4) WKY, respectively: The administration of the first CBD dose or its vehicle was preceded by systolic blood pressure (SBP) and mean arterial pressure (MAP) measurement in conscious rats by the non-invasive tail-cuff method (ADInstruments, Sydney, Australia). Diastolic blood pressure (DBP) was calculated from the equation DBP = (3xMBP - SBP)/2 (Geleta et al. 2016). Twenty four hours after the final dose the BP measurement was repeated. From part of the rats (10–11 animals per group) hearts or left atria were prepared for perfusion experiments (for basal parameters, see Table 1). For Western blots and histological analyses, cardiac tissue samples were collected from separate groups of rats (6 animals per group; for basal parameters, see Supplementary Table 1).

2.3. Isolated retrograde-perfused Langendorff heart

Experiments in vitro started 24 h after the last dose of CBD or its vehicle. Rats were anaesthetized with pentobarbital sodium (300 μmol/kg, *i.p.*) and injected with heparin (500 IU, *i.p.,* Polfa, Warsaw, Poland). After excision, hearts were weighed and inserted in a Langendorff system (Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) and experiments were conducted as described previously (Pędzińska-Betiuk et al., 2017). Diastolic stiffness was determined as the diastolic stiffness constant (κ, dimensionless), the slope of the linear relation between tangent elastic modulus (E, dyne/cm^2) and stress (σ, dyne/cm^2). The coronary perfusion pressure (CPP) was recorded with a second pressure transducer via a cannula in the aorta. Diastolic pressure was set to 8–10 mmHg by inflating the balloon accordingly. Hearts beat spontaneously and all parameters were monitored with the ISOHEART software (Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany). After 25 min of stabilization, hearts were perfused (peristaltic pump; Ascor, Warsaw, Poland) by increasing concentrations of isoprenaline (0.01 nM – 1 μM, Sigma-Aldrich, Munich, Germany) into the coronary arteries until a plateau was reached. Increasing concentrations of carbachol (0.01 μM - 1 μM, Merck, Darmstadt, Germany) were infused after reaching a steady state in response to isoprenaline (1 μM) (Ralay Ranaivo et al. 2004). Isoprenaline and carbachol were dissolved in distilled water (stock solutions) and further dilutions were made with Krebs solution. Experiments on were only performed if the initial left ventricular pressure (LVP) was >75 mmHg and arrhythmias were lacking. (1) Heart rate (HR,

Table 1
Influence of cannabidiol (CBD) on physiological parameters, cardiac structure and diastolic stiffness of deoxycorticosterone-salt (DOCA-salt) and spontaneously (SHR) hypertensive rats and sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls.

Parameters	n	SHAM	SHAM CBD	DOCA-salt	DOCA-salt CBD	WKY	WKY CBD	SHR	SHR CBD
		10	10	10	11	10	10	10	10
SBP (mmHg)									
day 0		126 ± 6	124 ± 6	148 ± 5$^{\#}$	153 ± 6	109 ± 5	110 ± 5	190 ± 6$^{\#\#\#}$	184 ± 8
day 14		119 ± 6	105 ± 5	152 ± 8$^{\#\#}$	168 ± 9	106 ± 4	112 ± 4	190 ± 6$^{\#\#\#}$	183 ± 5
DBP (mmHg)									
day 0		112 ± 6	109 ± 6	117 ± 5	121 ± 6	70 ± 5	73 ± 5	160 ± 5$^{\#\#\#}$	154 ± 9
day 14		107 ± 6	93 ± 5	129 ± 6$^{\#}$	143 ± 8	72 ± 4	74 ± 4	161 ± 7$^{\#\#\#}$	153 ± 6
MAP (mmHg)									
day 0		116 ± 6	114 ± 6	127 ± 4	132 ± 5	83 ± 5	85 ± 5	170 ± 5$^{\#\#\#}$	164 ± 9
day 14		111 ± 6	97 ± 5	136 ± 7$^{\#}$	151 ± 8	83 ± 4	87 ± 4	171 ± 6$^{\#\#\#}$	163 ± 6
HR (beats/min)									
day 0		372 ± 9	357 ± 8	333 ± 11$^{\#}$	341 ± 9	315 ± 3	338 ± 7	375 ± 25$^{\#\#}$	396 ± 6
day 14		351 ± 13	348 ± 19	291 ± 11$^{\#\#,\Delta}$	316 ± 12	311 ± 11	319 ± 4$^{\Delta}$	399 ± 9$^{\#\#\#}$	394 ± 7
Body weight (g)									
day 0		300 ± 14	288 ± 8	282 ± 10	283 ± 8	342 ± 8	337 ± 7	296 ± 9$^{\#\#\#}$	298 ± 8
day 14		321 ± 17$^{\Delta\Delta\Delta}$	314 ± 9$^{\Delta\Delta\Delta}$	296 ± 11$^{\Delta\Delta\Delta}$	300 ± 7$^{\Delta\Delta\Delta}$	368 ± 7$^{\Delta\Delta\Delta}$	359 ± 8$^{\Delta\Delta\Delta}$	325 ± 9$^{\#\#,\Delta\Delta\Delta}$	319 ± 8$^{\Delta\Delta\Delta}$
Tibia length (mm)		33.7 ± 0.4	34.5 ± 0.5	33.9 ± 0.4	34.0 ± 0.4	37.7 ± 0.3	37.3 ± 0.2	34.6 ± 0.4$^{\#\#\#}$	34.8 ± 0.4
Heart weight/body weight (mg/g)		4.28 ± 0.16	4.23 ± 0.21	5.08 ± 0.27$^{\#}$	4.97 ± 0.25	4.76 ± 0.29	4.71 ± 0.21	5.63 ± 0.21$^{\#}$	5.84 ± 0.13
Heart weight/tibia length (mg/mm)		40.9 ± 2.8	38.3 ± 1.9	44.4 ± 2.7	44.0 ± 2.7	46.5 ± 2.9	45.2 ± 2.4	52.6 ± 1.6	53.4 ± 1.6
LV + septum weight/body weight (mg/g)		2.18 ± 0.05	2.24 ± 0.11	2.93 ± 0.11$^{\#\#\#}$	2.99 ± 0.09	2.52 ± 0.08	2.56 ± 0.07	3.12 ± 0.06$^{\#\#\#}$	3.28 ± 0.07
LV + septum weight/tibia length (mg/mm)		20.6 ± 0.8	20.3 ± 1.00	25.6 ± 1.3$^{\#\#}$	26.5 ± 1.2	24.6 ± 0.8	24.7 ± 0.8	29.2 ± 0.5$^{\#\#\#}$	30.0 ± 0.6
RV weight/body weight (mg/g)		0.57 ± 0.02	0.55 + 0.02	0.63 ± 0.03	0.67 ± 0.03	0.72 ± 0.02	0.73 ± 0.04	1.00 ± 0.003$^{\#\#\#}$	0.86 ± 0.03**
RV weight/tibia length (mg/mm)		5.40 ± 0.28	4.96 ± 0.19	5.49 ± 0.30	5.98 ± 0.38	7.08 ± 0.33	7.01 ± 0.38	9.25 ± 0.25$^{\#\#\#}$	7.87 ± 0.36*
Diastolic stiffness constant (κ)		29.0 ± 4.8	30.1 ± 2.2	34.1 ± 3.5	35.2 ± 2.9	37.4 ± 1.9	26.6 ± 2.4*	47.2 ± 2.5$^{\#}$	42.9 ± 4.4

This table only contains data for those rats the isolated hearts and atria of which were used for functional in vitro experiments (Figs. 2-6; Tables 1-6). (Corresponding data for rats which were used for Western blots and histological studies are given in Supplementary Table 1). CBD (10 mg/kg) or vehicle was injected *i.p.* every 24 h for 14 days. Systolic (SBP), diastolic (DBP), mean arterial pressure (MAP) and heart rate (HR) were recorded before (day 0) the first dose of CBD or its vehicle and 14 days later, i.e. they represent in vivo data as opposed to the HR values in Figs. 2-6 and Tables 2-6. Heart and body weights were determined 24 h after the last dose of CBD or its vehicle. Diastolic stiffness was measured at the beginning of the Langendorff experiments. Weights of right and left ventricle (RV and LV with septum) were determined after the in vitro experiments. Means ± SEM; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ for DOCA-salt and SHR vs. SHAM and WKY; $^{*}P < 0.05$; $^{**}P < 0.01$ vs. values in rats not treated with CBD; one-way analysis of variance (ANOVA) with Bonferroni post hoc test; $^{\Delta}P < 0.05$; $^{\Delta\Delta\Delta}P < 0.001$ vs. values before CBD treatment (day 0 vs. day 14); Student's *t*-test for paired data.

beats/min) was used to estimate chronotropism; (2) inotropism was estimated in terms of LVP (mmHg, index of contractile activity) and maximal value of the first LVP derivative [$+(LVdP/dt)_{max}$; in mmHg/s, index of maximal LV contraction rate]; (3) lusitropism was evaluated on the basis of the maximal rate of LVP decline [$-(LVdP/dt)_{max}$; mmHg/s]; (4) the rate-pressure product (RPP: an index of cardiac work, which is defined as the product of left ventricular developed pressure (LVDP) and HR; mmHg x beats/min); (5) CPP (mmHg) served as an index of coronary contraction/dilatation. Changes in cardiac parameters are expressed as delta from the values obtained before the first concentration of isoprenaline or carbachol.

2.4. Isolated left atria

At the onset of isolated heart perfusion, the left atria were mounted in 10 ml organ baths and stimulated electrically (5 ms, 2 Hz) as described previously (Pędzińska-Betiuk et al., 2017; Weresa et al. 2019). Neither hypertension nor CBD affected basal values. Responses to particular cumulatively added agonists: CP55940 (1 nM – 30 μM, Tocris, Bristol, UK) and isoprenaline (0.01 nM – 10 μM) were determined as changes in the basal force of left atrium (decrease or increase) and expressed as a percentage of basal values. Stock solution of CP55940 was prepared in dimethyl sulphoxide (DMSO) and further diluted with Krebs solution. In that manner, the final concentration of DMSO in the tissue bath was <0.01%.

