



The effects of cannabidiol on the antigen-induced contraction of airways smooth muscle in the guinea-pig

A. Dudášová^{a,b}, S.D. Keir^{b,*}, M.E. Parsons^{a,†}, A. Molleman^{a,†}, C.P. Page^b

^aSchool of Life Sciences, University of Hertfordshire, CP Snow Building, Hatfield, UK

^bSackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, London, UK

ARTICLE INFO

Article history:

Received 4 September 2012

Received in revised form

7 February 2013

Accepted 8 February 2013

Keywords:

Airway obstruction

Cannabinoids

Guinea-pig bronchi

Sensory nerves

Mast cells

ABSTRACT

(–)- Δ^9 -Tetrahydrocannabinol has been demonstrated to have beneficial effects in the airways, but its psychoactive effects preclude its therapeutic use for the treatment of airways diseases. In the present study we have investigated the effects of (–)-cannabidiol, a non-psychoactive component of cannabis for its actions on bronchial smooth muscle *in vitro* and *in vivo*.

Guinea-pig bronchial smooth muscle contractions induced by exogenously applied spasmogens were measured isometrically. In addition, contractile responses of bronchial smooth muscle from ovalbumin-sensitized guinea-pigs were investigated in the absence or presence of (–)-cannabidiol. Furthermore, the effect of (–)-cannabidiol against ovalbumin-induced airway obstruction was investigated *in vivo* in ovalbumin-sensitized guinea-pigs.

(–)-Cannabidiol did not influence the bronchial smooth muscle contraction induced by carbachol, histamine or neurokinin A. In contrast, (–)-cannabidiol inhibited anandamide- and virodhamine-induced responses of isolated bronchi. A fatty acid amide hydrolase inhibitor, phenylmethanesulfonyl fluoride reversed the inhibitory effect of (–)-cannabidiol on anandamide-induced contractions. In addition, (–)-cannabidiol inhibited the contractile response of bronchi obtained from allergic guinea-pigs induced by ovalbumin. *In vivo*, (–)-cannabidiol reduced ovalbumin-induced airway obstruction.

In conclusion, our results suggest that cannabidiol can influence antigen-induced airway smooth muscle tone suggesting that this molecule may have beneficial effects in the treatment of obstructive airway disorders.

© 2013 Published by Elsevier Ltd.

1. Introduction

The main psychoactive constituent of cannabis, (–)- Δ^9 -tetrahydrocannabinol (Δ^9 -THC) has been found to have bronchorelaxant activity both in healthy subjects and subjects with asthma, but does not appear to be clinically beneficial in the acute treatment of respiratory diseases [1–4]. However, certain cannabinoids have also been shown to modulate inflammatory responses. Thus, in a murine model of asthma, Δ^9 -THC and another plant-derived

cannabinoid, cannabidiol, attenuated ovalbumin (chicken egg albumin) (OVA)-induced allergic airway responses, including IL-2 and Th2 cytokine (IL-4, IL-5 and IL-13) mRNA expression in lung tissue, serum IgE production and overproduction of mucus in the lungs [5]. Δ^9 -THC is a known potent psychoactive drug in humans and therefore use of such compounds as anti-inflammatory drugs [6] is difficult, although a recent study has identified that the beneficial effect of Δ^9 -THC in airway inflammation is independent of CB₁/CB₂ cannabinoid receptor activation [7].

It is now known that the non-psychoactive cannabinoid (–)-cannabidiol (CBD), exhibits a number of promising pharmacological properties [8] with the ability to interact with several molecular targets e.g. TRP channels and the enzymes involved in the inactivation of endocannabinoids, such as the fatty acid amide hydrolase (FAAH) [9]. In mice sensitized with OVA, CBD reduced the serum level of OVA-specific antibodies and in murine splenocytes, CBD also suppressed T cell proliferation and cytokine production [10] suggesting that it may be possible to find non-psychoactive cannabinoids as treatments for inflammatory diseases of the airways.

Abbreviations: AUC, area under the curve; CBD, (–)-cannabidiol; C_{dyn} , dynamic compliance; Δ^9 -THC, (–)- Δ^9 -tetrahydrocannabinol; FAAH, fatty acid amide hydrolase; NKA, neurokinin A; OVA, ovalbumin; PMSF, phenylmethanesulfonyl fluoride; R_L , total lung resistance.

* Corresponding author. Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, London, UK.

E-mail address: sandra.rudman@kcl.ac.uk (S.D. Keir).

† Deceased.

The OVA-sensitized and challenged guinea-pig has been widely used as a model for asthma in man because of similarities with human asthma [11]. In isolated bronchi obtained from OVA-sensitized guinea-pigs, Δ^9 -THC and a modified cannabinol, nabilone, did not influence antigen-induced bronchoconstriction [12]. However, there is no information on CBD's effect on contraction of airway smooth muscle, particularly contraction induced by allergen in allergic animals.

We have therefore investigated the functional pharmacology and potential mechanisms of action of CBD in isolated guinea-pig bronchi from healthy and OVA-sensitized animals. In addition, we have assessed the action of CBD against antigen-induced airway obstruction of allergic guinea-pigs *in vivo*.

2. Methods

2.1. Tissue preparation for *in vitro* experiments

Heston (strain 2) guinea-pigs (700–1000 g) of either sex were bred and kept in-house complying with the standard guidelines for animal care in the UK (University of Hertfordshire, UK). Guinea-pigs were killed by cervical dislocation, the lungs removed and placed in cold Krebs–Henseleit solution aerated with 95% O₂ and 5% CO₂. Two main bronchial rings (4–5 mm) were suspended under 0.5 g tension, in 10 ml organ baths with oxygenated Krebs–Henseleit solution at 37 °C, containing the cyclo-oxygenase inhibitor indomethacin (10 μ M). Tension was recorded isometrically in units of g using Dynamometer UF1 force transducers (Pioden Control, UK). The transducer signals were transformed in an analogue/digital converting board and recorded using PowerLab Chart Version 5.1 (AD Instruments, UK). In all the *in vitro* experiments, the contractile effect of each concentration of spasmogen was allowed to plateau at its maximum before the next concentration was added.

