Original Article

Vasodilatory effects of cannabidiol in human pulmonary and rat small mesenteric arteries: modification by hypertension and the potential pharmacological opportunities

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Objective: Cannabidiol (CBD) has been suggested as a potential antihypertensive drug. The aim of our study was to investigate its vasodilatory effect isolated in human pulmonary arteries (hPAs) and rat small mesenteric arteries (sMAs).

Methods: Vascular effects of CBD were examined in hPAs obtained from patients during resection of lung carcinoma and sMAs isolated from spontaneously hypertensive (SHR); 11-deoxycorticosterone acetate (DOCA-salt) hypertensive rats or their appropriate normotensive controls using organ bath and wire myography, respectively.

Results: CBD induced almost full concentration-dependent vasorelaxation in hPAs and rat sMAs. In hPAs, it was insensitive to antagonists of CB1 (AM251) and CB2 (AM630) receptors but it was reduced by endothelium denudation, cyclooxygenase inhibitors (indomethacin and nimesulide), antagonists of prostaglandin EP1 receptors (L161982, IP (Cay10441)), vanilloid TRPV1 (capsazepine) receptors and was less potent under KCl-induced tone and calcium (Cay10441), vanilloid TRPV1 (capsazepine) receptors and was less potent under KCl-induced tone and calcium-activated potassium channel (KCa) inhibitors (iberiotoxin, UCL1684 and TRAM-34) and in hypertensive, overweight patients was endothelium-dependent and mediated via KCa, Ca2+-activated K+ channels; l-NAME, Nω-nitro-o-arginine methyl ester; LPA, L-alpha-lysophosphatidylcholine; NO, nitric oxide; NOS, nitric oxide synthase; NSAIDs, nonsteroidal anti-inflammatory drugs; PGL2, prostacyclin; PPARγ, peroxisome proliferator-activated receptors γ; PPARY, prostanoid thromboxane receptor; rPA, rat pulmonary artery; SHAM, control sham-operated rats; SHR, spontaneously hypertensive rats; sMAs, small mesenteric arteries; SOD, superoxide dismutase; TP, thromboxane receptor potential vanilloid 1; WKY, Wistar–Kyoto rats.

Conclusion: The CBD-induced relaxation in hPAs that was reduced in hypertensive, obese and hypercholesteremic patients was endothelium-dependent and mediated via KCa and IP, EP4, TRPV1 receptors. The CBD effect in rats was CB1-sensitive and dependent on the hypertension model. Thus, modification of CBD-mediated responses in disease should be considered when CBD is used for therapeutic purposes.

Keywords: cannabidiol, cannabinoid CB1 receptor, endothelium, hypertension, mesenteric artery, prostacyclin IP receptor, prostanoid EP4 receptor, pulmonary artery, transient receptor potential vanilloid 1, vasorelaxation

Abbreviations: 2-AG, 2-arachidonoyl glycerol; abn-CBD, abnormal cannabinoid; ACE, angiotensin-converting enzyme; ARBs, angiotensin receptor blockers; AT1, angiotensin II receptor type 1; BMI, Body Mass Index; BP, blood pressure; CAD, coronary artery disease; CBD, cannabidiol; COPD, chronic obstructive pulmonary disease; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; CRCs, concentration-response curves; DETCA, sodium diethylthiocarbamate trihydrate; DMF, N,N-dimethylformamide; DMSO, dimethyl sulfoxide; DOCA, 11-deoxycorticosterone acetate; DOCA-salt, 11-deoxycorticosterone acetate, high salt diet treated and uninephrectomized; EP4, prostanoid EP4 receptor; FAAH, fatty acid amide hydrolase; hMAs, human mesenteric arteries; hPAs, human pulmonary arteries, Indo, indomethacin; IP, prostacyclin receptor; KCa, Ca2+-activated K+ channels; l-NAME, Nω-nitro-o-arginine methyl ester; LPA, L-alpha-lysophosphatidylcholine; NO, nitric oxide; NOS, nitric oxide synthetase; NSAIDs, nonsteroidal anti-inflammatory drugs; PGL2, prostacyclin; PPARγ, peroxisome proliferator-activated receptors γ; PPARY, prostanoid thromboxane receptor; rPA, rat pulmonary artery; SHAM, control sham-operated rats; SHR, spontaneously hypertensive rats; sMAs, small mesenteric arteries; SOD, superoxide dismutase; TP, thromboxane receptor potential vanilloid 1; WKY, Wistar–Kyoto rats.
INTRODUCTION

Cannabidiol (CBD) is a nonpsychotropic constituent of Cannabis sativa. It possesses tremendous therapeutic potential because of its anti-inflammatory, antioxidative, anticancer, neuroprotective, antinociceptive and many other beneficial properties [1–3]. CBD has already been approved by the US Food and Drug Administration (FDA) for the treatment of epilepsy [4], and internationally approved for the therapy of spasticity in multiple sclerosis, and has also received orphan designation for the treatment of neonatal hypoxia–ischaemic encephalopathy [5]. Moreover, it has been postulated to play a beneficial role in cardiovascular disorders including hypertension [1,6–10].

Indeed, acute and chronic CBD administration reduced increases in blood pressure (BP) induced by stress but not under control conditions [9,11]. Its potential antihypertensive role has been suggested based on its vasodilatory effects under in-vitro conditions in systemic vasculature. Furthermore, near-maximal and almost half-maximal relaxation has been demonstrated in rat [12] and human [5] small mesenteric arteries (sMAs), respectively. The vasodilatory effects of CBD in the human mesenteric artery were dependent on endothelium and potassium channels, the transient receptor potential vanilloid 1 (TRPV1) and the cannabinoid CB2 [5] receptors, and in rat aorta on peroxisome proliferator-activated receptors (PPARs), calcium channel inhibition and superoxide dismutase [13]. Moreover, CBD augmented the endothelium-dependent vasodilatation in response to acetylcholine in mesenteric arteries of Zucker augmented the endothelium-dependent vasodilatation in response to acetylcholine in mesenteric arteries of Zucker.

The concise guide to pharmacology 2017/18: G protein-coupled receptors [23].

MATERIALS AND METHODS

All of the protocols were approved by the Animal and Human Ethics Committee of the Medical University of Białystok, Poland (no. R-I-002/4/5/2015; 52/2015) and were performed in accordance with the European Directive (2010/63/EU). Animal studies were carried out in compliance with the replacement, refinement or reduction (the 3Rs). Male rats were obtained from the Centre for Experimental Medicine of the Medical University of Białystok (Poland). The tissue donors provided written informed consent for the use of their blood vessels.

The nomenclature of the molecular targets conforms to the concise guide to pharmacology 2017/18: G protein-coupled receptors [23].

Human tissue studies

Human lung tissue was obtained from 32 patients (21 men and 11 women, with a mean age of 65.2 ± 1.9 years) undergoing a lobectomy or pneumonectomy during the resection of lung carcinoma with a surgical margin of healthy tissue. Preoperative echocardiography revealed normal left and right ventricular function. A summary of control patient characteristics is shown in Table 1. Prior to the operation, all of the patients received treatment with cephalosporin and low-molecular-weight heparin. Seventy-two hours before surgery, patients stopped taking pain relief. This allowed time to metabolize and eliminate them from the organism.

The tissue from the surgical margin was transported to the laboratory within 30 min in cold (4°C) PSS solution (composition in mmol/l: NaCl, 139.2; KCl, 2.7; CaCl2, 1.8; MgCl2, 0.49; NaHCO3, 11.9; NaH2PO4, 0.4; glucose, 5.5) at pH 7.4.

Animals

Male rats housed in plastic cages had free access to water and food pellets and they were maintained under a 12 h/12 h light/dark cycle. We used age-matched rats with comparable primary (SHR; the most frequently studied genetic hypertensive model) and secondary DOCA-salt hypertension, in order to distinguish changes induced by hypertension from those related to any one particular hypertension model. The DOCA-salt model applied as a salt-rich diet is one of the main lifestyle factors leading to hypertension.