2.5. Western blotting

Total expression of adrenergic receptors was estimated in the left ventricle of cardiac muscle using the Western blot procedure (Remiszewski et al. 2020). In summary, samples were homogenized in radioimmunoprecipitation assay (RIPA) buffer with subsequent determination of protein concentration (bicinchoninic acid method). Next, all samples were reconstituted in Laemmli buffer and loaded (30 μg) onto CriterionTM TGX Stain-Free Precast Gels (Bio-Rad, Hercules, CA, USA). After electrophoresis proteins were transferred onto polyvinylidene difluoride (PVDF) membranes and blocked in Tris Buffer Saline Tween20 containing 5% (wt/vol) non-fat dry milk. In order to measure the total protein load in each sample lane a stain-free blot image was taken (ChemiDoc XRS; Bio-Rad, Hercules, CA, USA) for further quantification and normalization. After blocking, the membranes were incubated with primary antibodies: β_1 adrenergic receptor (1:500, cat no. Ab3442; Abcam, Cambridge, UK) and β_2 adrenergic receptor (1:500, cat no. Ab182136; Abcam, Cambridge, UK), which was followed by incubation with secondary antibodies conjugated with horseradish peroxidase (1:3000; Cell Signaling, Danvers, MA, USA). After visualization with chemiluminescence substrate (Clarity Western ECL Substrate; Bio-Rad, Hercules, CA, USA), the obtained signals were quantified densitometrically using the ChemiDoc visualization system XRS (Bio-Rad, Warsaw, Poland) and the Image Laboratory Software Version 6.0.1 (Bio-Rad, Hercules, CA, USA), respectively. Stain-free gels

and the total protein normalization method (Bio-Rad, Hercules, CA, USA) were applied to determine adrenergic receptor expression and the obtained changes were expressed as the fold change compared to the respective control group. Briefly, the first step was to create a multichannel image, which included stain-free and chemiluminescent blot images. The lanes established from the stain-free blot were copied and pasted to the corresponding chemiluminescent blot and the examined proteins bands were detected. The normalization channel was assigned as the stain free blot and all calculations were performed by the software (both normalization factor and normalized volumes). The chemiluminescent blot channel intensity values were adjusted for variation in the protein loading between the different lanes. Therefore, the total protein normalization included the comparison of the chemiluminescence signal in each lane to its corresponding stain-free lane.

2.6. Histological studies

Heart tissues, after fixation in 4% buffered formalin, dehydration and embedding in paraffin, were sliced into 4 μm thick slices and stained with hematoxylin and eosin (H + E). Histological staining was evaluated in an Olympus BX41 light microscope (Olympus 114 Corp., Tokyo, Japan) with an Olympus DP12 digital camera (Olympus 114 Corp., Tokyo, Japan). From each animal 6 sections of the heart were studied (three sections from the left and three from the right ventricle). Five randomly selected microscopic fields from each section (each field of 0.785 mm^2; magnification by 200×, 20× by the lens and 10× by the eyepiece) were documented. Subsequently, images were morphometrically evaluated by using the NIS-Elements Advanced Research software of Nikon (Tokyo, Japan). The width of 25 randomly selected cardiomyocytes was estimated in such fields in which the long axis of the cell in the cutting plane was oriented with a visible cell nucleus.

2.7. Statistical analysis

Results are given as the mean ± SEM (n = number of animals). Maximal effects of agonists (E_{max}) and their potencies [pEC_{50} values defined as the negative logarithm of the EC_{50} i.e. concentration exerting 50% of maximal effects (E_{max})] were evaluated from the particular concentration–response curves. Maximal effects refer to changes in % of basal values for isolated atria but to changes from baseline in absolute terms for Langendorff hearts; for carbachol-induced changes the level obtained after stimulation with isoprenaline 1 μM was considered as baseline. Statistical analysis was performed using Graph Pad Prism 5 (GraphPad Software, La Jolla, CA, USA). Inter-group statistical comparisons were made by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test for selected pairs of the entire data set. Post hoc tests were conducted only if F was significant, and there was no variance inhomogeneity. Student's t-test for paired and unpaired data was used as appropriate. Values were considered significant at $P < 0.05$.

3. Results

3.1. General

As shown in Table 1 and in Supplementary Table 1, before administration of CBD or its vehicle (day 0), SBP, diastolic blood pressure (DBP) and mean arterial pressure (MAP) were higher both in DOCA-salt (with the exception of DBP and MAP at day 0) and SHR than in their respective normotensive controls SHAM and WKY. However, hypertension was more pronounced in SHR than in DOCA-salt. Heart rate was lower in DOCA-salt but higher in SHR rats in comparison with SHAM and WKY, respectively. SHR weighed less than WKY whereas body weights of DOCA-salt and SHAM were comparable. Two-week administration of CBD or its vehicle did not modify SBP, DBP, MAP and HR in any group.

3.2. Influence of hypertension and chronic CBD administration on cardiac hypertrophy and diastolic stiffness

Cardiac hypertrophy was confirmed by higher (by about 20%) ratios of heart weight to body weight in DOCA-salt and SHR hearts in comparison to their respective normotensive controls (Table 1). These changes were accompanied by higher (by 20–35%) ratios of left ventricle + septum weight to body weights as well as left ventricle + septum weight to tibia length in both hypertensive models. Additionally, increases by 30–40% in the ratio of right ventricle weight to body weight and to tibia length in comparison to WKY were observed in SHR whereas both ratios did not differ between SHAM and DOCA-salt. CBD reduced the ratio of right ventricle weight to body weight and to tibia length in SHR by 15%.

Diastolic stiffness tended to increase in DOCA-salt and significantly increased in SHR by about 15 and 25%, compared to the respective normotensive rats (Table 1). Unexpectedly, CBD diminished diastolic stiffness in WKY by about 30% but only tended to decrease (by 9%) this parameter in SHR.

3.3. Influence of hypertension and CBD on width of cardiomyocytes

In comparison to SHAM and WKY, the width of cardiomyocytes isolated from DOCA-salt and SHR were increased by about 55% in left and by 25–30% in right ventricles (Fig. 1). Chronic CBD administration reduced the width of left ventricular myocytes from DOCA by 10% and of left and right ventricular myocytes from SHR by 11 and 7%, respectively. Unexpectedly, it increased the width of left and right ventricular myocytes of SHAM (by about 15 and 25%, respectively) without affecting those of WKY (Fig. 1).

3.4. Influence of hypertension and CBD on basal functional parameters of isolated hearts

Basal functional parameters of hearts isolated from DOCA-salt and SHAM were similar (Table 2). Cardiac parameters in SHR were comparable to the corresponding values in DOCA-salt but, except for HR and CPP, exceeded the corresponding values in WKY hearts by 30–40%. CBD failed to modify all basal parameters of normo- and hypertensive isolated hearts (Table 2).

3.5. Influence of hypertension and CBD on functional responses to isoprenaline and carbachol in isolated hearts

Isoprenaline (0.01 nM – 1 μM) concentration-dependently increased all cardiac parameters of hearts isolated from normotensive and hypertensive rats but decreased CPP (Figs. 2 and 3; Table 3). Its positive chronotropic effect was comparable in SHAM and DOCA-salt. Other parameters (LVP, $+(LVdP/dt)_{max}$, $-(LVdP/dt)_{max}$ and RPP) tended to decrease in DOCA-salt in comparison to SHAM by about 10, 15, 25 and 15%, respectively. Only the attenuation of relaxation [$-(LVdP/dt)_{max}$] and the increase in RPP induced by isoprenaline 0.1 μM reached a significant level (Fig. 2C, 3B). In hearts isolated from SHR, isoprenaline elicited lower increases in LVP and RPP and a lower decrement in $-(LVdP/dt)_{max}$ in comparison to WKY; the maximal responses to isoprenaline were reduced by about 50, 40 and 25%, respectively (Figs. 2 and 3). Moreover, the potency of isoprenaline for $+(LVdP/dt)_{max}$ was diminished in SHR (Table 3).

Chronic administration of CBD did not modify the concentration-response curves for isoprenaline in DOCA-salt and their normotensive controls. The only exceptions were an enhancement of the increases in HR and RPP induced in SHAM by isoprenaline 0.1 and 0.01 nM, respectively (Fig. 3A, B). In SHR it modified the positive lusitropic effect, i.e. $-(LVdP/dt)_{max}$, of isoprenaline only. Thus, it increased the potency of isoprenaline but diminished its maximal effect. Unexpectedly, CBD reduced the positive inotropic effect (LVP) of isoprenaline at high