2.2. Sensitization and challenge studies of isolated bronchial tissue

Sensitization to OVA was performed under a licence granted under the Animal Scientific Procedures Act, 1986. Heston guinea-pigs (700–1000 g) of either sex received an intraperitoneal (i.p.) injection of OVA (10 μ g) and aluminium hydroxide (100 mg) suspended in normal saline (1 ml). After 14 days, animals were challenged with OVA (1 mg ml⁻¹; i.p.). Mepyramine (1 mg kg⁻¹) was given 15 min before the challenge to prevent anaphylaxis. 21 days later tissue was prepared for *in vitro* experiments as outlined above. These tissues were only used to study the antigen-induced contractions of bronchi (section 2.4). The *in vitro* experiments assessing agonist-induced contractions (section 2.3 and 2.5) were performed on tissues from naïve guinea-pigs.

2.3. Agonist-induced contractions of isolated bronchial tissue

Cumulative concentration–response curves (1–100 μ M) for the endocannabinoids— anandamide and virodhamine were constructed only once on each bronchial tissue. The time between applications of each concentration was 10–20 min. The vehicle controls were performed in parallel. In other experiments, the airway smooth muscle tone was measured following administration of carbachol, capsaicin (10 nM–10 μ M), histamine (100 nM–100 μ M) or neurokinin A (NKA) (0.1 nM–10 μ M) alone or in the presence of 1 μ M CBD (the incubation time was 20 min). At the end of the experiment, the responsiveness to carbachol (10 μ M) was assessed.

2.4. Antigen-induced contractions of isolated bronchial tissue

OVA was added cumulatively to airway smooth muscle preparations obtained from allergic guinea-pigs (10 μ M–1 mM). One

bronchial ring per pair was tested in the presence of a drug of interest and OVA, with the second tissue being used as a paired control. The time between applications of each concentration was 3–10 min. The effects of pre-treatment with CBD (100 nM, 1 μ M and 10 μ M; 20 min), mepyramine (100 nM) and the 5-lipoxygenase inhibitor, MK886 (10 μ M) on the contractile response induced by OVA were also assessed. At the end of these experiments, the responsiveness to carbachol (10 μ M) was also assessed.

2.5. Compound 48/80-induced contractions of bronchial smooth muscle

As with allergen-induced airway smooth muscle contraction, only one bronchial ring per pair was exposed to the drug of interest and cumulative concentrations of the mast cell degranulator compound 48/80 (1–300 μ g ml⁻¹). The second bronchial ring was used as a control. The time between each concentration was 5–10 min. The effects of pre-treatment with CBD (1 μ M and 10 μ M), (100 nM) was assessed. At the end of the experiments, the responsiveness to carbachol (10 μ M) was also assessed.

In all experiments the incubation time with CBD and the inhibitors was 20 min apart from phenylmethanesulfonyl fluoride (PMSF) and MK886 where the pre-treatment time was for 30 and 40 min, respectively.

2.6. Antigen-induced airway obstruction in allergic guinea-pigs *in vivo*

Male Dunkin–Hartley guinea-pigs (400–600 g) were supplied by B & K Universal Ltd. (Hull, UK) for these experiments. Guinea-pigs were housed on-site for one week prior to experimentation [carried out in compliance with the Animals (Scientific Procedures) Act of 1986] and given free access to food and water. For passive sensitization anti-OVA guinea-pig plasma was prepared as described elsewhere [13] and injected intravenously via the saphenous vein of recipient naïve conscious guinea-pigs (1 ml·animal⁻¹). Lung function in response to allergen exposure was recorded 7–10 days later as described below.

2.7. Measurement of pulmonary function

Guinea-pigs were anaesthetised by i.p. injections of urethane (1.75 g kg⁻¹) and surgically prepared for the insertion of a tracheal cannula. Animals were ventilated (8 ml kg⁻¹; 60 breaths min⁻¹) through the tracheal cannula connected to a pneumotachograph and a pressure transducer (± 2 cm H₂O; model MP-45-14-871; Validyne Engineering, Northridge, CA, USA). Changes in airflow were measured using an automated lung function recording system (Pulmonary Monitoring System, version 5.0; Mumed, London, UK) and displayed in real time. The flow signal was integrated to give a measure of tidal volume. An intrathoracic cannula was inserted between the third and fourth, or the fourth and fifth intercostal space and connected to the negative side of the pressure transducer (± 2 cm H₂O; Validyne). The positive side of the transducer was connected to the side of the pneumotachograph proximal to the animal. The difference between mouth and thoracic pressure was used as a measure of transpulmonary pressure (TPP). Total lung resistance (R_L ; cm H₂O·litre⁻¹ s⁻¹) and dynamic lung compliance (C_{dyn} ; cm H₂O·litre⁻¹ s⁻¹) was calculated from flow, tidal volume and TPP by integration. The jugular vein was cannulated for the administration of drugs, with blood pressure being recorded via a pressure transducer attached through an arterial cannula inserted into the carotid artery. Airway obstruction was measured [as an increase in R_L and decrease in lung compliance (C_{dyn})] following administration of aerosolised saline (20 s) and then OVA

(10 mg ml⁻¹; 20 s) via a micropump nebuliser (Aeroneb Lab, Aerogen, USA) at 60 breaths min⁻¹. CBD or its vehicle (ethanol, tween 80 and saline, 1:1:18) was administered intravenously 20 min prior to nebulisation of saline and OVA.