DOCA-salt hypertension was induced in Wistar male rats (6–7 weeks old; weighing 260–300 g) as described previously [24]. Animals were anaesthetized by intraperitoneal (i.p.) injection of pentobarbital sodium (70 mg/kg). The right kidney was removed via a right lateral abdominal incision. After 1 week of recovery, hypertension was induced by subcutaneous injections of DOCA (25 mg/kg, 0.4 ml/kg) twice weekly for 4 weeks and replacement of drinking water with 1% NaCl solution. Control sham-operated rats (SHAM) were uninephrectomized and received the vehicle for DOCA (N, N-dimethylformamide) twice weekly and drank tap water.

The following four experimental groups were created: hypertensive DOCA-salt (4 weeks after the onset of DOCA-salt administration); 12–14-week-old male SHR (250–320 g) and their respective normotensive controls: SHAM (4 weeks after the onset of vehicle for DOCA-salt...
Cannabinoid-mediated vasorelaxations

TABLE 1. Patient characteristics and post hoc analysis of its effects on the vasorelaxant responses to the cannabidiol-induced control concentration–response curves in human pulmonary artery with intact endothelium

| Characteristic            | n   | Range (mean ± SEM) | pEC50 | Rmax (%) |
|---------------------------|-----|--------------------|-------|----------|
| Ethnicity                 | 21  | Polish white       | 5.0 ± 0.1 | 93.0 ± 3.7 |
| Male                      | 15  |                    | 4.9 ± 0.1 | 91.2 ± 4.6 |
| Female                    | 6   |                    | 4.8 ± 0.1 | 86.5 ± 7.9 |
| Age (years)               |     |                    |       |          |
| Younger (< 60)            | 21  | 51–81 (66.5 ± 1.4) | 4.9 ± 0.2 | 91.9 ± 8.9 |
| Older (> 60)              | 6   |                    | 4.8 ± 0.1 | 86.9 ± 8.9 |
| BMI (kg/m²)               | 21  | 18.8–30.6 (24.6 ± 0.7) | 5.0 ± 0.1 | 93.2 ± 4.8 |
| Lean (<26)                | 14  |                    | 4.4 ± 0.1 | 76.6 ± 5.0 |
| Overweight (>26)          | 7   |                    |         |          |
| Smokers                   | 15  |                    | 4.9 ± 0.1 | 90.9 ± 3.9 |
| No smokers                | 6   |                    | 4.8 ± 0.2 | 91.5 ± 7.3 |
| SBP (mmHg)                | 21  | 102–205 (135 ± 4)  | 4.8 ± 0.1 | 93.6 ± 4.8 |
| DBP (mmHg)                | 21  | 132–57 (81 ± 3)    | 4.2 ± 0.3 | 93.1 ± 5.5 |
| Normotensive              | 13  |                    | 4.7 ± 0.1 | 94.2 ± 4.2 |
| Hypertensive              | 8   |                    | 4.9 ± 0.2 | 91.6 ± 9.0 |
| No COPD                   | 17  |                    | 5.0 ± 0.1 | 83.5 ± 10.7 |
| COPD                      | 4   |                    | 4.7 ± 0.1 | 87.2 ± 5.3 |
| No CAD                    | 17  |                    | 4.8 ± 0.1 | 91.2 ± 3.8 |
| CAD                       | 4   |                    | 4.9 ± 0.1 | 95.5 ± 7.6 |
| Non-diabetic              | 18  |                    | 5.0 ± 0.1 | 91.2 ± 3.8 |
| Diabetic                  | 3   |                    | 5.1 ± 0.1 | 87.1 ± 5.4 |
| Normocholesterinaemia     | 13  |                    | 4.9 ± 0.1 | 95.1 ± 3.1 |
| Hypercholesterinaemia     | 8   |                    | 4.9 ± 0.1 | 87.1 ± 4.0 |
| No α1 adrenoceptor antagonists | 18 |             | 4.8 ± 0.1 | 103.1 ± 3.9 |
| α1 adrenoceptor antagonists | 3  |                | 4.7 ± 0.2 | 102.6 ± 9.7 |
| No β blockers             | 13  |                    | 5.1 ± 0.1 | 87.1 ± 5.4 |
| β blockers                | 8   |                    | 4.9 ± 0.1 | 95.1 ± 3.1 |
| No ACE inhibitors         | 17  |                    | 4.9 ± 0.1 | 87.1 ± 4.0 |
| ACE inhibitors            | 4   |                    | 4.9 ± 0.1 | 103.1 ± 3.9 |
| No NSAIDs                 | 12  |                    | 4.9 ± 0.1 | 85.3 ± 4.9 |
| NSAIDs                    | 9   |                    | 4.7 ± 0.1 | 96.6 ± 4.7 |
| No statins                | 16  |                    | 4.9 ± 0.1 | 87.7 ± 4.2 |
| Statins                   | 5   |                    | 4.6 ± 0.1 | 99.0 ± 6.1 |
| No PPIs                   | 14  |                    | 5.0 ± 0.1 | 86.4 ± 4.3 |
| PPIs                      | 7   |                    | 4.9 ± 0.1 | 99.5 ± 5.2 |
| AT1 antagonists           | 2   |                    | N.D.   | N.D.     |
| Calcium channel blockers  | 2   |                    | N.D.   | N.D.     |
| Hypoglycaemic medication  | 2   |                    | N.D.   | N.D.     |

ACE, angiotensin-converting enzyme; AT1, angiotensin receptor type 1; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; NSAIDs, nonsteroidal anti-inflammatory drugs; PPIs, proton pump inhibitors.

Values of pEC50 and Rmax for vasodilatory effects of CBD are based on the concentration–response curves and represent mean percentage relaxations of the isometric contraction induced by U46619 and, with the standard error of the mean (SEM) fit to nonlinear Regression (Curve Fit) (Prism Version 5; GraphPad Software, California, USA). n represents the number of patients.

*P < 0.05; **P < 0.01; compared with the respective control group as determined by Student’s t-test for unpaired data.

administration) and Wistar–Kyoto rats (WKY; 290–370 g). Rats were assigned randomly to the different experimental groups. The SBP was recorded in conscious rats by the noninvasive tail-cuff method using the Rat Tail Blood Pressure Monitor from Hugo Sachs Elektronik-Harvard Apparatus (March–Hugstetten, Germany). SBP (in mmHg) were higher in SHR (190 ± 6, n = 11, P < 0.001) and DOCA-salt animals (206 ± 8, n = 11, P < 0.001) than in their respective controls (WKY 94 ± 3, n = 12, SHAM 125 ± 7, n = 12).

The mesenteric arterial bed was removed from rats anesthetized with pentobarbital sodium (70 mg/kg; intraperitoneally) and placed into a cold Krebs–Henseleit solution with the following composition (in mmol/l): NaCl 118, KCl 4.8; CaCl2 2.5; MgSO4 1.2; NaHCO3 24; KH2PO4 1.2; glucose 11; EDTA 0.03 at pH 7.4.

Vessel preparation

The isolation of lobar and segmental hPA branches and sMAs have been described previously [19,25]. The lobar and segmental hPA branches were cleaned from the lung parenchyma and cut into rings (from the middle portion of each artery; 3–5 mm length and 2–4 mm outer diameter). They were suspended on stainless steel wires in 10 ml organ baths containing Tyrode’s solution and muscle tensions were recorded using a force-displacement transducer (PIM 100RE; BIO-SYS-TECH, Białystok, Poland) and displayed on a computer.

From the rat mesenteric arterial bed, 2 mm long segments of the third-order of the superior artery were dissected from surrounding adipose tissue. Isolated sMAs (250 μm internal diameter) were mounted in a
Molvun–Halpern type wire myograph (Multi Wire Myograph System DMT 620M; Danish Myo Technology, Aarhus, Denmark) filled with 5 ml Krebs–Henseleit solution. Muscle tension was recorded by the software LabChart 7.3.7 Pro (ADInstruments, Hastings, UK).