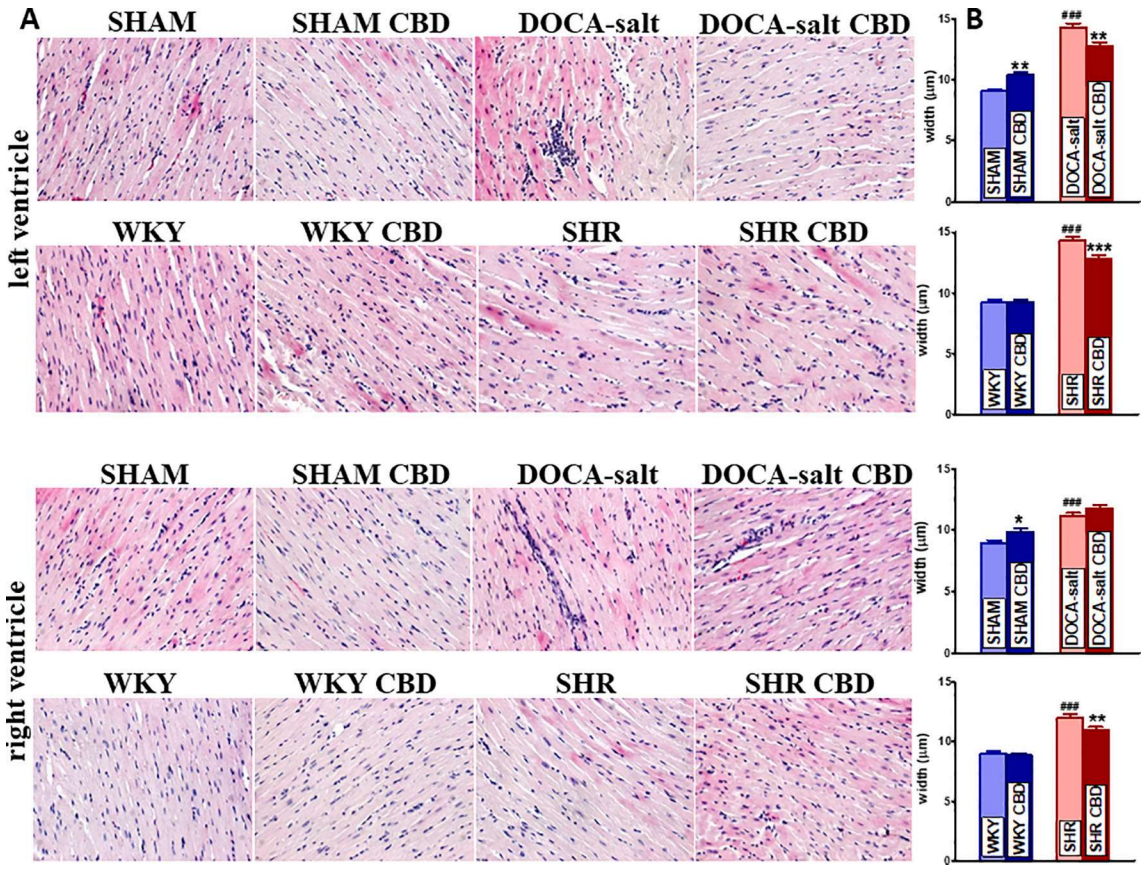

Fig. 1. Representative cardiac sections (A) and influence of cannabidiol (CBD) on the width of cardiomyocytes (B) in the left and right ventricles of hearts isolated from deoxycorticosterone (DOCA-salt) and spontaneously (SHR) hypertensive rats and from sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls (magnification 200×). CBD (10 mg/kg) or its vehicle was injected i.p. every 24 h for 14 days. Hearts were prepared 24 h after the final injection of CBD or its vehicle. Mean ± SEM (of 6 animals per group). $^{*}P < 0.05$; $^{**}P < 0.01$;$^{***}P < 0.001$, significant effect of CBD, $^{###}P < 0.001$, DOCA-salt and SHR significantly different from SHAM and WKY, respectively; one-way analysis of variance (ANOVA) with Bonferroni post hoc test.

Table 2
Influence of cannabidiol (CBD) or its vehicle on basal parameters of hearts isolated from deoxycorticosterone-salt (DOCA-salt) and spontaneously (SHR) hypertensive rats and sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls.

Parameters	n	SHAM	SHAM CBD	DOCA-salt	DOCA-salt CBD	WKY	WKY CBD	SHR	SHR CBD
	–	10	10	10	11	10	10	10	10
HR (beats/min)		301 ± 13	297 ± 10	273 ± 8	275 ± 10	260 ± 7	267 ± 6	257 ± 8	260 ± 7
LVP (mmHg)		108 ± 6	109 ± 5	107 ± 9	112 ± 7	84 ± 4	87 ± 4	109 ± 8$^{##}$	111 ± 3
+(LVdP/dt)$_{max}$ (mmHg/s)		2983 ± 119	3135 ± 189	2950 ± 173	3203 ± 135	2186 ± 150	2293 ± 186	3032 ± 227$^{##}$	2869 ± 115
-(LVdP/dt)$_{max}$ (mmHg/s)		-2125 ± 115	−2336 ± 145	−2130 ± 235	−2200 ± 154	−1703 ± 97	−1866 ± 121	−2394 ± 223$^{##}$	−2296 ± 109
RPP (mmHg/beats/min)		29,163 ± 1973	29,617 ± 2137	25,914 ± 2265	27,577 ± 1797	20,591 ± 1225	21,367 ± 1254	26,052 ± 2175$^{##}$	25,801 ± 1217
CPP (mmHg)		75 ± 5	86 ± 5	76 ± 5	79 ± 4	81 ± 4	86 ± 3	77 ± 4	70 ± 3

Units are given in brackets. CBD 10 mg/kg or its vehicle were injected *i.p.* every 24 h for 14 days. Hearts were isolated 24 h after the final dose of CBD or its vehicle. Data are given as the means ± SEM. $^{##}P < 0.01$ for SHR vs. WKY; one-way analysis of variance (ANOVA) with Bonferroni post hoc test. HR - heart rate, LVP - left ventricular pressure, maximum rate of positive +(LVdP/dt)$_{max}$ and negative -(LVdP/dt)$_{max}$ changes in LVP, RPP - rate pressure product, CPP - coronary perfusion pressure.

concentrations by about 30% in WKY. However, the potency of isoprenaline was not affected (Fig. 2A', Table 3).

The isoprenaline-induced decrease in CPP (vasodilatation of the coronary arteries) was abolished by hypertension (both DOCA-salt and SHR) but not altered by CBD (Fig. 3C, C', Table 3).

Basal parameters of isolated hearts before infusion of the first concentration of carbachol represent the cardiac activity in the presence of the highest concentration of isoprenaline (1 μM) (Table 4). On the rule, hypertension or CBD treatment did not affect basal parameters. There were two exceptions. CPP was elevated by 42% in DOCA-salt in comparison to SHAM; −(LVdP/dt)$_{max}$ in SHR treated with CBD was lower by 29% in comparison to untreated SHR.

Increasing concentrations of carbachol (0.01 μM - 1 μM) diminished all increases in cardiac parameters induced by isoprenaline 1 μM but increased CPP (Figs. 4 and 5, Table 5). Despite the strong decrease in heart rate, the hearts were still working. The decrease in LVP in DOCA-

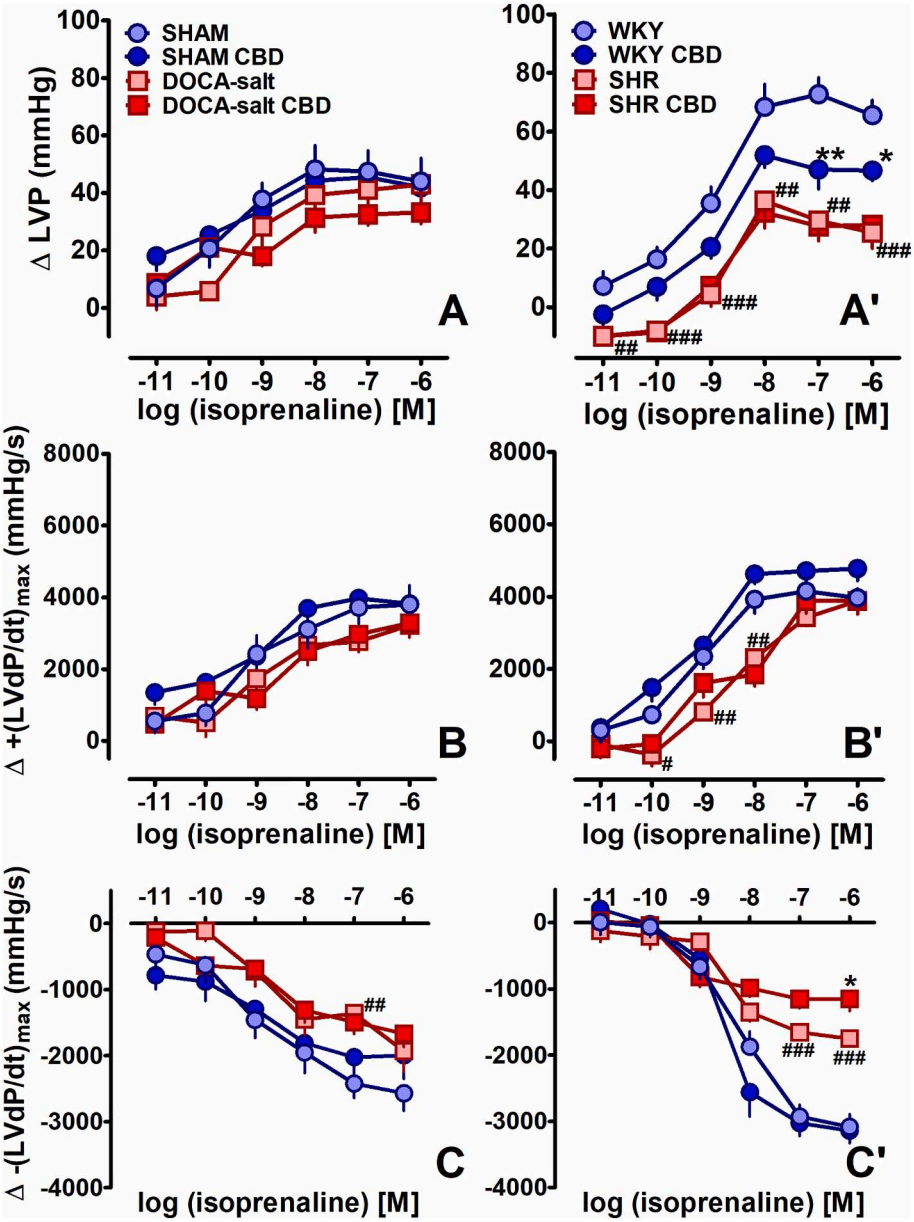

Fig. 2. Influence of cannabidiol (CBD) on the isoprenaline-induced changes in LVP (A, A'), $+(LVdP/dt)_{max}$ (B, B′) and $-(LVdP/dt)_{max}$ (C, C′) of hearts isolated from deoxycorticosterone (DOCA-salt) and spontaneously (SHR) hypertensive rats and sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls. CBD (10 mg/kg) or vehicle was injected *i.p.* every 24 h for 14 days. Values shown are changes from baseline (Table 2). Data are given as the means ± SEM of 10–11 rats. $^{*}P < 0.05$; $^{**}P < 0.01$, significant effect of CBD. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$, DOCA-salt and SHR significantly different from SHAM and WKY, respectively; one-way analysis of variance (ANOVA) with Bonferroni post hoc test. LVP - left ventricular pressure; $+(LVdP/dt)_{max}$, $-(LVdP/dt)_{max}$ - maximum rate of positive and negative changes in LVP, respectively.

salt was less prominent in comparison to SHAM by about 35% in the case of E_{max}, respectively; for HR, $+(LVdP/dt)_{max}$, $-(LVdP/dt)_{max}$ and RPP the difference (by about 25%) did not reach a significant level. In SHR, the maximal decreases in LVP and $-(LVdP/dt)_{max}$ were smaller by about 30% in comparison to WKY (Figs. 4 and 5, Table 5). The carbachol-induced increase in CPP was slightly attenuated in DOCA-salt (significant at 10 nM and 0.3 μM) but not modified in SHR (Fig. 5, Table 5).