2.8. Drugs

All compounds were obtained from Sigma–Aldrich (Poole, UK), unless stated otherwise. Anandamide [N-(2-hydroxyethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide], CBD [2-[(1R,6R)-3-Methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol], and virodhamine [O-(2-aminoethyl)-5Z,8Z,11Z,14Z-eicosatetraenoate] were obtained from Tocris-Cookson (Bristol, UK). CBD for the *in vivo* experimentation was kindly provided by GW Pharmaceuticals (UK).

2.9. Data analysis

Values are expressed as a percentage (%) of the mean maximal contraction induced by carbachol (10 µM) ± standard error of the mean (sem) or as g contraction ± sem and *n* denotes the number of animals. Mean values of the two sets of data were compared using Student's unpaired two-tailed *t* test. Data obtained with OVA was evaluated using Student's paired two-tailed *t* test. Differences between mean values were considered significant if *P* ≤ 0.05. Curves were fitted using non-linear regression analysis using GraphPAD Prism statistical software (GraphPAD software, CA, USA). With respect to the airway obstruction, the area under the curve (AUC) of % increase in *R*_L over 20 min and % decrease in *C*_{dyn}, respectively,

was calculated. Student's paired two-tailed *t* test was implemented to identify significant differences between the AUC data.

3. Results

3.1. Effects of CBD on concentration–response curves to capsaicin, endocannabinoids and on FAAH inhibition

The cannabinoid ligand CBD, at the concentration of 1 µM or 10 µM did not contract bronchial rings at baseline. Previous experience with the non-selective CB₁/CB₂ agonist WIN55212-2 used in the electrical-field stimulation study provided the rationale for the incubation time (20 min) and the concentration of CBD (1 µM) used in this manuscript. However, the possible interaction of CBD with other spasmogens was tested using the TRPV₁ receptor agonist capsaicin (10 nM–10 µM) or the endocannabinoids, anandamide or virodhamine (1 µM–100 µM) in isolated guinea-pig bronchi. In the presence of CBD (1 µM) the contractile responses (expressed as g contractions ± sem) to capsaicin were not significantly different from the control responses (Fig. 1A). In contrast, CBD at the same concentration (1 µM) significantly inhibited contractile responses to anandamide and virodhamine in isolated bronchial rings. The responses to both endocannabinoids were significantly reduced in the presence of CBD (Fig. 1B and C). The presence of the FAAH inhibitor, PMSF (20 µM) failed to evoke any change in anandamide-induced contractions (*n* = 5, Fig. 1D). In contrast, PMSF (20 µM) reversed the inhibitory action of CBD (1 µM), whilst there was no difference between contractile responses to anandamide only

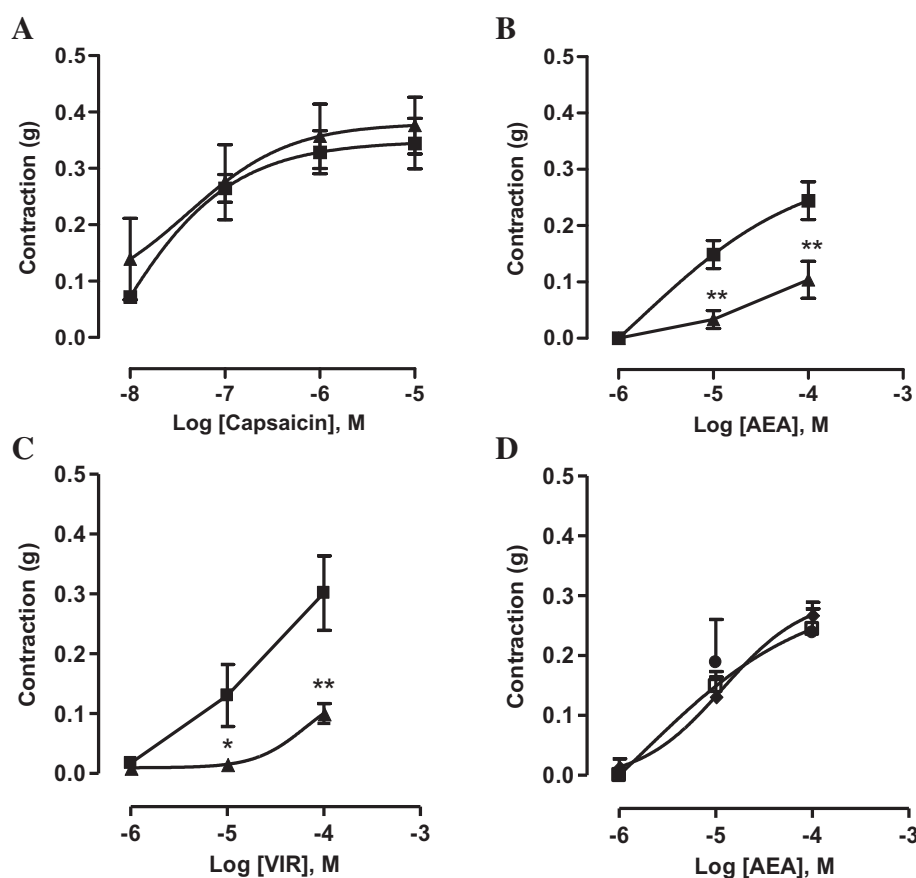


Fig. 1. Cumulative concentration–response curves of guinea-pig bronchial rings to (A) capsaicin (B) anandamide (C) virodhamine [(■) *n* = 6] alone or in the presence of CBD [(▲) 1 µM] (D) anandamide [(□) *n* = 6] alone or in the presence of PMSF [(●) 20 µM, *n* = 5] or in combination with PMSF (20 µM) and CBD (1 µM) [(◆) *n* = 5]. Each point represents the mean and vertical lines represent sem. **P* ≤ 0.05 and ***P* ≤ 0.01 vs. controls.