The hPAs and sMAs were allowed to equilibrate for 90 and 45 min (resting tension ~20–25 mN; ~2.5 mN, respectively) and were gassed continuously with 95% O₂ and 5% CO₂ at 37 °C, pH 7.4. After the equilibration period, all rings were exposed to high KCl (60 mmol/l [19] and 120 mmol/l [25], respectively) to establish tissue viability. Then, the integrity of the vessel endothelium was checked for submaximal preconstriction of hPAs and sMAs with (+)–phenylephrine (10 μmol/l) followed by the induction of at least 80% relaxation in response to acetylcholine (1 and 10 μmol/l, respectively). Before certain experiments, the endothelium was removed by gently rubbing off the intima. A successful endothelial denudation was confirmed by the absence of acetylcholine-induced vasorelaxation.

**Functional studies**

In each individual preparation, only one concentration–response curve (CRC) was determined. All experiments were performed in paired vessels, that is, the effect of a drug or of endothelium denudation was studied in one vessel and another vessel from the same patient or animal, which served as a control.

**Vasorelaxation study**

Human pulmonary arteries and rat sMAs and were submaximally constricted with U46619 (0.01 μmol/l; a prostanoid TP receptor agonist) and (+)-phenylephrine (10 μmol/l, respectively; concentrations approximately equivalent to their EC₆₀). After a stable level of tone was maintained, CRCs were generated by the cumulative application of CBD or its vehicle. To determine the mechanisms of the vasorelaxant effects of CBD, tissues were pretreated for 30 min with either the following antagonists or inhibitors (the respective concentrations are given in parentheses): AM251 (1 μmol/l; cannabinoid CB₂ receptor antagonist); AM630 (1 μmol/l; cannabinoid CB₁ receptor antagonist); O-1918 (10 μmol/l; antagonist of endothelium-dependent relaxation to acetylcholine; see Discussion); capsaicin (1 μmol/l; vanilloid TRPV1 receptor antagonist), Cay10441 (10 μmol/l; prostanoid IP receptor antagonist) [19], RN1734 (20 μM; vanilloid TRPV4 receptor antagonist) [26]; L161982 (1 μmol/l; prostanoid EP₃ receptor antagonist); nimesulide (10 μmol/l; cyclooxygenase COX-2 inhibitor); sodium diethyldithiocarbamate trihydrate (DETCa; 300 μmol/l; SOD inhibitor) [16]; indomethacin (INDO; 10 μmol/l; cyclooxygenase COX-1/COX-2 inhibitor); AN₀-nitro- l-arginine methyl ester (l-NAME; 300 μmol/l; nitric oxide synthase inhibitor); and UCL1684 (0.1 μmol/l; TRAM-34 (10 μmol/l), and ibertoxotin (0.1 μmol/l), which are small (KCa), respectively [27]. To assess the contribution of PPARy activation, experiments were performed in the presence of the PPARy antagonist GW9662 (1 and 10 μmol/l) [5,28] added 10 min prior to preconstriction. Following this, only one concentration of CBD (10 μmol/l) was administered in order to examine its time-dependent effect.

In control tissues of both series, the effects of the appropriate volume of the respective vehicle of inhibitors or antagonists, ethanol (for CBD, O-1918, GW9662, nimesulide, Cay10441, RN1734), or dimethyl sulfoxide (DMSO; for TRAM-34), on the relaxation to CBD were also tested when the final bath concentration was equal to or exceeded 0.1% (v/v). U46619 (0.01 μmol/l) and KCl (60 mmol/l) induced comparable increases in tone of the endothelium-intact and endothelium-denuded hPAs (11.5 ± 0.1 mN, n = 105, 9.5 ± 0.2 mN, n = 6 and 10.8 ± 0.2 mN, n = 15, respectively). A 30 min incubation with CBD 10 μmol/l, the antagonists, inhibitors or vehicles did not modify the U46619-induced contractions.

The mean tensions generated (in mN) by phenylephrine (10 μmol/l; a concentration approximately equivalent to its EC₆₀) in sMAs were similar between all normotensive WKY, SHAM and hypertensive SHR and DOCA-salt groups (7.1 ± 1.0, n = 30; 6.9 ± 0.6, n = 29; 7.7 ± 0.7, n = 29; 7.1 ± 0.9, n = 27, respectively). Endothelium removal, the vehicle for CBD and receptor antagonists did not change the basal tone.

**Vasoconstriction study**

To examine the potential effect of CBD on vasoconstriction increasing concentrations of U46619 in isolated hPAs or phenylephrine in sMAs some rings were treated for 30 min with one concentration (hPA 10 μmol/l; sMAs 1 μmol/l, a concentration approximately equivalent to its EC₆₀) of CBD or its vehicle and then a concentration–response curve for U46619 or phenylephrine were constructed.

**RNA isolation and gene expression analysis**

Rat sMAs were isolated from hypertensive SHR, DOCA-salt and normotensive WKY and SHAM animals (pooled from six rats from each group). The RNA isolation and Curt1 and Curt2 gene expression analysis were performed according to methodology previously described [27,29]. Tissue samples were immediately flash-frozen in liquid nitrogen and stored at −80 °C. Next, up to 5 mg of tissue was finely ground with a chilled stainless-steel mortar and pestle. Total RNA was purified using NucleoSpin RNA XS (Macherey-Nagel GmbH and Co., Düren, Germany) with carrier RNA and rDNase treatment, according to the manufacturer’s protocol. Spectrophotometric measurements (A260/A280) were made to assess the quantity and quality of the extracted RNA (NanoPhotometer, Implen, Germany). Synthesis of the cDNA was performed using the PrimeScript RT Reagent Kit (Takara) following the manufacturer’s instructions. Briefly, 500 ng of purified total RNA was used in a 10 μl reaction mixture containing random octamers, oligo dT-16 primers, dNTPs and PrimeScript Reverse Transcriptase. cDNA (2 μl) served as a template for real-time qPCR reactions. Amplification of the product was performed using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, California, USA). A set of predesigned primer pairs for Curt1 and Curt2 were purchased from Bio-Rad (PrimePCR PreAmp for SYBR Green Assay: Curt1 and Curt2...
Cannabidiol-mediated vasorelaxations

In the immunohistochemical study, the EnVision method was used according to Baranowska-Kuczko et al. [25] using an antibody against the cannabinoid CB2 receptor [1: 1000; rabbit Anti-CB2 (no cat. ab23703); Abcam, Cambridge, UK], cannabinoid CB1 receptor [1: 200; rabbit Anti-CB1 (no cat. ab3561); Abcam], TPRV1 [1: 1000; rabbit Anti-TPRV1 (no cat. bs-1931R); Bioss Antibodies, Woburn, Massachusetts, USA] and GPR18 [1: 1000; rabbit Anti-GPR18 (no cat. ab150618); Abcam]. The specificity of all antibodies included a negative control, in which the antibodies were replaced by normal rabbit serum (Vector Laboratories, Burlingame, California, USA) at the respective dilution (no staining), and a positive control, where the tissue recommended by the antibody manufacturer was used for staining, which was prepared: for CB1 from human cerebellum, CB2 human tonsil and TPRV1 human endometrial cancer and for GPR18 from human testis. All control reactions performed gave positive results. The obtained results of immunohistochemical staining were evaluated using the relative quantification method modified by Pfaffl [32].

**Immunohistochemistry**

In the immunohistochemical study, the EnVision method was used according to Baranowska-Kuczko et al. [25] using an antibody against the cannabinoid CB2 receptor [1: 1000; rabbit Anti-CB2 (no cat. ab23703); Abcam, Cambridge, UK], cannabinoid CB1 receptor [1: 200; rabbit Anti-CB1 (no cat. ab3561); Abcam], TPRV1 [1: 1000; rabbit Anti-TPRV1 (no cat. bs-1931R); Bioss Antibodies, Woburn, Massachusetts, USA] and GPR18 [1: 1000; rabbit Anti-GPR18 (no cat. ab150618); Abcam]. The specificity of all antibodies included a negative control, in which the antibodies were replaced by normal rabbit serum (Vector Laboratories, Burlingame, California, USA) at the respective dilution (no staining), and a positive control, where the tissue recommended by the antibody manufacturer was used for staining, which was prepared: for CB1 from human cerebellum, CB2 human tonsil and TPRV1 human endometrial cancer and for GPR18 from human testis. All control reactions performed gave positive results. The obtained results of immunohistochemical staining were evaluated using the relative quantification method modified by Pfaffl [32].