Chronic CBD treatment significantly reduced the negative inotropic effect (LVP and RPP) of high concentrations of carbachol in WKY hearts by approximately 25% of the E_{max} in comparison to hearts isolated from untreated WKY. In SHR, CBD diminished the E_{max} of the negative lusitropic effect of carbachol (−(LVdP/dt)max) by about 40%. Moreover, CBD reduced the carbachol-induced increases in CPP in hearts both from DOCA-salt and SHR in comparison to hearts from untreated rats (by approximately 50% and 40% for E_{max}, respectively) (Figs. 4 and 5, Table 5). None of the pEC_{50} values was modified by hypertension or CBD (Table 5).

3.6. *Influence of hypertension and CBD on isoprenaline-induced cardiostimulatory and CP55940-induced cardioinhibitory effects in isolated left atria*

As shown in Table 6, the initial contractile forces of isolated left atria (before the first concentration of isoprenaline) were comparable in all experimental normotensive and hypertensive groups. Isoprenaline (0.01 nM – 10 μM) concentration-dependently increased the force of contractions. Its potency was comparable in SHAM and WKY, but its efficacy was higher by about 60% ($P < 0.05$) in WKY than in SHAM. Hypertension did not alter the isoprenaline effect in DOCA-salt in comparison to SHAM (Fig. 6, Table 6). However, the positive inotropic effect of high concentrations of isoprenaline (0.1–10 μM) tended to be higher in WKY than in SHR. CBD did not alter (or only marginally altered) the inotropic effects of isoprenaline in atria isolated from DOCA-salt, SHR and their corresponding controls (Fig. 6, Table 6).

CP55940 (1 nM - 30 μM) concentration-dependently decreased the force of contractions of left atria with similar efficacy (about 55–60%) in WKY and SHAM but with higher potency ($P < 0.001$) in WKY than in

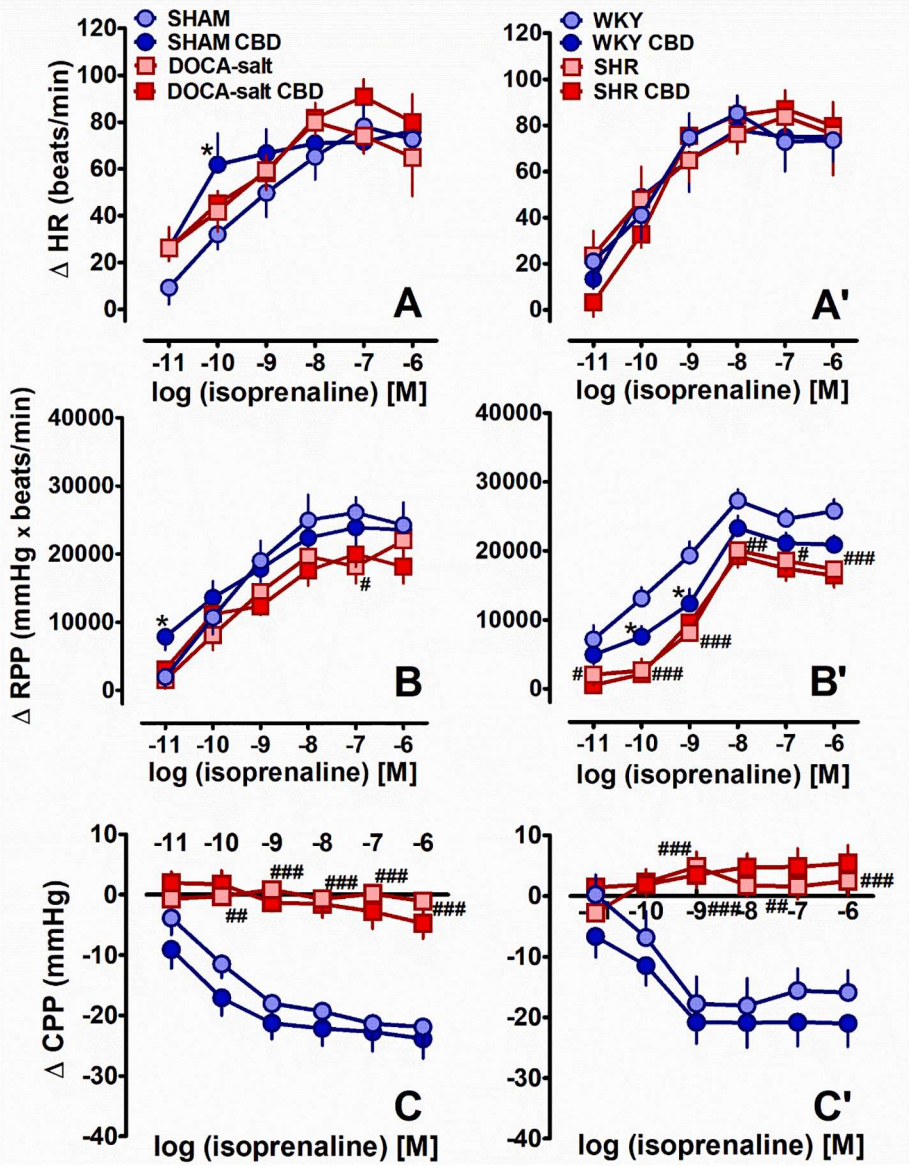

Fig. 3. Influence of cannabidiol (CBD) on the isoprenaline-induced changes in HR (A, A'), RPP (B, B′) and CPP of hearts isolated from deoxycorticosterone (DOCA-salt) and spontaneously (SHR) hypertensive rats and sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls. CBD (10 mg/kg) or vehicle was injected *i.p.* every 24 h for 14 days. Values show changes from baseline (Table 2). Data are given as the means ± SEM of 10–11 rats. $^{*}P < 0.05$, significant effect of CBD. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$, DOCA-salt and SHR significantly different from SHAM and WKY, respectively; one-way analysis of variance (ANOVA) with Bonferroni post hoc test. HR - heart rate, RPP - rate pressure product, CPP - coronary perfusion pressure.

SHAM. The negative inotropic effect of CP55940 was slightly lower in DOCA-salt than in SHAM (significant difference for E_{max} only), but markedly weaker in SHR than in WKY (by about 40% in the case of E_{max}). Chronic CBD treatment enhanced the maximal negative inotropic effect of CP55940 in DOCA-salt by about 50% (for E_{max} values, see Table 6, Fig. 6). In atria isolated from SHR, CBD slightly shifted the concentration–response curve for CP55940 to the right (Fig. 6, for pEC_{50}, see Table 6).

3.7. Influence of hypertension and CBD on cardiac β_1- and β_2-adrenoceptor expression in left ventricles

As shown in Fig. 7, the density of β_1-adrenoceptors in left ventricle tended to decrease (by about 20%) in DOCA-salt and significantly decreased in SHR (by about 40%). CBD decreased the β_1-adrenoceptor expression in both normotensive groups (by about 35%) as well as in DOCA-salt (by about 50%) without affecting the level of this receptor in SHR. The expression of β_2-adrenoceptors was modified neither by hypertension nor by CBD (Fig. 7).

4. Discussion

4.1. General

The question whether chronic CBD administration has a beneficial effect on structural and functional changes of hypertensive heart disease was examined on rats with primary (SHR) and secondary (DOCA-salt) hypertension. SHR is a genetic hypertensive model which resembles the hypertension phenotypes observed in humans and in which most antihypertensive drugs are active (Lerman et al. 2019). The uninephrectomized DOCA-salt model was chosen because salt-rich diet is one of the main factors leading to severe hypertension with some features of human low-renin hypertension (Lerman et al. 2019). CBD at a dose of 10 mg/kg *i.p.* was given for 14 days. The maximum recommended human maintenance dose of CBD for the treatment of drug-resistant epileptic seizures is 20 mg/kg (Ewing et al. 2019). We used 10 mg/kg since it diminished BP and HR in anaesthetized SHR and their normotensive controls (Kossakowski et al. 2019) and the stress-induced increases in BP and HR in conscious rats (Resstel et al. 2009) and humans (i.e., ~600 mg/70 kg; Jadoon et al. 2017). Additionally, chronic administration of this dose had a cardioprotective effect in mouse cardiomyopathy

Table 3
Influence of cannabidiol (CBD) on the isoprenaline (0.01 nM - 1 μM)-induced changes in parameters of hearts isolated from deoxycorticosterone-salt (DOCA-salt) and spontaneously (SHR) hypertensive rats and their respective normotensive controls sham-operated (SHAM) and Wistar-Kyoto (WKY) rats.

Parameters	n	SHAM		SHAM CBD		DOCA-salt		DOCA-salt CBD	
		10		10		10		11	
		pEC_{50}	E_{max}	pEC_{50}	E_{max}	pEC_{50}	E_{max}	pEC_{50}	E_{max}
HR (beats/min)		9.3 ± 0.4	78 ± 9	10.8 ± 0.9	76 ± 11	9.6 ± 0.5	80 ± 5	9.1 ± 0.3	91 ± 7
LVP (mmHg)		9.7 ± 0.4	48 ± 8	9.3 ± 0.6	45 ± 7	9.2 ± 0.3	43 ± 6	8.7 ± 0.5	33 ± 4
+$(LVdP/dt)_{max}$ (mmHg/s)		9.1 ± 0.3	3806 ± 532	8.8 ± 0.4	3976 ± 431	8.9 ± 0.3	3224 ± 315	8.3 ± 0.3	3285 ± 402
-$(LVdP/dt)_{max}$ (mmHg/s)		8.9 ± 0.3	−2569 ± 270	8.8 ± 0.5	−2023 ± 310	8.8 ± 0.3	−1931 ± 312	8.5 ± 0.3	−1668 ± 196
RPP (mmHg x beats/min)		9.7 ± 0.3	26,073 ± 2250	9.4 ± 0.4	23,886 ± 2729	9.6 ± 0.4	21,996 ± 4344	10.0 ± 0.3	19,997 ± 1860
CPP (mmHg)		9.9 ± 0.3	−22 ± 3	10.2 ± 0.5	−24 ± 3	–	–	–	–
		WKY		**WKY CBD**		**SHR**		**SHR CBD**	
	n	10		10		10		10	
HR (beats/min)		9.9 ± 0.3	85 ± 8	10.2 ± 0.4	78 ± 10	9.9 ± 0.5	84 ± 9	9.8 ± 0.2	87 ± 8
LVP (mmHg)		8.9 ± 0.2	73 ± 6	9.0 ± 0.2	52 ± 4*	8.9 ± 0.2	36 ± $6^{\#\#\#}$	9.0 ± 0.2	32 ± 5
+$(LVdP/dt)_{max}$ (mmHg/s)		9.0 ± 0.2	4153 ± 311	9.0 ± 0.2	4776 ± 345	8.3 ± $0.1^{\#}$	3878 ± 245	8.1 ± 0.2	3890 ± 386
-$(LVdP/dt)_{max}$ (mmHg/s)		8.2 ± 0.1	−3082 ± 189	8.5 ± 0.1	−3141 ± 186	8.4 ± 0.2	−1751 ± $98^{\#\#\#}$	9.4 ± 0.2***	−1155 ± 139*
RPP (mmHg x beats/min)		9.4 ± 0.2	27,314 ± 1601	9.0 ± 0.2	23,319 ± 1749	8.9 ± 0.2	20,061 ± $1732^{\#\#}$	9.1 ± 0.2	19,247 ± 1653
CPP (mmHg)		10.0 ± 0.5	−18 ± 5	9.9 ± 0.5	−21 ± 4	–	–	–	–