($n = 6$, Fig. 1D) and contractile responses to anandamide with CBD ($1 \mu\text{M}$) ($n = 5$, Fig. 1D).

3.2. Effects of CBD on concentration–response curves to contractile agents

Furthermore, CBD at a concentration of $1 \mu\text{M}$ did not affect the contractile responses (expressed as a % of the mean response to $10 \mu\text{M}$ of carbachol $\pm\text{sem}$) induced by carbachol (10 nM – $10 \mu\text{M}$) (Fig. 2A), histamine (1 nM – $100 \mu\text{M}$) (Fig. 2B) or NKA (0.1 nM – $10 \mu\text{M}$) (Fig. 2C) in guinea-pig bronchial rings.

3.3. Effects of CBD on antigen-induced contractions

CBD at concentrations of 100 nM (Fig. 3A) and $10 \mu\text{M}$ (Fig. 3C), did not produce a significant reduction of the allergen-induced contractions (expressed as g contractions $\pm\text{sem}$). However, at $10 \mu\text{M}$, CBD actually produced an enhancement of OVA-induced contractions. In contrast, CBD at $1 \mu\text{M}$ significantly inhibited the airway smooth muscle contraction evoked by OVA [$23.13\% \pm 13.50$ ($n = 6$) vs. paired controls: 84.10 ± 1.36 ($n = 6$)] (Fig. 3B). Mepyramine at 100 nM also produced a significant reduction of the contractions evoked by OVA [$27.29\% \pm 10.62$ ($n = 5$) vs. paired controls: $66.43\% \pm 5.08$ ($n = 5$) respectively] (Fig. 3D). The 5-lipoxygenase inhibitor, MK-886 ($10 \mu\text{M}$) produced significant inhibition of the contraction evoked by OVA ($100 \mu\text{g ml}^{-1}$) [$0.22 \text{ g} \pm 0.03$ ($n = 5$) vs. paired controls: $0.37 \text{ g} \pm 0.05$ ($n = 5$)] (Fig. 3E).

3.4. Effects of CBD on compound 48/80-induced contractions

Pre-treatment with 1 or $10 \mu\text{M}$ of CBD did not result in a significant change in the contraction induced by compound 48/80 (Fig. 4A and B respectively). Interestingly, a significant inhibition of the contraction induced by compound 48/80 ($100 \mu\text{g ml}^{-1}$) was

observed in the presence of CBD ($1 \mu\text{M}$) when the data were evaluated in g of contraction $\pm\text{sem}$ [$0.09 \text{ g} \pm 0.03$ ($n = 4$) vs. paired controls: $0.21 \text{ g} \pm 0.07$ ($n = 4$)] (data not shown). 100 nM Mepyramine significantly shifted the concentration–response curve for compound 48/80-induced contractions to the right ($n = 4$, data not shown).

3.5. Effects of CBD on OVA-induced airway obstruction in passively sensitized guinea-pigs

The intravenous administration of both CBD (1 mg kg^{-1}) and its vehicle (ethanol, tween 80 and saline, 1:1:18) 20 min prior to inhalation evoked changes in the lung function of allergic guinea-pigs (Table 1). However, there was no significant difference in the increase in total R_L or in the decrease in C_{dyn} in response to intravenous administration of CBD or its vehicle in anesthetized guinea-pigs. In addition, the intravenous administration of CBD (1 mg kg^{-1}) or its vehicle produced a fall (% decrease) in blood pressure (CBD: $17.45 \pm 5.88\%$; vehicle: $13.62 \pm 2.73\%$), which recovered to baseline after 4 min.

However, airway obstruction, as measured by a decrease in C_{dyn} ($\text{cm H}_2\text{O} \cdot \text{litre}^{-1} \text{ s}^{-1}$), in response to aerosolised OVA (10 mg ml^{-1} ; 20 s) was significantly modulated by CBD treatment (1 mg kg^{-1}) [-0.04 ± 0.49 ($n = 5$) vs. paired controls -2.48 ± 0.79 ($n = 5$)]. Vehicle-treated animals used as controls showed a significantly greater decrease in C_{dyn} (analysed as AUC 0–20 min % decrease in C_{dyn}) compared with CBD-treated animals (Fig. 5).

4. Discussion

The present findings demonstrate for the first time that CBD has the ability to abrogate antigen-induced airway obstruction *in vivo* which is supported by our observations that CBD can influence antigen-induced contraction of airway smooth muscle *in vitro* obtained from allergic guinea-pigs. In addition, CBD inhibited the