**Table 2. Influence of various antagonists and inhibitors and endothelium denudation on the vasorelaxant effects of cannabidiol in the U46619- or KCl1-preconstricted human pulmonary arteries**

| Group | Pharmacological activity | n  | pEC25 | pEC50 | Rmax |
|-------|-------------------------|----|-------|-------|------|
| CONTROL | - | 21 | 5.8±0.1 | 5.0±0.1 | 90.3±3.7 |
| -ENDOTHELIUM | -endothelial nitric oxide synthase | 6 | 5.0±0.1*** | N.D. | 37.4±9.7*** |
| l-NAME (300) | (-) | 6 | 5.7±0.2 | 5.0±0.1 | 80.1±12.0 |
| DETCA (300) | (-) superoxide dismutase | 6 | 5.8±0.2 | 5.2±0.1 | 78.5±11.3 |
| INDOMETACIN (10) | (-) cyclooxygenase 2 | 5 | 5.2±0.2*** | N.D. | 45.0±13.6*** |
| NIMESUDE (10) | (-) cytochrome c | 5 | 4.9±0.1*** | N.D. | 26.8±6.8*** |
| Cay10441 (10) | (-) prostacyclin IP receptor | 5 | 4.9±0.1*** | N.D. | 38.6±6.8*** |
| L161982 (1) | (-) prostaglandin EP2 receptor | 7 | 5.1±0.1*** | N.D. | 42.2±11.0*** |
| IBERIOTOXIN (0.1) | (-) large conductance Ca2+-activated potassium channel (KCa1.1) | 5 | 5.0±0.2** | N.D. | 39.0±7.0*** |
| UCL1634 (0.1) + TRAM-34 (10) | (-) intermediate (KCa2.3) and small (KCa2.3) conductance Ca2+-activated potassium channel, respectively | 7 | 4.9±0.2*** | N.D. | 40.7±10.4*** |
| AM251 (1) | (-) cannabinoid CB1 receptor | 5 | 5.6±0.2 | 5.3±0.1 | 64.2±9.4 |
| AM630 (1) | (-) cannabinoid CB2 receptor | 6 | 5.6±0.2 | 5.1±0.1 | 72.6±14.2 |
| O1918 (10) | (-) endothelium-dependent relaxation to cannabinoids | 5 | 5.4±0.1 | 4.9±0.1 | 72.3±9.4 |
| CAPSAZEPINE (1) | (-) vanilloid TRPV1 receptor | 5 | 5.5±0.2 | N.D. | 46.1±1.2*** |
| RN1734 (20) | (-) vanilloid TRPV4 receptor | 6 | 5.8±0.2 | 5.0±0.1 | 96.5±9.6 |
| KCl (60 mmol/l) | | 5 | N.D. | N.D. | 21.8±5.8*** |

Values are based on the concentration–response curves shown in Figs. 1–6. Data are expressed as mean ± SEM. n represents the number of patients. DETCA, sodium diethyldithiocarbamate trihydrate; ENDO, endothelium; l-NAME, N'-nitro-l-arginine methyl ester; ND, not determined. Molar concentrations (μmol/l) of chemicals are provided in parentheses.

**P<0.05.
**

**P<0.01.

**P<0.001 compared with the control group as determined by one-way ANOVA followed by Dunnett’s post hoc test or Student’s t-test for unpaired data.

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the control curve were calculated on the basis of the EC$_{25}$ values. EC$_{25}$ values were transformed into pEC$_{25}$ values (the negative logarithms of EC$_{25}$ values, respectively). The antagonistic potency (pA$_2$) of the latter was calculated from the equation

$$pA_2 = \log (CR - 1) - \log[B],$$

where [B] is the molar concentration of Cay10441 or L161982 and CR is the concentration ratio of the EC$_{25}$ values of CBD in the presence and absence of an antagonist.

When two or more treatment groups were compared with the same control, a one-way analysis of variance (ANOVA) followed by Dunnett’s test was used. A Student’s t-test for unpaired data was used wherever appropriate. Data were analyzed using GraphPad Prism version 5.00 for Windows, GraphPad Software. Differences were considered to be statistically significant at P less than 0.05.

**Drugs**

Cremophor EL, DOCA, dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF) and Tween-80; pentobarbitone sodium (Biowet, Puławy, Poland) and sodium chloride (NaCl) (Chempur, Piekary Śląskie, Poland).

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (Munich, Germany). U46619, CBD, GW9662, L161982, O-1918, TRAM-34, AM251, and AM630 were bought from Tocris Bioscience (Bristol, UK). Cay10441 was purchased from Cayman Chemical Co. (Ann Arbor, Michigan, USA). Stock solutions of CBD, U46619, GW9662, AM251, O-1918, capsazepine, nimesulide, Cay10441 and RN1734 were made to 10 mmol/l in ethanol; AM630, L161982, UCL 1684 and TRAM-34 were made to 10 mmol/l in dimethylsulfoxide. Their final concentrations were prepared by dilutions with deionized water, which adjusted the final concentrations of ethanol or DMSO 0.1% v/v or less with the exception of RN1734 (ethanol final concentration 0.2% v/v). Stock solutions of L-NAME (300 mmol/l), phenylephrine and acetylcholine (all 10 mmol/l) were made in distilled water. Indomethacin was dissolved in 0.5 mol/l NaHCO$_3$ and DETCA directly into Krebs.

**RESULTS**

**Influence of cannabidiol on the preconstricted human pulmonary arteries**

U46619 induced a concentration-dependent contraction of hPA rings (pEC$_{50}$ = 8.4 ± 0.1, R$_{max}$ = 101.5 ± 1.7, n = 6). CBD 10 μmol/l and its vehicle did not modify the CRC of U46619 (pEC$_{50}$ = 8.4 ± 0.1, R$_{max}$ = 98.8 ± 1.8, n = 6, pEC$_{50}$ = 8.4 ± 0.1, R$_{max}$ = 98.5 ± 1.9, n = 5, respectively, Fig. 1a).

CBD (0.1–30 μmol/l) but not its vehicle caused almost full relaxation of the hPAs preconstricted with U46619 (Fig. 1b), the pEC$_{25}$ and pEC$_{50}$ values were 5.8 and 5.0, respectively (Table 2). The vehicle controls with CBD were comparable (ethanol, pEC$_{50}$ = 5.0 ± 0.1, n = 5; DMSO, 5.0 ± 0.1, n = 6; water, pEC$_{50}$ = 4.9 ± 0.1, n = 10). They are presented in the Figures as one merged control group (pEC$_{50}$ = 5.0 ± 0.1, n = 21). The vasodilatation induced by CBD was gradual in onset. Thus, it took 80 min to construct the whole CRCs (for the original traces see Fig. 1c).

The vasodilator responses to CBD varied between patients (the maximal response to CBD ranged from 63 to 120% relaxation), so post hoc analysis was performed to establish any relationships between CBD responses and patient characteristics (see Table 1). The potency of CBD was reduced by hypertension (vs. normotension; pEC$_{50}$ = 4.1 ± 0.3, n = 8 vs. 4.9 ± 0.1, n = 13, P < 0.05; Fig. 1d), obesity (vs. lean; pEC$_{50}$ = 4.4 ± 0.1, n = 7 vs. 5.0 ± 0.1, n = 14, P < 0.01; Fig. 1e) and hypercholesteremia (vs. normocholesteremia; pEC$_{50}$ = 4.4 ± 0.2, n = 8 vs. 5.0 ± 0.1, n = 13, P < 0.01; Fig. 1f), whereas the maximal response was diminished only in obese patients (R$_{max}$ = 76.6 ± 5.0, n = 7 vs. 93.2 ± 4.8, n = 14, P < 0.05; Fig. 1e). Moreover, the vasorelaxation to lower concentrations of CBD was attenuated in those taking β-blockers (vs. no β-blockers; pEC$_{25}$ = 5.9 ± 0.1, n = 8 vs. 5.5 ± 0.1, n = 15, P < 0.01; no differences in pEC$_{50}$ values).