Units are given in brackets. CBD 10 mg/kg or its vehicle were injected *i.p.* every 24 h for 14 days. Hearts were isolated 24 h after the final dose of CBD or its vehicle. Values are based on the concentration-response curves shown in Figs. 2 and 3. Maximal effects (E_{max}) represent maximum changes from baseline in absolute terms. Data are given as the means ± SEM. *$P < 0.05$, ***$P < 0.001$ vs. the respective values in rats not treated with CBD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, SHR vs. WKY; one-way analysis of variance (ANOVA) with Bonferroni post hoc test; for CPP Student's *t*-test for unpaired data. HR - heart rate, LVP - left ventricular pressure, the maximum rate of positive +$(LVdP/dt)_{max}$ and negative -$(LVdP/dt)_{max}$ changes in LVP, RPP - rate pressure product, CPP - coronary perfusion pressure.

Table 4
Initial parameters before carbachol infusion (values obtained after stimulation with 1 μM of isoprenaline) of hearts isolated from deoxycorticosterone-salt (DOCA-salt) and spontaneously (SHR) hypertensive rats and sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls.

Parameters	n	SHAM	SHAM CBD	DOCA-salt	DOCA-salt CBD	WKY	WKY CBD	SHR	SHR CBD
		10	10	10	11	10	10	10	10
HR (beats/min)		373 ± 13	374 ± 12	338 ± 8	335 ± 15	334 ± 12	342 ± 18	333 ± 19	339 ± 11
LVP (mmHg)		152 ± 13	151 ± 8	150 ± 13	145 ± 9	149 ± 7	134 ± 8	134 ± 5	139 ± 6
+$(LVdP/dt)_{max}$ (mmHg/s)		6789 ± 628	6558 ± 451	6174 ± 443	6466 ± 489	6151 ± 313	7069 ± 397	6909 ± 360	6759 ± 321
-$(LVdP/dt)_{max}$ (mmHg/s)		−4693 ± 369	−4332 ± 360	−4061 ± 447	−3868 ± 308	−4785 ± 253	−5007 ± 134	−4145 ± 222	−2949 ± 210***
RPP (mmHg/beats/min)		53,415 ± 4632	53,204 ± 3738	47,910 ± 4267	45,720 ± 3418	46,359 ± 2669	42,257 ± 1655	43,397 ± 2176	42,230 ± 1907
CPP (mmHg)		53 ± 3	62 ± 5	75 ± $6^{\#\#}$	74 ± 4	70 ± 4	70 ± 4	80 ± 3	76 ± 5

Units are given in brackets. CBD 10 mg/kg or its vehicle were injected *i.p.* every 24 h for 14 days. Hearts were isolated 24 h after the final dose of CBD or its vehicle. Data are given as the means ± SEM. ***$P < 0.001$ SHR vs. SHR CBD; $^{\#\#}P < 0.01$ DOCA-salt vs. SHAM; one-way analysis of variance (ANOVA) with Bonferroni post hoc test. HR - heart rate, LVP - left ventricular pressure, maximum rate of positive +$(LVdP/dt)_{max}$ and negative -$(LVdP/dt)_{max}$ changes in LVP, RPP - rate pressure product, CPP - coronary perfusion pressure.

induced by doxorubicin (Hao et al. 2015) or by diabetes (Rajesh et al. 2010), decreased cardiac and plasma oxidative stress in SHR and DOCA-salt (Remiszewski et al. 2020), reduced rat pulmonary hypertension (Sadowska et al. 2020) and improved the endothelium-dependent vasorelaxation in mesenteric arteries of diabetic rats (Wheal et al. 2017). In the present paper, functional alterations were studied in vitro in Langendorff hearts in which contractility is examined simultaneously with heart rate and coronary response. The β-adrenoceptor agonist isoprenaline and the cholinesterase-resistant muscarinic receptor agonist carbachol were used to mimic the cardiac effects of the sympathetic and parasympathetic system, respectively. Carbachol was examined in isoprenaline-pretreated hearts since (1) cardioinhibitory vagal effects are more marked under sympathetic tone (Coote 2013) and (2) parasympathetic nerves affect ventricular function not only directly but also indirectly by counteracting the β-adrenergic action (so-called accentuated antagonism) (Coote 2013) observed also in the failing human left ventricle (Newton et al. 1996). Our experiments were extended to paced left atria, in which changes in the positive and negative inotropic effects induced by isoprenaline and the cannabinoid receptor agonist CP55940 were considered, respectively.

4.2. Changes related to hypertension

We obtained hemodynamic, structural and functional changes which are typical of hypertension. Systolic blood pressure in SHR and DOCA-salt was higher than in their respective controls. The increase in SBP was more marked for SHR than for DOCA-salt; this could be expected since we had reduced the administration of DOCA plus salt from 6 weeks (which increased SBP to 220 mmHg) (Pędzińska-Betiuk et al. 2017) to 4 weeks to obtain more comparable levels of hypertension in SHR and DOCA-salt. Like in our previous paper (Remiszewski et al. 2020), HR was lower in DOCA-salt but higher in SHR rats in comparison to their respective controls. The degree of cardiac hypertrophy (heart weight/body weight; left ventricle plus septum/body weight; left ventricle plus septum/tibia length) was comparable in DOCA-salt and SHR. This held also true for the width of left and right ventricle cardiomyocytes; right ventricle hypertrophy and an increase in diastolic stiffness occurred in SHR only. Left ventricular β_1-adrenoceptor density and positive inotropic and lusitropic effects of isoprenaline were reduced in SHR and tended to be reduced in DOCA-salt.

While the effects of hypertension on cardiostimulatory effects of β-adrenergic stimulation are relatively well established, its influence on

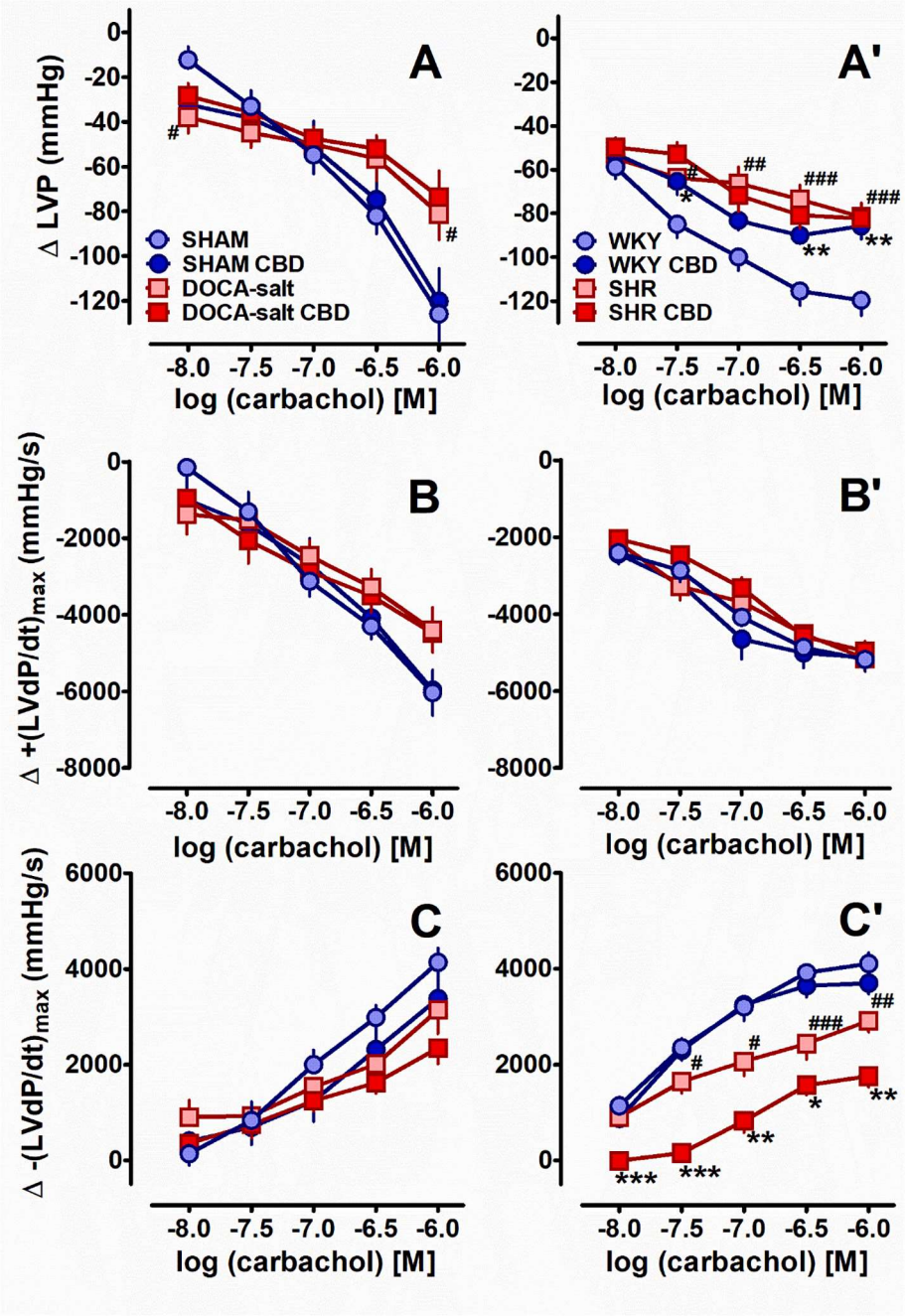

Fig. 4. Influence of cannabidiol (CBD) on the carbachol-induced changes in LVP (A, A'), +(LVdP/dt)$_{max}$ (B, B′) and -(LVdP/dt)$_{max}$ (C, C′) of hearts isolated from deoxycorticosterone (DOCA-salt) and spontaneously (SHR) hypertensive rats and sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls. CBD (10 mg/kg) or vehicle was injected *i.p.* every 24 h for 14 days. Values shown are changes from the values obtained after stimulation of the heart with isoprenaline 1 μM (Table 4). Data are given as the means ± SEM of 10–11 rats. $^{*}P < 0.05$; $^{**}P < 0.01$, $^{***}P < 0.001$ significant effect of CBD, $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$, DOCA-salt and SHR significantly different from SHAM and WKY, respectively; one-way analysis of variance (ANOVA) with Bonferroni post hoc test. LVP - left ventricular pressure; +(LVdP/dt)$_{max}$, −(LVdP/dt)$_{max}$ - maximum rate of positive and negative changes in LVP, respectively.