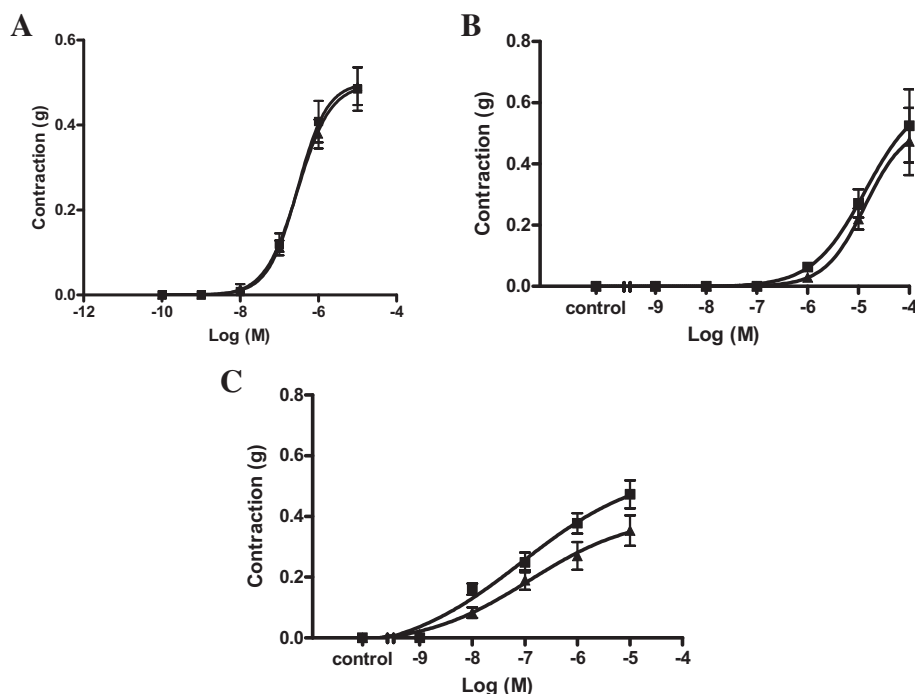


Fig. 2. Cumulative concentration–response curves of guinea-pig bronchial rings to (A) carbachol (B) histamine [(■) $n = 4$] and (C) NKA [(■) $n = 5$] alone or in the presence of CBD [(▲) $1 \mu\text{M}$]. Each point represents the mean and vertical lines represent sem.

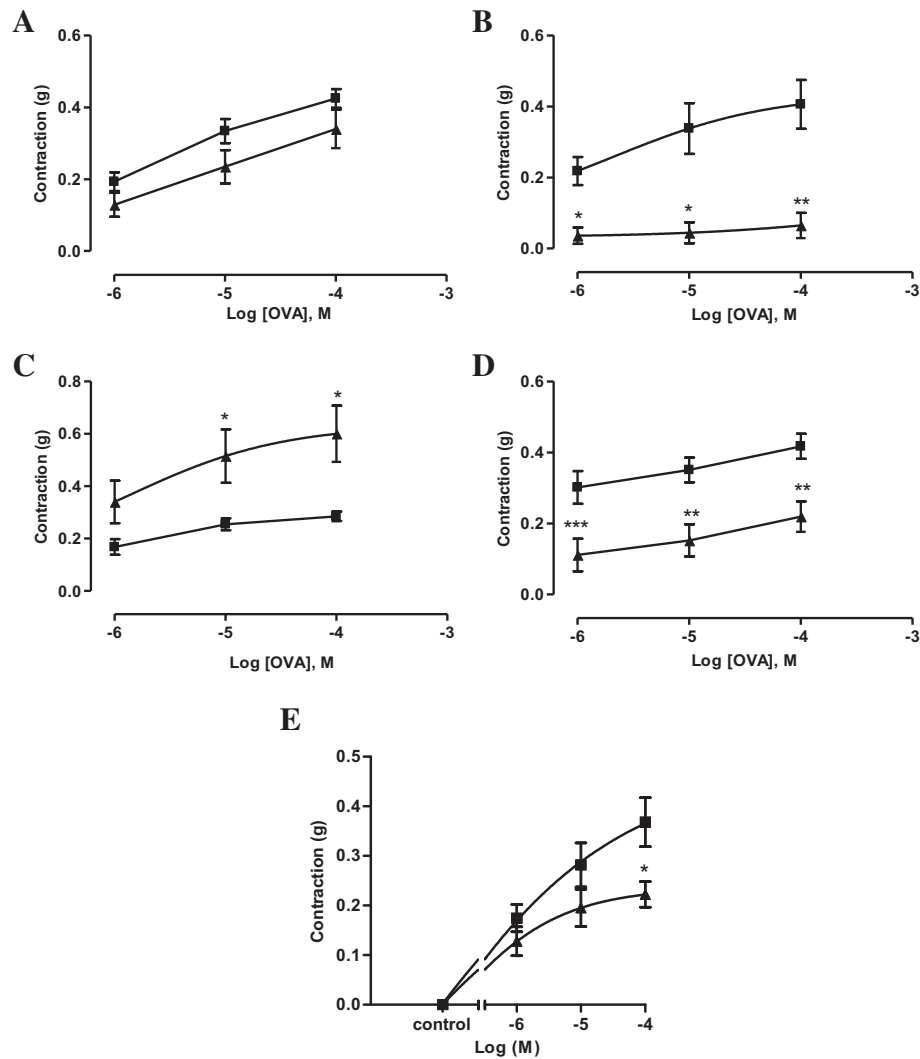


Fig. 3. Cumulative concentration–response curves to OVA of sensitized guinea-pig bronchial rings alone (■) or in the presence of CBD at (A) 100 nM (B) 1 μM (C) 10 μM [(▲) $n = 6$], mepyramine (D) [(▲) 100 nM, $n = 5$] or MK-886 (E) [(▲) 10 μM, $n = 5$]. Each point represents the mean and vertical lines represent sem. * $P \leq 0.05$ and ** $P \leq 0.01$ vs. controls.

contractile effects of the endocannabinoids, but was unable to inhibit the contraction induced by the TRPV₁ agonist capsaicin or the NK₂ agonist NKA or the directly acting spasmogens, carbachol and histamine. These results, coupled with the fact that CBD had no direct effect on airway smooth muscle tone, suggest that the ability

of CBD to influence antigen-induced contractile responses *in vitro* and *in vivo* is via mechanism(s) unrelated to a direct effect on airway smooth muscle.