The following parameters, concurrent diseases and cardiovascular therapy had no influence on the CBD-evoked relaxation: sex, age, smoking, prevalence of chronic obstructive pulmonary disease, type 2 diabetes and angiotensin-converting enzyme inhibitors, α$_2$-adrenolytics statins, nonsteroidal anti-inflammatory drugs (NSAIDs and proton pump inhibitors (PPIs)) (for pEC$_{50}$ and R$_{max}$ see Table 1). In a few cases, coronary artery disease, angiotensin receptor blockers,
calcium channel blockers and hypoglycaemics virtually did not change CBD-mediated effects, however patient numbers were too small for adequate statistical analysis.

Influence of endothelium denudation and inhibitors of cyclooxygenase, nitric oxide synthetase and superoxide dismutase pathways and antagonists of prostacyclin and prostanoid receptors on the cannabidiol-induced vasorelaxation in human pulmonary arteries

The endothelium removal reduced the potency and efficacy of CBD (Fig. 2a). Inhibition of COX-1 or COX-2 activity using indomethacin (10 μmol/l, nonselective) or nimesulide (10 μmol/l; COX-2), reduced the vasorelaxant effects of CBD at 30 μmol/l by about 50 and 70%, respectively, and shifted the CRC for CBD to the right by a factor of 4 and 8, respectively (Fig. 2b). Therefore, further experiments were performed to test prostanoids (or other metabolite) pathways. The IP receptor antagonist Cay10441 (10 μmol/l) as well as the EP2 receptor antagonist L161982 (1 μmol/l), shifted the CRC for CBD to the right by a factor of 6 and 8, respectively (Fig. 2c). The effect elicited by the highest concentration of CBD was reduced in both cases by about 55% (for pEC25 and Rmax values, see Table 2).

Influence of KCa channel inhibitors on the cannabidiol-induced vasorelaxation in human pulmonary arteries

In order to determine if potassium channels contribute to the vasodilatory effects of CBD, some experiments were carried out in high KCl-preconstricted arteries. The vasorelaxation induced by CBD at a concentration of 30 μmol/l was reduced by about 75% in high KCl-preconstricted arteries compared with those preconstricted with U46619 (Fig. 2d). Pretreatment with the selective inhibitors of large (KCa 1.1, iberiotoxin, 0.1 μmol/l) and given together small (KCa 2.3, UCL1684, 0.1 μmol/l) and intermediate (KCa 3.1, TRAM-34, 10 μmol/l) conductance KCa shifted the CRC for CBD (determined for the U46619-preconstricted hPAs) to the right by a factor of 6, and 8, respectively. The effect elicited by the highest concentration of CBD was reduced in both cases by about 55% (for pEC25 and Rmax values, see Table 2 and Fig. 2c).

Influence of AM251, AM630, capsazepine, O-1918 and RN1734 on the cannabidiol-induced vasorelaxation in human pulmonary arteries

The antagonists of the CB1 receptor, AM251 (1 μmol/l) and CB2 receptor, AM630 (1 μmol/l) and endothelium-dependent

FIGURE 1 Influence of cannabidiol (10 μmol/l) on the concentration–response curve of U46619-induced contraction (a), its vasodilatory effect in endothelium-intact isolated human pulmonary arteries (b; c – representative original trace) and post hoc analysis of the patients comorbidities on cannabidiol-mediated vasorelaxation (d–f). *P less than 0.05 according to Student’s t-test. The results are presented as the mean ± SEM of n tissue samples for each curve. See Table 1 for n and the statistical analysis (b). The control curve for cannabidiol vehicle [ethanol, 0.1% v/v (a) and 0.001 – 0.3% v/v final concentration (b), respectively]. In a few cases, SEM is smaller than or equal to the size of symbols. Arrows show the moment of application of a particular concentration of CBD. CBD, cannabidiol; hPAs, human pulmonary arteries.
relaxation to cannabinoids, O-1918 (10 μmol/l) and the inhib-
itor of TRPV4, RN1734 (20 μmol/l)did not change the CRCs for
CBD (Fig. 3a and b). The only exception was inhibition by
AM251 (by about 30%) of the vasodilatory response to the
highest concentration of CBD (30 μmol/l; Fig. 3a). The TRPV1
antagonist capsazepine (10 μmol/l), shifted the CRC for CBD
to the right by a factor of 2 and reduced the relaxant effect of
the highest concentration of CBD (30 μmol/l) by about 50%
(Fig. 3b). For pEC50, pEC25, and Rmax values, see Table 2.
Immunohistochemical staining revealed the presence of CB1, CB2 and
TRPV1 receptors in endothelial and smooth muscle cells of the walls of the hPAs (Fig. 3c–e, respectively).

**Influence of GW9662 on the time-dependent cannabidiol-induced vasorelaxation in human pulmonary arteries**

A single concentration of CBD (10 μmol/l; approximately equivalent to its pEC60) but not its vehicle, ethanol
caused an initial vasorelaxation of 22.4±7.8% at 15 min,
developing to 67.3±7.4% at 120 min (n = 8). The PPARγ
antagonist GW9662 at concentrations of 1 and 10 μmol/l
tended towards and significantly reduced, the respective

**FIGURE 2** Concentration–response curve of the cannabidiol-induced vasorelaxation after endothelium removal (-ENDO) or 30 min incubation (before precontraction)
with inhibitors/antagonists of various enzymes, receptors or ion channels in hPAs. Panel a: nitric oxide synthase – N²-nitro-arginine methyl ester (L-NAME) 300 μmol/l;
superoxide dismutase – DETCA 300 μmol/l. Panel b: COX-2 – nimesulide 10 μmol/l, COX-1/COX-2 - indomethacin (INDO) 10 μmol/l; IP3 receptor – Cay10411 10 μmol/l;
EP2 receptor – L161982 1 μmol/l. Panel c: large conductance Ca2+-activated potassium channels (KCa1.1) – iberiotoxin 0.1 μmol/l; small (KCa2.3) – UCL1684 0.1 μmol/l
with intermediate conductance Ca2+-activated potassium channels – (KCa3.1) TRAM-34 10 μmol/l. Results are expressed as the percentage relaxation of the isometric contraction induced by U46619 and, in the case of the open hexagons, of the contraction induced by KCl 60 mmol/l. Values are presented as the mean ± SEM of n tissue samples for each curve. See Table 2 for n and the statistical analysis. In a few cases, SEM is smaller than or equal to the size of symbols. SEM, standard error of the mean.

**FIGURE 3** Mechanisms of the cannabidiol-induced vasorelaxation in isolated human pulmonary arteries. Concentration–response curve of the cannabidiol-induced vaso-
relaxation after 30 min incubation (before precontraction) with antagonists of CB1 AM251 (1 μmol/l), CB2 AM630 (1 μmol/l) (a) and endothelium-dependent relaxation to
free radicals O-1918 (10 μmol/l), TRPV1 capsazepine (1 μmol/l) and TRPV4 RN1734 (20 μmol/l) (b) in endothelium-intact hPAs. The results are presented as the mean ± SEM of n tissue samples for each curve. In a few cases, SEM is smaller than or equal to the size of symbols. SEM, standard error of the mean.
time-dependent relaxation induced by CBD 10 μmol/l. Thus, its vasodilatory effects at 120 min were 49.5 ± 7.2% (n = 3) and 40.7 ± 6.6% (n = 5; P < 0.05), respectively (Fig. 4a; for the original traces of control and GW9662 10 μmol/l vasodilatory effect in endothelium-intact isolated hPAs (b) The control–time curve for cannabidiol vehicle (ethanol, 0.1% v/v) was also shown. Arrow shows the moment of application of CBD and/or vehicle. ANOVA, analysis of variance; CBD, cannabidiol; hPAs, human pulmonary arteries; SEM, standard error of the mean.