parasympathetic modulation is less clear. We found that carbachol had negative chronotropic and inotropic effects in isoprenaline-stimulated perfused hearts confirming previous results obtained on rats (MacDonell and Diamond 1997; Ralay Ranaivo et al. 2004) and mice (Gödecke et al. 2001) and that the above effects were accompanied by a negative lusitropic effect of carbachol. We are the first to show that the cardioinhibitory effects were diminished or tended to decline in both models of hypertension (except for HR and + (LVdP/dt)$_{max}$ in SHR).

We also found that the isoprenaline-induced vasodilatation of coronary arteries in SHAM and WKY was completely prevented by hypertension, probably due to a loss of endothelium function (Tran et al. 2020). Carbachol caused vasoconstriction of coronary arteries in both normotensive rat models. The vasoconstriction was attenuated in DOCA-salt but not affected in SHR. One might have expected that carbachol, by releasing NO from the endothelium, causes coronary dilatation, at least in normotensive rats. However, vasoconstriction of coronary arteries in response to acetylcholine or carbachol (Ateş and Kaygisiz 1998; Hoover and Neely 1997; Nasa et al. 1997) was observed previously in spontaneously beating rat isolated hearts. Experiments with appropriate antagonists show that in this response muscarinic M2 or M3 receptors (both of which occur in human coronary arteries (Kalsner 1989; Saternos et al. 2018)) and vasoconstrictor prostaglandins derived from the endothelium are involved (Ateş and Kaygisiz 1998; Hoover and Neely 1997; Nasa et al. 1997). Moreover, one should keep in mind, that vasodilator responses to Ach or carbachol mainly occur in coronary arteries preconstricted by vasoconstrictor agents (for literature, see Nasa et al. 1997) and in our hands carbachol was infused after reaching a steady state in response to the vasodilator isoprenaline (1 μM). Thus, the initial level of basal tone was probable too low to allow for a vasodilator response to carbachol. We can probably exclude an endothelium-

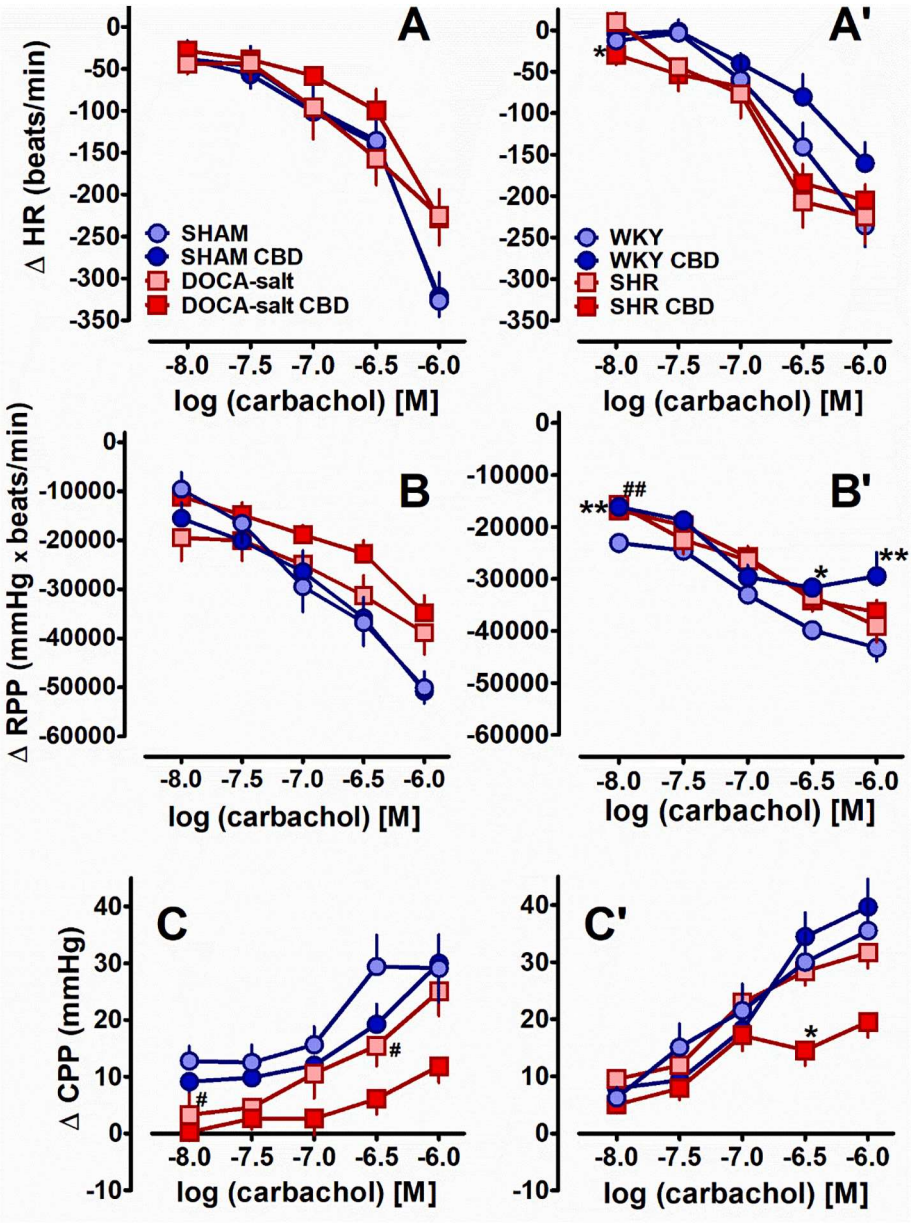

Fig. 5. Influence of cannabidiol (CBD) on the carbachol-induced changes in HR (A, A'), RPP (B, B′) and CPP (C, C′) of hearts isolated from from deoxycorticosterone-salt (DOCA-salt) and spontaneously (SHR) hypertensive rats and sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls. CBD (10 mg/kg) or vehicle was injected *i.p.* every 24 h for 14 days. Values shown are changes from the values obtained after stimulation of the heart with isoprenaline 1 μM (Table 4). Data are given as the means ± SEM of 10–11 rats. $^{*}P < 0.05$; $^{**}P < 0.01$ significant effect of CBD, $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ DOCA-salt and SHR significantly different from SHAM and WKY respectively; one-way analysis of variance (ANOVA) with Bonferroni post hoc test. HR - heart rate, RPP - rate pressure product, CPP - coronary perfusion pressure.

dependent component(s) of the carbachol-induced increases in CPP since the endothelium-dependent decrease in CPP stimulated by isoprenaline was completely abolished in SHR and DOCA-salt rats.

In harmony with our previous results (Pędzińska-Betiuk et al. 2017) the negative inotropic effect of the cannabinoid receptor agonist CP55940 on left atria was diminished both by primary and secondary hypertension. Cannabinoids decrease or increase rat (Sterin-Borda et al. 2005) and human (Bonz et al. 2003) cardiac contractility via CB_1 and CB_2 receptors, respectively. The hypertension-related decline in the negative inotropic effect of CP55940 probably resulted from the reduction of cardiac CB_1 receptor density in DOCA-salt and SHR (Remiszewski et al. 2020).

4.3. Cannabidiol-induced changes in hypertension and in normotension

Two-week administration of CBD 10 mg/kg led to two distinct positive effects occurring in both hypertension models. Firstly, CBD reduced the carbachol-induced increases in CPP in hearts of DOCA-salt and SHR by 40–50%. CBD is known to possess vasodilatory properties (for review: Stanley et al. 2013). However, CBD neither modified the vasoconstrictory effect of carbachol in both normotensive controls nor did it affect the vasodilatory effect of isoprenaline in normotension and hypertension. Thus, we can exclude differences in basal tone, a direct vasodilatory influence of CBD, its interaction with β-adrenoceptors or intact endothelium as potential mechanisms or sites of CBD action. Our results are reminiscent of the study by Wheal et al. (2017) in which 7-day treatment with CBD (10 mg/kg/day) diminished the contractile response to high concentrations of acetylcholine in mesenteric arteries from Zucker diabetic fatty (but not lean) rats (Wheal et al. 2017) suggesting that CBD exerts its beneficial vascular effects only in the presence of vascular dysfunction. The effect of CBD may be related to an increase in vasodilatory endocannabinoids or adenosine (e.g. Pisanti et al. 2017). Since chronic CBD administration enhanced the levels of vasodilatory endocannabinoids in DOCA-salt as opposed to SHR (Remiszewski et al. 2020), enhancement of adenosine levels seems to be the more plausible explanation (Durst et al. 2007; Walsh et al. 2010; Gonca and Darıcı, 2015). Importantly, CBD has been shown to decrease the incidence of ventricular arrhythmias and/or to reduce infarct size

Table 5
Influence of cannabidiol (CBD) on the carbachol (0.01 μM - 1 μM)-induced changes in parameters of hearts isolated from deoxycorticosterone-salt (DOCA-salt) and spontaneously (SHR) hypertensive rats and their respective normotensive controls sham-operated (SHAM) and Wistar-Kyoto (WKY) rats.