While most of the pharmacological effects of Δ^9 -THC appear to be mediated via cannabinoid CB₁ and CB₂ receptors, some of CBD's

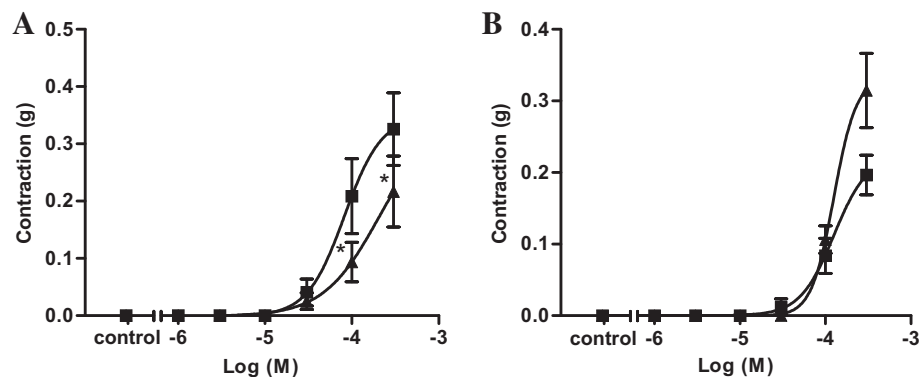


Fig. 4. Cumulative concentration–response curves to compound 48/80 of non-sensitized guinea-pig bronchial rings alone (■) or in the presence of CBD at (A) 1 μM and (B) 10 μM [(▲) $n = 4$]. Each point represents the mean and vertical lines represent sem.

Table 1
Effects of CBD and its vehicle on the lung function of allergic GPs.

	% Increase in R_L	% Decrease in C_{dyn}	n
Vehicle	35.60 ± 4.72	20.40 ± 4.94	5
CBD	24.40 ± 6.23	20.00 ± 3.56	5

Lung function was monitored for 20 min and the change was calculated as a % increase in R_L above baseline and as a % decrease in C_{dyn} below baseline (the individual animal's own baseline value), respectively. CBD (1 mg kg⁻¹) or its vehicle (ethanol, tween 80 and saline, 1:1:18) was applied intravenously. The data are expressed as mean ± sem.

actions have been suggested to be mediated via multiple targets including TRP channels or via molecules of the endocannabinoid system such as FAAH [9,14–18]. CBD has been reported to act as a weak agonist ($EC_{50} = 3.5 \pm 0.3 \mu M$) at human TRPV₁ receptors in HEK293 cells over-expressing these receptors [9,14]. However, despite the expression of TRPV₁ receptors in guinea-pig airways [19,20] that mediate capsaicin-induced airway smooth muscle contraction [21–23], capsaicin-induced contractions were not affected by CBD at 1 μM in this study, providing no evidence of any detectable interaction between CBD and TRPV₁ receptors in guinea-pig bronchi. We have also excluded any interaction of CBD with M₃, H₁ or NK₂ receptors in bronchial smooth muscle as carbachol-, histamine- or NKA-induced contractions were not inhibited by CBD in the present experiments. In contrast, we have demonstrated that CBD at 1 μM could significantly inhibit the responses to the endocannabinoids, anandamide and virodhamine. CBD displays low affinity at both CB₂ [8] and CB₁ receptors and CB₁ receptors appear to be involved in the ability of anandamide to influence airways [25]. Furthermore, CB₁ receptors appear to be involved in the presynaptic inhibition of noradrenaline release in the guinea-pig lung [26]. It will be important to assess whether the inhibitory effect of CBD against anandamide/virodhamine-induced contraction is through an action on CB receptors, including whether CBD can act via the recently described 'atypical' cannabinoid receptor, the orphan G protein coupled receptor GPR55 [27].

A possible involvement of the endocannabinoid metabolic enzyme pathway in the inhibitory action of CBD in isolated guinea-pig bronchi was demonstrated because, whilst the non-specific FAAH inhibitor, PMSF failed to potentiate anandamide-induced bronchoconstriction [21–23], in the presence of CBD, PMSF was able to reverse the inhibitory action of CBD (1 μM) against anandamide-induced smooth muscle contraction, although caution must be used in interpreting these experiments with PMSF as it is

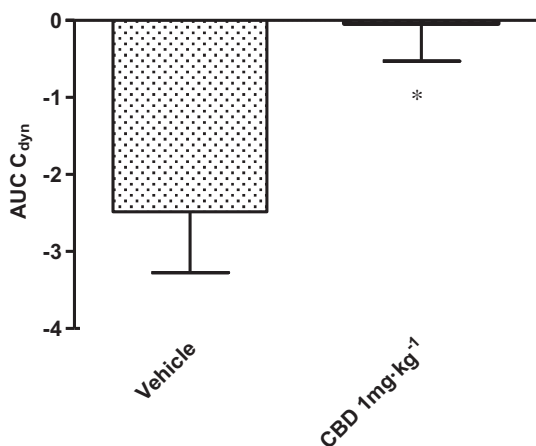


Fig. 5. Bar graph representing the airway obstruction (AUC 0–20 min % decrease in C_{dyn}) to aerosolised OVA (10 mg ml⁻¹; 20 s) in the presence of CBD (1 mg kg⁻¹, intravenously, n = 5) or its vehicle (ethanol, tween80 and saline, 1:1:18, n = 5) in allergic guinea-pigs. Vertical lines represent sem. * $P \leq 0.05$ vs. control.

also an inhibitor of serine proteases. The use of a more selective inhibitor of FAAH would help clarify this activity and clearly it would be of interest to see whether this drug also inhibits OVA-induced contraction as Massi et al. [24] proposed FAAH mediation of the anti-tumour activity of CBD in mice.