Influence of hypertension and receptor antagonists on vasodilatory effects of cannabidiol in rat endothelium-intact and denuded small mesenteric arteries

CBD (1 μmol/l) did not modify the CRC of phenylephrine in all groups (WKY pEC50 = 5.5 ± 0.1, Rmax = 93.5 ± 8.0, n = 6 vs. CBD pEC50 = 5.6 ± 0.1, Rmax = 107.2 ± 10.7, n = 6; SHR pEC50 = 5.6 ± 0.1, Rmax = 102.7 ± 7.8, n = 7 vs. CBD pEC50 = 5.3 ± 0.2, Rmax = 107.7 ± 4.8, n = 7; SHAM pEC50 = 5.9 ± 0.1, Rmax = 109.6 ± 9.4, n = 5 vs. CBD pEC50 = 5.7 ± 0.2, Rmax = 110.2 ± 10.7, n = 6; DOCA-salt pEC50 = 5.8 ± 0.1, Rmax = 119.5 ± 8.5, n = 6 vs. CBD pEC50 = 5.6 ± 0.2, Rmax = 122.0 ± 9.4, n = 6; Fig. 5a and b).

CBD (0.1–30 μmol/l) but not its vehicle produced a concentration-dependent acute, almost full relaxation of endothelium-intact phenylephrine-preconstricted sMAs isolated from hypertensive (SHR and DOCA-salt) and normotensive animals (WKY and SHAM) (Fig. 5c and d). The maximal relaxation (Rmax ~90%) was comparable in all experimental groups. The vasorelaxant potency of CBD was stronger in DOCA-salt than in respective normotensive controls, and in SHR. In contrast, in SHR, CBD was less potent as a vasodilator than in WKY (for pEC50 values, see Table 3).

Interestingly, in endothelium-intact sMAs of SHR and DOCA-salt, the relaxation response to CBD was CB1-dependent, as the blockade of CB1 receptors by AM251 (1 μmol/l) resulted in a significant 3-fold and 4-fold rightward displacement of the relaxation response, respectively, and reduction of its potency but not its maximal effects. In DOCA-salt animals, it was additionally sensitive to the CB2-receptor antagonist, AM630 (1 μmol/l) with attenuation in relaxant potency, and to endothelium removal that caused both reduced affinity and efficacy. The TRPV1 receptor antagonist capsazepine (1 μmol/l) and AM251 (1 μmol/l) did not change the CRC for CBD in endothelium intact and denuded sMAs of hypertensive rats, respectively. None of the antagonists or endothelium denudation changed the potency and efficacy of CBD in normotensive controls (for respective pEC50 and Rmax values, see Table 3 and Fig. 6).
FIGURE 5 Influence of cannabidiol (1 μmol/l) on the concentration–response curve of phenylephrine-induced contraction (a and b), and its vasodilatory effects (c and d) in endothelium-intact isolated small mesenteric arteries from normotensive control (a and c, WKY; b and d, SHAM) and hypertensive (a and c, SHR; b and d, DOCA-salt) rats. The results are presented as the mean ± SEM of n tissue samples for each curve. See Table 3 for n and the statistical analysis (c and d). The control–time curve for cannabidiol vehicle (ethanol, 0.001–0.3% v/v final concentration, n = 5 in each group). In a few cases, SEM is smaller than or equal to the size of symbols. SHR, spontaneously hypertensive rats; SEM, standard error of the mean; SHAM, control sham-operated rats; WKY, Wistar–Kyoto.

FIGURE 6 Mechanisms of the cannabidiol-induced vasorelaxation in isolated rat small mesenteric arteries. Concentration–response curve of the cannabidiol-induced vasorelaxation after 30 min incubation (before precontraction) with antagonists of CB1 AM251 (1 μmol/l; a–h), CB2 AM630 (1 μmol/l; a–d), TRPV1 capsazepine (1 μmol/l; a–d), in endothelium-intact (a–d) and endothelium-denuded (e–h) rat sMAs. The results are presented as the mean ± SEM of n tissue samples for each curve. See Table 3 for n and the statistical analysis. In a few cases, SEM is smaller than or equal to the size of symbols. SEM, standard error of the mean; sMAs, small mesenteric arteries.
Influence of hypertension on Cnr1 and Cnr2 gene expression in rat small mesenteric arteries

Expression of Cnr1 and Cnr2 genes were analyzed by real-time qPCR (Fig. 7a and b). It has been shown that Cnr1 expression was up-regulated in the case of both SHR and DOCA-salt rats in comparison to controls, with over 2-fold and 3-fold increases, respectively (Fig. 7a). The corresponding effect was observed regarding the Cnr2 gene in sMAs of DOCA-salt animals (Fig. 7b).

Influence of hypertension on immunostaining of CB1, CB2, transient receptor potential vanilloid 1 and GPR18 receptors in rat small mesenteric arteries

CB1, CB2, TRPV1 and GPR18 receptor immunostaining revealed their presence in the endothelial and smooth muscle cells of the walls of the sMAs in all groups with the exception of GPR18 immunostaining in WKY and SHAM. A more intense staining of CB1 and GPR18 receptors was observed in endothelium and smooth muscle cells of sMAs of SHR and DOCA-salt hypertensive animals (Fig. 8).

DISCUSSION

The present study demonstrated a potent influence of CBD in the regulation of vascular tone in pulmonary circulation of human and that it could be modified by disease, including hypertension. The vasorelaxant effects of CBD in hPAs preconstricted with thromboxane analogue, U46619 is endothelium-dependent and include the following mechanisms: IP, EP4, TRPV1, PPARγ receptors and KCa channels. We have also shown species-specific, vascular-bed-specific and hypertension model differences in response to CBD, as in phenylephrine-preconstricted rat sMAs, CBD-evoked vasorelaxation was enhanced in DOCA-salt but diminished in SHR compared with respective normotensive controls. Moreover, in rats but not in humans, CB1 receptors could play potential protective role in both models of hypertension. Our findings provide evidence that CBD could be a potential relevant vasorelaxant; however, modification of its responses in disease should be considered whenever CBD is used for therapeutic purposes.

Cannabinoid causes strong relaxation of human pulmonary arteries via complex mechanisms

We are the first to demonstrate the vasodilatory effect of CBD on the pulmonary circulation. Thus, CBD induced a concentration-dependent, slowly developed full relaxation (at a concentration of 30 μmol/l) in isolated endothelium-intact hPAs precontracted with U46619. It neither affected basal tone nor the U46619-induced vasoconstriction by itself. The pEC50 value for its vasodilatory response was in the mid-micromolar range (~5.0). Importantly, it could be extrapolated to in-vivo concentrations that decreased BP [9,11] and are achieved in serum after oral administration of 800 mg [33] in humans. Moreover, a high oral dose reaching 1280 mg/day of CBD was used in psychiatric patients without any side effects [34].

Although mechanisms are responsible for the vasodilatory effect of CBD in hPAs? Firstly, it is to a large extent endothelium-dependent as its removal reduced the maximal effect of the highest concentration used (30 μmol/l) by about 60%. Thus, all further experiments were carried out in rings with an intact endothelium. We found that the CBD-induced relaxation was reduced by about 50–70% by the nonselective COX inhibitor indomethacin, the selective COX-2 inhibitor nimesulide, the IP receptor antagonist Cay10441 and the EP4 receptor antagonist L161982, demonstrating that CBD caused vasorelaxation via an arachidonic acid COX/COX-2-derived metabolite acting at IP and/or EP4 receptors. On the other hand, we excluded the possibility that it is related to a direct antagonistic effect of CBD at prostanooid TP receptors as CBD at a concentration that evoked ~60% relaxation (10 μmol/l) did not influence the CRC of U46619. Similarly, the vasodilatory effects of anandamide in hPAs and rPAs were mediated via IP receptors [19,20] and EP4 receptors participated in the
enhancement by CBD and acetylcholine-evoked [16] and 2-AG-evoked relaxation in rat and hMA [35]. On the other hand, the effects of CBD in hPAs were not related to activation of NO and/or SOD as incubation with L-NAME or DETCA did not modify vascular responses to CBD.

