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

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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.

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, cannabinol, 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 CB_1/CB_2 cannabinoid receptor activation [7].

It is now known that the non-psychotropic 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.

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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% O_2 and 5% CO_2. 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 μM). 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 μg) 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 μM) 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 μM), histamine (100 nM-100 μM) or neurokinin A (NKA) (0.1 nM–10 μM) alone or in the presence of 1 μM CBD (the incubation time was 20 min). At the end of the experiment, the responsiveness to carbachol (10 μM) 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 μM–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 μM and 10 μM; 20 min), mepyramine (100 nM) and the 5-lipoxygenase inhibitor, MK886 (10 μM) on the contractile response induced by OVA were also assessed. At the end of these experiments, the responsiveness to carbachol (10 μM) 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 μg 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 μM and 10 μM), (100 nM) was assessed. At the end of the experiments, the responsiveness to carbachol (10 μM) 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 H_2O: 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 H_2O; 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 H_2O·$litre^{-1}$ s^{-1}) and dynamic lung compliance (C_{dyn}; cm H_2O·$litre^{-1}$ 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