We have however demonstrated that antigen-induced contractions are partially mediated by both histamine and leukotrienes confirming other studies in guinea-pig and man [11,28–32]. The H₁ antagonist mepyramine and the 5-lipoxygenase inhibitor MK886 significantly inhibited the OVA-induced contraction, validating our assay. It was of interest that CBD (but only at 1 μM) substantially and significantly inhibited ovalbumin-induced contraction of airway smooth muscle *in vitro*. This is, as far as we are aware, the first demonstration that a cannabinoid can alter antigen-induced responses of airway tissue. In fact, there is only one other report of a cannabinoid acting in this regard which shows that Δ^9 -THC and nabilone had no effect in a guinea-pig model of asthma [12]. However, in contrast to these observations with 1 μM CBD, a higher concentration of CBD (10 μM) actually potentiated OVA-induced contractions, although only when the data were presented as absolute g of contractions. Due to the large variability of OVA-induced responses between experimental data sets, it was found more appropriate to standardise the data by expressing them as a % of the mean maximal contraction to carbachol (10 μM). In an attempt to confirm that the anti-allergic activity of CBD may be via an effect on mast cells, we examined the effect of CBD on the non-allergic challenge of isolated bronchi using the non-immunological mast cell secretagogue, compound 48/80. A significant reduction in compound 48/80 (100 μg ml⁻¹)-induced mast cell degranulation was also observed, but only with CBD at 1 μM suggesting that CBD at 1 μM may act to inhibit mast cell degranulation.

Our observations suggest that the effect of CBD on antigen-induced responses is complex and our results are supported by the only report investigating the effect of CBD on mast cell function which showed that higher concentrations of CBD (3–10 μM) activated mast cells, but in a CB receptor independent manner [33]. Thus CBD has the ability to both inhibit and stimulate mast cells, depending on whether low or high concentrations of this cannabinoid are present. Furthermore, we can rule out any effect of 1 μM CBD on OVA-induced contractions being via any direct effects of histamine on airway smooth muscle as CBD did not inhibit histamine-induced contraction of isolated guinea-pig bronchi.

In this study we also used the model of OVA-induced airway obstruction in passively sensitized guinea-pigs [13] to discern the effects of CBD on allergen-induced airways obstruction *in vivo*. The 1 mg kg⁻¹ dose of CBD significantly reduced OVA-induced airway obstruction as detected by an inhibition of the reduction in C_{dyn} . This bronchoprotective effect of CBD against allergen-induced bronchoconstriction supports other work developing a bronchoprotective effect of the synthetic cannabinoid receptor agonists, WIN55212-2 and JWH133 against neurogenic airway inflammation in the guinea-pig [34,35] and is consistent with our *in vitro* observations that CBD can influence OVA-induced contraction of isolated guinea-pig bronchi.

Overall, our data provide evidence that CBD can inhibit allergen-induced contraction of airway smooth muscle, as well as reduce antigen-induced airway obstruction *in vivo*. The observed anti-allergic activity of CBD may be partly related to an effect of CBD on mast cell degranulation, although this needs to be further investigated. Our results have implications for the development of cannabinoid-based treatments for allergic airways disease.

Conflict of interest

The authors state no conflict of interest.

Acknowledgements

We thank Dr. Cliff Whelan for his valuable help and support given during the PhD study of the first author at the University of Hertfordshire.