Secondly, two sets of experiments suggest the involvement of potassium channels in the effect of CBD. The relaxation to CBD was significantly attenuated in hPAs preconstricted with a high concentration of K\(^+\) that not only blocks outward potassium channels but also in addition, leads to membrane depolarization. The latter phenomenon may interfere with the quantitative contribution of ion channels to the vasodilator effect of CBD. Accordingly, CBD-induced responses were strongly, by a comparable degree, inhibited by iberiotoxin (K\(_{Ca1.1}\) blocker) and TRAM-34 (K\(_{Ca2.3}\) blocker) given in the presence of UCL1684 (K\(_{Ca2.3}\) blocker), indicating a major role for K\(_{Ca}\) in the vascular action of CBD in endothelium-intact hPAs. It is generally accepted [36] that K\(_{Ca3.1}\) and K\(_{Ca2.3}\) channels are located in the endothelium and K\(_{Ca1.1}\) in the smooth muscle. However, as we used iberiotoxin in endothelium-intact hPAs, we cannot exclude a minor role for endothelial K\(_{Ca1.1}\) that has been recently suggested in CB1/CB2 receptor-independent cannabinoid-induced vasodilation [37]. Similarly to the present results, CBD and its synthetic analogue abn-CBD relaxed endothelium-intact hMAs and hPAs via potassium [5] and K\(_{Ca}\) channels [21], respectively.

Thirdly, we have determined the possible involvement of cannabinoid-sensitive receptors in the CBD-induced relaxation of hPAs. CBD might activate them directly or indirectly leading to enhancement of endocannabinoid tone, as it is known to inhibit fatty acid amide hydrolase (FAAH; an enzyme responsible for the degradation of endocannabinoid anandamide activity and transport [38]) and to activate phospholipase A2 with subsequent elevation in arachidonic acid-derived products.

The expression of CB1-receptors has been determined in hPAs (present study, [39]). CBD has a low affinity at CB1 receptors, including human receptors [38]. Accordingly, in our study, AM251 failed to modify the vasorelaxation induced by CBD 0.1–10 \(\mu\)mol/l pointing to a CB1 receptor-independent mechanism. The inhibitory effect of AM251 against the response to the highest CBD concentration 30 \(\mu\)mol/l, results probably from nonspecific and also CB1-independent actions of CBD, such as increasing membrane fluidity demonstrated for CBD 30 \(\mu\)mol/l [38,40], a negative allosteric modulation [41] and/or enhancement of endocannabinoid tone [38].

CBD has also been demonstrated to activate TRPV1 [42]. Indeed, we demonstrated that in hPAs TRPV1 receptors are involved in the vasodilatory response to CBD, as their agonist capsazepine reduced the vasodilatory effect of CBD and they are expressed in the endothelium and smooth muscle cells of hPAs.

![FIGURE 8](image_url)

**FIGURE 8** Representative micrographs of immunohistochemical staining of CB1, CB2, TRPV1 and GPR18 receptors in cross-sections of rat small mesenteric arteries from normotensive control (WKY; SHAM) and hypertensive (SHR; DOCA-salt) rats. Scale bars: 50 \(\mu\)m. DOCA-salt, 11-deoxycorticosterone acetate-salt; SHR, spontaneously hypertensive rats; SHAM, control sham-operated rats; WKY, Wistar–Kyoto.
The PPARγ receptors mediate a slowly developed relaxation of hPAs (e.g. rosiglitazone maximum relaxation 75%, pEC50 5.5 [28]). CBD is considered to be a PPARγ ligand capable of eliciting time-dependent relaxation in rat-isolated aorta, maximally by about 50% [13]. In our hands, CBD (10 μmol/l) evoked a nearly 70% time-dependent relaxation in hPAs that was not significantly (by 25%) and significantly (by 40%) reduced by the potent PPARγ antagonist, GW9662. 1 and 10 μmol/l, respectively.

We excluded the potential involvement of CB2 and TRPV4 receptors in the CBD-induced relaxation of hPAs as they were not altered by the respective antagonists AM630, and RN1734. In addition, we have examined the vasodilatory effect of CBD in the presence of O-1918 known to antagonize endothelial-dependent vasodilator effects of various cannabinoids including anandamide and abn-CBD (see below). However, in our hands, O-1918 failed to modify the response to CBD in hPAs. Thus, we can exclude the involvement of the following mechanisms connected with this antagonist: still questionable endothelial atypical cannabinoid receptors, the Na+/Ca2+ exchanger or GPR18 receptors (according to [37,43]). The latter despite the fact that CBD is a weak GPR18 partial agonist with pEC50 51.1 μmol/l [44] and we determined the expression of GPR18 in the endothelium of hPAs. O-1918 has also been demonstrated to inhibit KCa1.1 channels [45,46] but in cell cultures only.

The CBD-induced relaxation of hPAs in the current study was comparable with that demonstrated by us previously in endothelial-intact hPAs relaxed by endocannabinoids, that is, anandamide [19], 2-AG [39] and virodhamine [47], the CBD analogue abn-CBD [21] and LPI [27]. In all cases, we noticed almost full relaxation, with similar respective pEC50 values: 5.0, 5.4, 5.1, 4.8 and 6.4, the involvement of endothelium, potassium channels and sensitivity to O-1918 (not examined in the case of 2-AG). The COX metabolites mediated the relaxation induced by unstable anandamide and virodhamine but not by stable abn-CBD. CB2 receptors were involved in responses to 2-AG only (see above) and CB2 receptors were excluded in the actions of anandamide and virodhamine.

Our results demonstrated that the vasodilatory effect of CBD in human arteries is dependent on the vascular bed, as it relaxed hMAs with similar potency (pEC50 was 5.1) but only in about 40% [5]. The difference in efficacy results probably comes from the enhanced physiological role of the endothelium in the regulation of smooth muscle tone in the pulmonary circulation compared with mesenteric arteries [19,39,27]. However, both responses involved similar mechanisms, that is, endothelium-dependency, involvement of TRPV1 receptors and via inhibition of potassium efflux. In addition, they were not modified by AM630, O-1918 and l-NAME. There are following differences between the CBD-induced relaxation in hPAs and in hMAs. Thus, in hMAs, CBD-induced vasorelaxation was reduced by two CB2 receptor antagonists (including AM251). It also gradually increased with time, but it was not inhibited by PPARγ antagonism [5], confirming that these receptors are involved in the relaxation of conduit vessels, such as rat aorta [13], but not in small arteries. Moreover, indomethacin was ineffective in hMAs excluding the involvement of COX metabolites in the vasodilatory effect of CBD.

Cannabidiol-mediated vasorelaxations

The post hoc analysis revealed that the potency of the CBD-induced relaxation of hPAs was reduced by hypertension, obesity and hypercholesterolemia and in patients taking β-blockers compared with respective controls. The efficacy was diminished by obesity only. The patient age, sex, smoking habits and comorbidities, such as chronic obstructive pulmonary disease and type 2 diabetes did not influence the potency of CBD in hPAs. Similarly, hypercholesterolemia and β-blockers reduced the CBD responses in hMAs [5]. However, these data of comorbidities and the medication taken showed only rough approximations. It is not yet known whether this might result from drug interactions or the disorder for which the medication is being taken. Hypertension, obesity and hypercholesterolemia are well known important risk factors for endothelial dysfunction [25] that in this study could impair the CBD-mediated vasorelaxation. Interestingly, patients taking and not taking NSAIDs exhibited the same degree of CBD-induced vasodilatation in spite of evident attenuation of the CBD-induced vasodilatation by selective and nonselective COX inhibitors. However, one should keep in mind, that 72 h before surgery patients stopped taking pain relief, that is, the time was sufficient for drug metabolization and elimination and in our hands, COX inhibitors were given in vitro 30 min before CBD.

Influence of hypertension on the cannabidiol-mediated vasorelaxation in rat small mesenteric arteries

We decided to examine the vasodilatory response to CBD in experimental hypertension as CBD is suggested to have antihypertensive potency. We applied the same models of primary (SHR) and secondary (DOCA-salt) hypertension in which we noticed previously different effects of chronic FAAH inhibition on the vasodilator effect of methanandamide (the stable anandamide analogue; see below).