Parameters	n	10		10		10		11	
		pEC_{50}	E_{max}	pEC_{50}	E_{max}	pEC_{50}	E_{max}	pEC_{50}	E_{max}
		SHAM		SHAM CBD		DOCA-salt		DOCA-salt CBD	
HR (beats/min)		5.3 ± 1.6	−327 ± 34	5.4 ± 0.7	−321 ± 24	6.5 ± 0.4	−225 ± 35	5.4 ± 1.1	−228 ± 35
LVP (mmHg)		6.5 ± 0.2	−126 ± 12	6.1 ± 0.5	−120 ± 15	5.8 ± 1.2	−81 ± 11$^{\#}$	7.0 ± 0.5	−74 ± 12
+$(LVdP/dt)_{max}$ (mmHg/s)		6.9 ± 0.2	−6033 ± 592	6.5 ± 0.3	−5960 ± 526	6.6 ± 0.4	−4406 ± 604	7.1 ± 0.3	−4476 ± 504
-$(LVdP/dt)_{max}$ (mmHg/s)		6.9 ± 0.2	4137 ± 298	6.5 ± 0.4	3380 ± 595	6.2 ± 0.6	3146 ± 501	6.7 ± 0.3	2343 ± 328
RPP (mmHg x beats/min)		6.9 ± 0.3	−50,053 ± 3212	6.4 ± 0.3	−50,783 ± 4042	6.4 ± 0.6	−38,839 ± 4419	6.2 ± 0.4	−34,758 ± 3514
CPP (mmHg)		6.7 ± 0.5	29.5 ± 5.5	6.0 ± 0.6	30.0 ± 5.1	6.4 ± 0.5	25 ± 4.3	6.0 ± 0.9	11.9 ± 2.9*
		WKY		**WKY CBD**		**SHR**		**SHR CBD**	
	n	10		10		10		10	
HR (beats/min)		6.3 ± 0.3	−235 ± 23	6.2 ± 0.4	−160 ± 25	6.9 ± 0.3	−224 ± 32	6.7 ± 0.3	−205 ± 19
LVP (mmHg)		7.6 ± 0.3	−120 ± 7	7.7 ± 0.4	−90 ± 3**	6.8 ± 0.6	−81 ± 6$^{\#\#\#}$	7.2 ± 0.4	−82 ± 7
+$(LVdP/dt)_{max}$ (mmHg/s)		7.2 ± 0.2	−5176 ± 296	7.5 ± 0.4	−5128 ± 315	7.2 ± 0.3	- 5153 ± 342	6.9 ± 0.2	−4966 ± 259
-$(LVdP/dt)_{max}$ (mmHg/s)		7.5 ± 0.2	4108 ± 227	7.9 ± 0.3	3701 ± 228	7.3 ± 0.3	2914 ± 236$^{\#\#}$	7.0 ± 0.2	1758 ± 230**
RPP (mmHg x beats/min)		6.9 ± 0.2	−43,242 ± 2605	7.5 ± 0.4	−31,655 ± 1601**	6.9 ± 0.3	−39,033 ± 3094	7.0 ± 0.2	−36,336 ± 2210
CPP (mmHg)		7.1 ± 0.4	35 ± 5	6.7 ± 0.2	40 ± 5	7.1 ± 0.2	32 ± 3	7.5 ± 0.5	19 ± 3**

Units are given in brackets. CBD 10 mg/kg or its vehicle were injected *i.p.* every 24 h for 14 days. Hearts were isolated 24 h after the final dose of CBD or its vehicle. Values are based on the concentration-response curves shown in Figs. 4 and 5. Maximal effects (E_{max}) represent maximum changes from the values (in absolute terms) obtained after stimulation of the heart with the 1 μM of isoprenaline. Data are given as the means ± SEM. *$P < 0.05$; **$P < 0.01$ vs. the respective values in rats not treated with CBD; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$, DOCA-salt and SHR vs. SHAM and WKY, respectively; one-way analysis of variance (ANOVA) with Bonferroni post hoc test. HR - heart rate, LVP - left ventricular pressure, the maximum rate of positive +$(LVdP/dt)_{max}$ and negative -$(LVdP/dt)_{max}$ changes in LVP, RPP - rate pressure product, CPP - coronary perfusion pressure.

Table 6
Influence of cannabidiol (CBD) on the isoprenaline (0.01 nM – 10 μM)-induced positive and the CP55940 (1 nM – 30 μM)-induced negative inotropic effects in left atria isolated from deoxycorticosterone-salt (DOCA-salt) and spontaneously (SHR) hypertensive rats and sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls.

	Basal value (mN)	pEC_{50}	E_{max}	n	Basal value (mN)	pEC_{50}	E_{max}	n	Basal value (mN)	pEC_{50}	E_{max}	n	Basal value (mN)	pEC_{50}	E_{max}	n
Group	SHAM				SHAM CBD				DOCA-salt				DOCA-salt CBD			
isoprenaline	2.3 ± 0.1	7.7 ± 0.1	119 ± 9	10	2.3 ± 0.1	7.8 ± 0.1	150 ± 12	10	2.4±0.1	8.0 ± 0.1	152 ± 19	10	2.3 ± 0.1	7.9 ± 0.1	151 ± 20	11
CP55940	2.4 ± 0.1	6.1 ± 0.1	−55 ± 3	10	2.4 ± 0.1	5.7 ± 0.1	−53 ± 6	10	2.4 ± 0.1	5.9 ± 0.2	−38 ± 5$^{\#}$	10	2.4 ± 0.1	6.1 ± 0.2	−56 ± 4*	11
	WKY				**WKY CBD**				**SHR**				**SHR CBD**			
isoprenaline	2.2 ± 0.1	7.9 ± 0.1	189 ± 24	10	2.3 ± 0.1	7.9 ± 0.2	213 ± 40	9	2.3 ± 0.1	8.2 ± 0.2	131 ± 26	9	2.4 ± 0.01	8.0 ± 0.2	133 ± 23	9
CP55940	2.4 ± 0.1	7.5 ± 0.1	−62 ± 2	10	2.4 ± 0.1	7.3 ± 0.2	−57 ± 4	9	2.4 ± 0.1	6.8 ± 0.4	−37 ± 7$^{\#}$	9	2.3 ± 0.1	5.2 ± 0.3***	−39 ± 6	9

Values are based on the concentration-response curves shown in Fig. 6. CBD 10 mg/kg or its vehicle were injected *i.p.* every 24 h for 14 days. Hearts were isolated 24 h after the final dose of CBD or its vehicle. Maximal effects (E_{max}) are expressed in % of basal values. Data are given as the means ±SEM. *$P < 0.05$; ***$P < 0.001$, significant effect of CBD; $^{\#}P < 0.05$, DOCA-salt and SHR significantly different from SHAM and WKY, respectively; one-way analysis of variance (ANOVA) with Bonferroni post hoc test.

induced by ischemia/reperfusion in rats (Durst et al. 2007; Walsh et al. 2010; Gonca and Darıcı, 2015) and rabbits (Feng et al. 2015) and those beneficial effects may be partially related to its influence on the effect of CBD on CPP.

Secondly, CBD reduced the increased width of cardiomyocytes of the left (in both hypertension models) and right ventricle (SHR only). Left ventricular hypertrophy is one of the main structural changes that characterizes hypertensive heart disease (Nwabuo and Vasan 2020). Unfortunately, the reduction of the width of the cardiomyocytes was not associated with an attenuation of left ventricular hypertrophy despite the beneficial influence of CBD on cardiac and plasma oxidative stress (Remiszewski et al. 2020). Only a decrease in right ventricle hypertrophy in SHR was found. Beneficial effects of chronic CBD administration on cardiac fibrosis have so far been described in mice autoimmune myocarditis (Lee et al. 2016) and diabetic cardiomyopathy (Rajesh et al. 2010) resulting mainly from its anti-inflammatory and anti-oxidative properties. However, cardiac hypertrophy was not assessed in any of the above papers.

To the best of our knowledge, we were the first to examine the influence of CBD on cardiostimulatory and cardioinhibitory effects induced by stimulation of β-adrenoceptors (in isolated hearts and left atria) and muscarinic receptors (in isolated hearts) in hypertension, respectively. In SHR, CBD administration did not restore but further aggravated the hypertension-induced reduction of the positive and negative lusitropic effects induced by isoprenaline and carbachol, respectively. The additional reduction of the negative lusitropic effect of carbachol probably resulted from its low basal value induced by isoprenaline. To explain the additional reduction of the positive lusitropic effect a direct influence of CBD on β-adrenoceptors can be excluded since CBD has no sufficient β-adrenoceptor affinity (Hillard and Bloom 1982). Chronic inhibition of FAAH (fatty acid amide hydrolase, i.e., an endocannabinoid-degrading enzyme) which normalized (atria) and enhanced (isolated hearts) the positive ino- and chronotropic effects of isoprenaline (Pędzińska-Betiuk et al. 2017), is also an unlikely explanation. However, CBD not only inhibits FAAH but also antagonizes GPR55 receptors (e.g. Pisanti et al. 2017). GPR55 deletion in mice has