References

- [1] Shapiro BJ, Tashkin MD, Vachon L. Tetrahydrocannabinol as a bronchodilator. Why bother? *Chest* 1977;71:558–9.
- [2] Tashkin DP, Reiss S, Shapiro BJ, Calvarese B, Olsen JL, Lodge JW. Bronchial effects of aerosolized Δ^9 -tetrahydrocannabinol in healthy and asthmatic subjects. *Am Rev Respir Dis* 1977;115:57–65.
- [3] Hartley JP, Nogrady SG, Seaton A. Bronchodilator effect of Δ^1 -tetrahydrocannabinol. *Br J Clin Pharm* 1978;5:523–5.
- [4] Graham JDP. The bronchodilator action of cannabinoids. In: Mechoulam A, editor. *Cannabinoids as therapeutic agents*. Boca Raton: FL: CRC Press; 1986. p. 147–58.
- [5] Jan TR, Farraj AK, Harkema JR, Kaminski NE. Attenuation of the ovalbumin-induced allergic airway response by cannabinoid treatment in A/J mice. *Toxicol Appl Pharmacol* 2003;188:24–35.
- [6] Costa B. On the pharmacological properties of Δ^9 -tetrahydrocannabinol (THC). *Chem Biodivers* 2007;4:1664–77.
- [7] Braun A, Engel T, Aguilar-Pimentel JA, Zimmer A, Jakob T, Behrendt H, et al. Beneficial effects of cannabinoids (CB) in a murine model of allergen-induced airway inflammation: role of CB1/CB2 receptors. *Immunobiol* 2011;216:466–76.
- [8] Mechoulam R, Peters M, Murillo-Rodriguez E, Hanuš L. Cannabidiol-recent advances. *Chem Biodivers* 2007;4:1678–92.
- [9] De Petrocellis L, Ligresti A, Schiano-Moriello A, Allara M, Bisogno T, Petrosino S, et al. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *Br J Pharmacol* 2011;163:1479–94.
- [10] Jan TR, Su ST, Wu HY, Liao MH. Suppressive effects of cannabidiol on antigen-specific antibody production and functional activity of splenocytes in ovalbumin-sensitized BALB/c mice. *Int Immunopharmacol* 2007;7:773–80.
- [11] Muccitelli RM, Tucker SS, Hay DW, Torphy TJ, Wasserman MA. Is the guinea-pig trachea a good in vitro model of human large and central airways? Comparison on leukotriene-, methacholine-, histamine- and antigen-induced contractions. *J Pharmacol Exp Ther* 1987;243:467–73.
- [12] Orzelek-O'Neil RM, Goodman FR, Forney RB. The effects of Δ^9 -tetrahydrocannabinol and nabilone on the isolated guinea-pig bronchus. *Toxicol Appl Pharmacol* 1980;54:493–550.
- [13] Keir S, Boswell-Smith V, Spina D, Page CP. Mechanism of adenosine-induced airways obstruction in allergic guinea-pigs. *Br J Pharmacol* 2006;147:720–8.
- [14] Bisogno T, Hanuš L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol* 2001;134:845–52.
- [15] Costa B, Giagnoni G, Franke C, Trovato AE, Colleoni M. Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the non-psychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. *Br J Pharmacol* 2004;143:247–50.
- [16] Costa B, Trovato AE, Comelli F, Giagnoni G, Colleoni M. The non-psychoactive agent cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. *Eur J Pharmacol* 2007;556:75–83.
- [17] De Petrocellis L, Vellani V, Schiano-Moriello A, Marini P, Magherini P, Orlando P, et al. Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastin type-8. *J Pharmacol Exp Ther* 2008;325:1007–15.
- [18] Qin N, Neeper MP, Liu Y, Hutchinson TL, Lubin ML, Flores CM. TRPV₂ is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. *J Neurosci* 2008;28:6231–8.
- [19] Kagaya M, Lamb J, Robbins J, Page CP, Spina D. Characterization of the anandamide induced depolarization of guinea-pig isolated vagus nerve. *Br J Pharmacol* 2002;137:39–48.
- [20] Watanabe N, Horie S, Michael GJ, Spina D, Page CP, Priestley JV. Immunohistochemical localization of vanilloid receptor subtype 1 (TRPV₁) in the guinea-pig respiratory system. *Pulm Pharmacol Therap* 2005;18:187–97.
- [21] Craib SJ, Ellington HC, Pertwee RG, Ross RA. A possible role of lipoxygenase in the activation of vanilloid receptors by anandamide in the guinea-pig bronchus. *Br J Pharmacol* 2001;134:30–7.
- [22] Tucker RC, Kagaya M, Page CP, Spina D. The endogenous cannabinoid agonist, anandamide stimulates sensory nerves in guinea-pig airways. *Br J Pharmacol* 2001;132:1127–35.
- [23] Andersson DA, Adner M, Högestätt ED, Zygmunt PM. Mechanism underlying tissue selectivity of anandamide and other vanilloid receptor agonists. *Mol Pharmacol* 2002;62:705–13.
- [24] Massi P, Valenti M, Vaccani A, Gasperi V, Perletti G, Marras E, et al. 5-Lipoxygenase and anandamide hydrolase (FAAH) mediate the antitumor activity of cannabidiol, a non-psychoactive cannabinoid. *J Neurochem* 2008;104:1091–100.
- [25] Calignano A, Katona I, Desarnaud F, Giuffrida A, La Rana G, Mackie K, et al. Bidirectional control of airway responsiveness by endogenous cannabinoids. *Nature* 2000;408:96–101.
- [26] Vizi ES, Katona I, Freund TF. Evidence for presynaptic cannabinoid CB₁ receptor-mediated inhibition of noradrenaline release in the guinea-pig lung. *Eur J Pharmacol* 2001;431:237–44.
- [27] Sharif H, Abood ME. Pharmacological characterization of GPR55, a putative cannabinoid receptor. *Pharmacol Ther* 2010;126:301–13.
- [28] Dahlén SE, Hansson G, Hedqvist P, Björck T, Granström E, Dahlén B. Allergen challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with the release of leukotrienes C₄, D₄ and E₄. *Proc Natl Acad Sci* 1983;80:1712–6.
- [29] Lindström EG, Grundström N, Hammarström S, Andersson RG. Relationship between histamine, lipoxygenase and cyclooxygenase products in antigen-induced contraction in guinea-pig tracheal tube preparation. *Pharmacol Toxicol* 1992;71:216–20.
- [30] Björck T, Dahlén SE. Leukotrienes and histamine mediate IgE-dependent contractions of human bronchi: pharmacological evidence obtained with tissues from asthmatic and non-asthmatic subjects. *Pulm Pharmacol* 1993;6:87–96.
- [31] Roquet A, Dahlén B, Kumlin M, Ihre E, Anström G, Binks S, et al. Combined antagonism of leukotrienes and histamine produces predominant inhibition of allergen-induced early and late phase airway obstruction in asthmatics. *Am J Respir Crit Care Med* 1997;155:1856–63.
- [32] Chen G, Zhou D, Kim JT, Wu Z, Lee LY, Kang BC. Contractile responses to allergen and cholinergic stimulation in bronchus and trachea of cockroach allergen-sensitized guinea-pigs. *Int Arch Allergy Immunol* 1998;116:132–9.
- [33] Del Giudice E, Rinaldi L, Passarotto M, Facchinetti F, D'Arrigo A, Guiotto A, et al. Cannabidiol, unlike synthetic cannabinoids, triggers activation of RBL-2H3 mast cells. *J Leu Biol* 2008;81:1–11.
- [34] Cui YY, D'Agostino B, Risse PA, Marrocco G, Naline E, Zhang Y, et al. Cannabinoid CB₂ receptor activation prevents bronchoconstriction and airway oedema in a model of gastro-oesophageal reflux. *Eur J Pharmacol* 2007;573:206–13.
- [35] Fukuda H, Abe T, Yoshihara S. The cannabinoid receptor agonist WIN55,212-2 inhibits antigen-induced plasma extravasation in guinea-pig airways. *Int Arch Allergy Immunol* 2010;152:295–300.