In normotensive rats, acute CBD did not affect basal tone and the phenylephrine-induced vasoconstriction but caused the concentration-dependent full relaxation of sMAs preconstricted with phenylephrine with similar mid-micromolar potency as demonstrated previously in rat sMAs [12] and hMAs [5]. Importantly, CBD caused full relaxation of rat sMAs (present study [12]) but only almost half-maximal relaxation of hMAs [5], confirming the suggestion of Stanley et al. [5] that CBD efficacy might be reduced in human mesenteric vasculature.

We are the first to demonstrate the influence of hypertension on the vascular response to CBD. Interestingly, it was enhanced in DOCA-salt hypertension but reduced in SHR. The reactivity of the CBD-mediated relaxation by endothelium removal in DOCA-salt but not in SHR argues for its possible crucial role in compensatory mechanisms in secondary hypertension, like DOCA-salt [48]. Similarly, despite comparable values of enhanced blood pressure in both models of hypertension in comparison with respective controls secondary [25] and primary [49,50] hypertension enhanced and reduced the vasodilator responses to methanandamide and/or anandamide in rat sMAs and/or the mesenteric vascular bed, respectively; endocannabinoids levels were lower in SHR and higher in DOCA-salt [51]; one
should keep in mind that CBD is antagonist of anandamide degradation), pressor responses of MAs to periarterial nerve stimulation and to exogenous noradrenaline infusion were higher in SHR and lower or without change in DOCA-salt, respectively [52]. Thus, various genetic and environmental triggers or key pathophysiologic mechanisms characterized both models of hypertension [53] might determine opposite responses to CBD determined by us in SHR and DOCA-salt.

Importantly, none of the mechanisms involved in the CBD-induced vasorelaxation in hypertension was detected in normotension despite the fact that all the receptors described below were expressed in sMAs of normotensive and hypertensive rats (present study, [25,49]). Thus, CB1 receptors were involved in the CBD-induced vasorelaxation as it was reduced by their antagonist AM251 and increased Cnr1 expression was observed in sMAs in both models of hypertension. Their up-regulation, shown by the higher expression level and their involvement in methanandamide-induced relaxation and potential protective role, has been previously demonstrated in sMAs isolated from primary [49] and secondary [25] hypertensive rats. Moreover, the enhancement and a potential protective effect of CB1 receptor-mediated presynaptic inhibition of the sympathetic nerve endings innervating resistance vessels of pithed rats was noticed in DOCA-salt hypertension [54] but not in SHR [55]. In contrast to hPAs, in rat sMAs, AM251 reduced CBD’s potency, suggesting that CBD might rather act directly at CB1 receptors in this vascular bed. Interestingly, in both models of hypertension, they are expressed in the endothelium and the smooth muscle, but AM251 reduced the CBD effect in endothelium-intact arteries only. In contrast, in hMAs CBD acted at CB1 receptors that are present on both the endothelium and smooth muscle [5].

The CBD-induced relaxation was diminished by the CB2 receptor antagonist AM630 in DOCA-salt, but not in SHR. This is in line with the increased Cnr2 expression and CB2 immunostaining observed in DOCA-salt only. Similarly, AM630 diminished the CBD-stimulated improvement of the acetylcholine-evoked vasorelaxation in sMAs of diabetic rats, resulting probably from the off-target effect of AM630 or the allosteric effect of CBD on CB2 receptors [16]. Thus, we could not exclude that these phenomena appeared also in hypertension.

In contrast to responses to CBD in hPAs (see above) and in hMAs [5], the TRPV1 receptor antagonist capsazepine failed to produce the vasodilator effect of CBD in normotensive and hypertensive rats. This is in agreement with the present and former studies, which have demonstrated unchanged TRPV1 receptor expression and/or no alteration in the TRPV1-sensitive component of cannabinoid-mediated vasorelaxant effects in DOCA-salt rats (methanandamide [25]) or in SHR (anandamide [50]).

Limitations and dichotomous cannabidiol pharmacology

It is important to mention the following limitations of our study. Polypharmacological profiles of CBD [38,56] makes it impossible to precisely determine its vasodilatory mechanism of action. The above was probably the reason that CBD was originally introduced to cardiovascular system pharmacology as the antagonist of the currently questionable endothelial atypical cannabinoid receptor [12,20,21,47] that given in nonpreconstricted arteries did not produce any or only slight vasodilatation. However, currently it is known as a potent vasodilator [5,12,13], but this effect is observed in preconstricted arteries. In the literature, there still exists two opposite pharmacological descriptions that complicated the final conclusions. The efforts to re-evaluate discrepancies between the studies on the pharmacological profile of CBD were made and described in detail by Bondarenko [43,37]. The lipophilic nature of CBD and other cannabinoi ligands could allow them (especially in μmol/l concentrations) to incorporate into membranes or associate with lipid rafts [37]. We have done our best to use the selective ligands of particular antagonists (e.g. according to [23]). However, AM251 has also been suggested to directly inhibit KCa1.1 channel activity independently of cannabinoid receptors [37] and/or as a mixed CB1/GPR18 antagonist [57]. The complex effects of O-1918 were described above. Thus, the confirmation of the role of CB1, PPARγ and/or GPR18 receptors in the CBD-mediated vasorelaxation warrants further investigation and application of more selective antagonists combined with genetic tools.

Conclusion and perspectives

In conclusion, our results demonstrate that CBD caused a full concentration-dependent and endothelium-dependent relaxation of hPAs. This was mediated through IP, EP4 and TRPV1 receptors and KCa channels. Moreover, CBD produced a time-dependent slowly developing decrease in the tone of endothelium-intact hPAs sensitive to the PPARγ antagonist, GW9662. Pulmonary hypertension is a life-threatening condition that lacks effective therapy. The following facts underline the possibility that CBD might represent a future goal/option in the therapy of this disease that had been previously suggested to abn-CBD [22]: the strong vasorelaxant efficacy of CBD in hPAs is dependent among others on IP receptors, prostacyclin analogues belong to the frontline therapy against pulmonary hypertension [58] and inhalation is the main route of cannabinoid administration. The vasorelaxant effect of CBD was reduced by comorbidities, including hypertension, obesity and hypercholesterolemia and in patients taking β-blockers. In addition, response to CBD is species-specific, vascular-bed-specific and hypertension model differences, as in phenylephrine-preconstricted rat sMAs CBD-evoked vasorelaxation was enhanced in DOCA-salt but diminished in SHR compared with respective normotensive controls and the protective role of CB1 receptors has been suggested in animal models of hypertension but not in human. In light of the evidence that CBD could reduce BP in stressed patients, our data supplies a further rationale that the cardiovascular system could be indeed a valid therapeutic target for CBD and that preclinical responses to CBD will translate into the human cardiovascular system. Thus, CBD-derived therapies after years might have potential for the evaluation and development of CBD-based drugs not only for neuronal
disorders but also in cardiovascular diseases. Importantly, CBD is a nonpsychotropic drug and it has been demonstrated to offer well tolerated therapy. Overall, our results suggest that although the beneficial effects of CBD in cardiovascular disorders are known, additional studies are required to fully understand its potential contributions in the clinical setting [1].

ACKNOWLEDGEMENTS

This study was supported by the National Science Centre (Poland) (grant no. NCN 2015/19/B/NZ7/02270); and by the Medical University of Białystok (grant no. N/ ST/ZB/17/003/2213). We wish to thank Mrs. I. Malinowska (Poland) (grant no. NCN 2015/19/B/NZ7/02270); and by the National Science Centre (Poland) (grant no. NCN 2015/19/B/NZ7/02270). The study was supported by the National Science Centre (Poland) (grant no. NCN 2015/19/B/NZ7/02270). The authors declare that there is no conflict of interest.

Previous presentations of part of this work: Baranowska-Kuczko M, Kozłowska H, Kloza M, Karpinska O, Toczek M, Kozłowski M, et al. Cannabidiol-induced vasorelaxation in isolated human pulmonary and small mesenteric arteries of hypertensive rat - preliminary study. In: 26th Annual Symposium on Cannabinoids, International Cannabinoid Research Society. Book of abstracts, 2016; pp. 1–19.

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Conflicts of interest

There are no conflicts of interest.

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