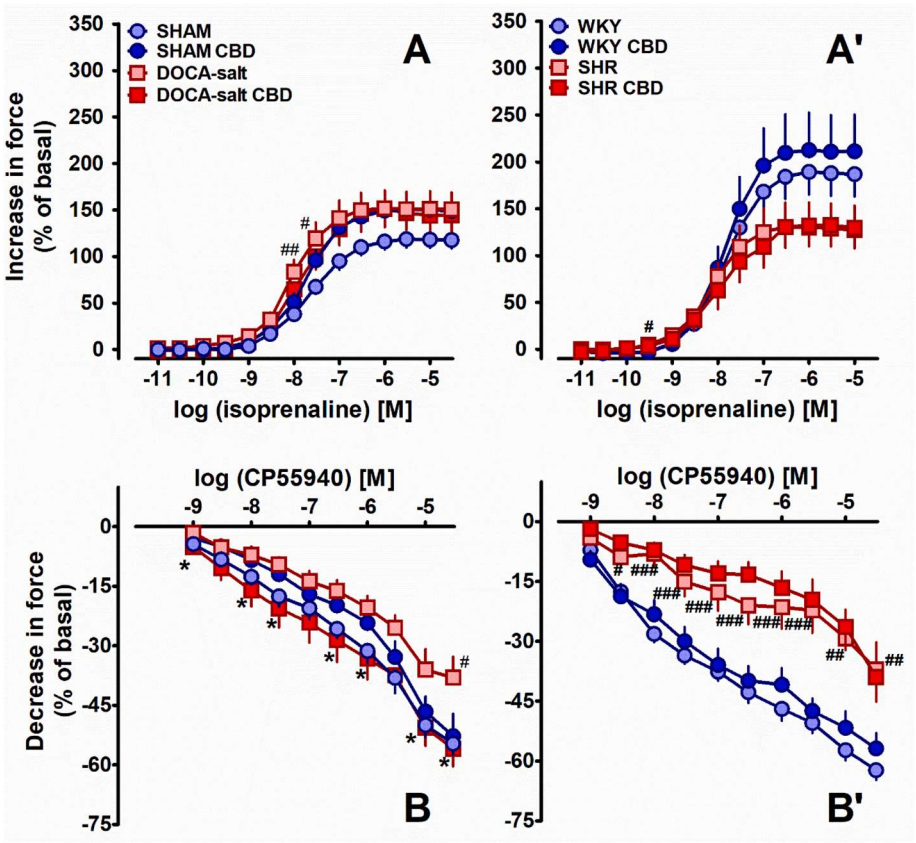

Fig. 6. Influence of cannabidiol (CBD) on the isoprenaline-induced (A, A') increases and on the CP55940-induced (B, B′) decreases in contractile force of left atria isolated from deoxycorticosterone (DOCA-salt) and spontaneously (SHR) hypertensive rats and sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls. The vehicle for CP55940 decreased atrial contractility force by about 5–10% in all experimental groups. CBD (10 mg/kg) or vehicle was injected *i.p.* every 24 h for 14 days. Data are expressed in % of basal values (Table 6). Data are given as the means ± SEM of 9–11 rats; $^{*}P < 0.05$, significant effect of CBD; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$, DOCA-salt and SHR significantly different from SHAM and WKY, respectively; one-way analysis of variance (ANOVA) with Bonferroni post hoc test.

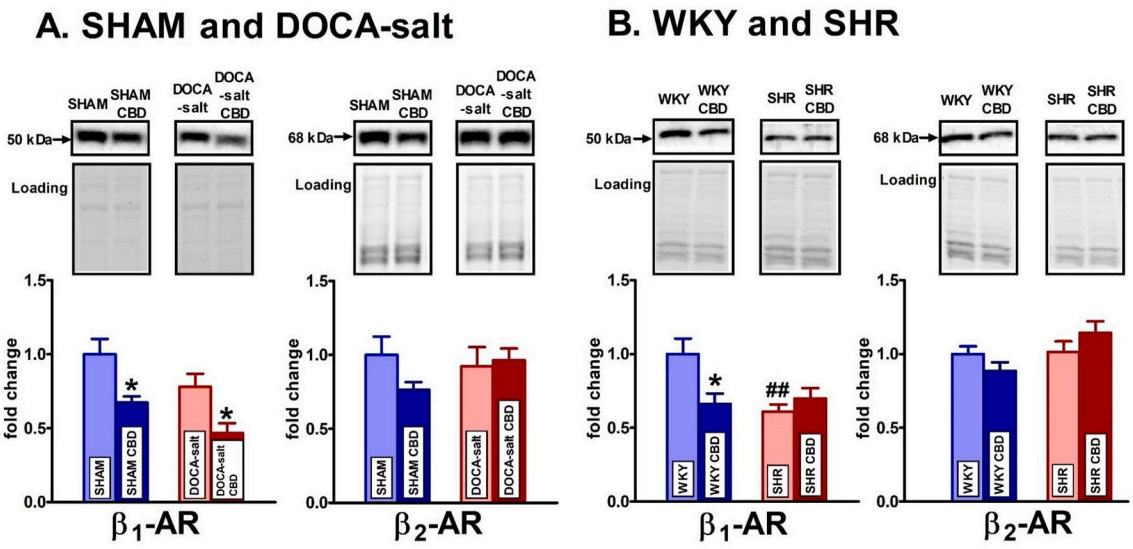

Fig. 7. Influence of cannabidiol (CBD) or its vehicle on the expression of β_1- and β_2-adrenoceptors in left ventricle isolated from deoxycorticosterone (DOCA-salt) and spontaneously (SHR) hypertensive rats and sham-operated (SHAM) and Wistar-Kyoto (WKY) rats, their respective normotensive controls. CBD (10 mg/kg) or vehicle was injected *i.p.* every 24 h for 14 days. Hearts were prepared 24 h after the final injection. Receptor protein was determined by Western blots and given as fraction of the value in the normotensive control (first of the four columns). Representative examples are presented in the upper part of the figure. Additionally, representative images using stain-free gel technology (that allows for total protein visualization and quantification) are shown as a loading control (Loading). For statistical evaluation (lower part of the figure), data are expressed as the mean ± SEM and given as fraction of the value in the normotensive control (first of the four columns); $n = 5$–6. $^{*}P < 0.05$, significant effect of CBD; $^{\#\#}P < 0.01$ vs. WKY; one-way analysis of variance (ANOVA) with Bonferroni post hoc test.

been shown to impair adrenoceptor-mediated inotropic effects in left ventricle including the positive lusitropic influence of dobutamine (Walsh et al. 2014) and the reduction of the positive lusitropic effect by CBD in the present study may be related to its antagonistic influence on GPR55 receptors as well.

Cannabidiol differently modified the hypertension-related decrease of the negative inotropic effect of CP55940 in the two models. Thus, it restored this effect in DOCA-salt to the level of SHAM but further

decreased the potency of CP55940 in SHR. The mechanism of this differential effect of CBD is unclear but cannot result from binding to CB_1 or CB_2 receptors involved in the effect of CP55940 on rat atrial contractility (Sterin-Borda et al. 2005), since CBD (i) possesses very low affinity at these receptors (e.g. Pisanti et al. 2017) and (ii) did not modify their densities in cardiac tissues of DOCA-salt and SHR (Remiszewski et al. 2020).

Although a two-week administration of CBD 10 mg/kg *i.p.* had some structural, functional (present study) and biochemical effects (reduction of cardiac and plasma oxidative stress) (Remiszewski et al. 2020), it did not affect BP and HR in both hypertensive models and their normotensive controls in the present and our previous study (Remiszewski et al. 2020). CBD displayed vasodilatation of coronary arteries but this vascular bed is too small to affect systemic BP. Moreover, the absence of or slight influence of CBD on basal or β-adrenoceptor- or muscarinic Ach receptor-stimulated parameters of isolated hearts excludes a cardiac effect as another potential factor that could lead to a decrease in BP and HR. The simultaneous occurrence of hyper- and hypotensive effects described in the Introduction (Kossakowski et al. 2019) may be responsible for the lack of a hypotensive response to CBD when administered once both to male (Kossakowski et al. 2019) or female (Graham and Li 1973) rats or over a time period of 14 days. Although in young healthy male volunteers a single oral dose of CBD (600 mg ≈ 8 mg/kg) decreased resting BP and the stress-induced increase in BP by about 2–5 mmHg this effect was retained after seven days of treatment only in stressed but not in non-stressed people (Jadoon et al. 2017; Sultan et al. 2020). Summarizing, CBD does not seem to possess antihypertensive activity in animals and humans.

CBD is generally recognized as a safe drug for use in humans (Iffland and Grotenhermen 2017). With respect to its side effects (Chesney et al. 2020; Dos Santos et al. 2020; Ewing et al. 2019; Pauli et al. 2020), it may be of interest that, in WKY, CBD decreased diastolic stiffness and the positive and negative inotropic effects elicited by isoprenaline and carbachol, respectively. In SHAM, CBD increased the width of left and right ventricular cardiomyocytes whereas a decrease in the density of left ventricular β_1-adrenoceptors occurred both in WKY and SHAM. We cannot exclude that these effects are associated with the increased cardiac and plasma lipid peroxidation observed on the respective normotensive rats in our previous study (Remiszewski et al. 2020).

4.4. Limitations of the study

The current study was limited to examination of the potential cardiac therapeutic effects of two weeks of CBD 10 mg/kg administration in SHR and DOCA-salt hypertensive rats. Thus, we cannot exclude that there would be more beneficial cardiac effects of CBD if higher doses of CBD were used and/or the time period of its administration were extended. Moreover, since a cannabinoid CB_1 receptor antagonist had a hypotensive effect in SHR but caused hypertension in rats with renin-angiotensin-aldosterone system (RAAS)-dependent hypertension (for review, see: Malinowska et al. 2019) and hypertension is sex-dependent (Lerman et al. 2019; Kamon et al. 2020) we cannot exclude that the use of rats with RAAS hypertension or of female rats may unmask a more robust therapeutic cardiac CBD activity (for review, see: Shayesteh et al. 2019).

The effect of chronic CBD administration on animal models of heart diseases has so far been studied in five papers (Durst et al. 2007; Rajesh et al. 2010; Fouad et al. 2013; Hao et al. 2015; Lee et al. 2016), in most of which beneficial effects of preventive but not therapeutic use of CBD were found. Only in the paper by Rajesh et al. (2010) chronic CBD treatment (20 mg/kg *i.p.* for 4 weeks) was able also to reverse myocardial alterations observed in mice diabetic cardiomyopathy although the effect was less marked in the therapeutic than in the preventive paradigm. Thus, the question remains whether CBD per se is suited for therapeutic purposes in heart disease.

5. Conclusions

Chronic CBD administration in the progression arrest paradigm although not lowering BP in two rat models of hypertension (Remiszewski et al. 2020) has some positive effects on hypertensive heart disease, including a reduction of the carbachol-induced vasoconstriction of coronary arteries and of the width of cardiomyocytes in left ventricle. On the other hand, it fails to affect left ventricular hypertrophy and even aggravates the impaired positive and negative lusitropic effects elicited by isoprenaline and carbachol, respectively. In normotensive hearts CBD can lead to untoward structural and functional effects (possibly related to increased lipid peroxidation) (Remiszewski et al. 2020). This phenomenon should be taken into consideration if CBD is used as a medicine in humans.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.taap.2020.115368.

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