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ScienceDirect
European Journal of Pharmacology 573 (2007) 206–213
www.elsevier.com/locate/ejphar

Cannabinoid CB_2 receptor activation prevents bronchoconstriction and airway oedema in a model of gastro-oesophageal reflux

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Received 7 April 2006; received in revised form 1 June 2007; accepted 12 June 2007
Available online 3 July 2007

Abstract

Cannabinoids have been shown to inhibit sensory nerve activation in guinea-pigs and humans. Their effects are mediated by specific activation of two types of receptors, named CB_1 and CB_2. The purpose of this study was to investigate the effects of WIN 55,212-2, (*R*)-(+)-[2,3-dihydro-5methyl-3-[(4-morpholino)methyl]pyrrolo-[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphthyl)methanone, a non selective agonist of cannabinoid receptors, and JWH 133, (6a*R*,10a*R*)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[b,d]pyran a selective cannabinoid CB_2 receptor agonist, on the sensory nerve component of intraoesophageal (i.oe.) HCl-induced airway microvascular leakage and bronchoconstriction in guinea-pigs. We also tested the effect of WIN 55,212-2 on substance P-induced plasma extravasation and bronchoconstriction. Airway microvascular leakage and bronchoconstriction induced by i.oe. HCl was inhibited by the cannabinoid CB_1/CB_2 agonist WIN 55,212-2 (0.3–3 mg/kg i.p.) in a dose-dependent manner (maximal inhibition at the dose of 3 mg kg^{-1}, $P<0.01$). The effect of WIN 55,212-2 was inhibited by a cannabinoid CB_2 receptor antagonist SR 144528, [*N*-[(1S)-endo-1,3,3-trimethylbicyclo[2,2,1] heptan-2yl]-5-(-4-chloro-3-methylphenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide], but not by a CB_1 receptor antagonist, SR 141716, [*N*-(piperidin-1yl)-5-(-4-chlorophenyl)-1-(2,4dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride]. The cannabinoid CB_2 agonist JWH 133 (0.3–3 mg/kg i.p.) mimicked the inhibitory effect of WIN 55,212-2 on HCl-induced microvascular leakage. Under similar conditions, WIN 55,212-2 (1 mg kg^{-1} i.p.) was unable to counteract the airway microvascular leakage and bronchoconstriction induced by substance P. These results suggest that inhibition by WIN 55,212-2 of airway plasma extravasation and bronchoconstriction induced by i.oe. HCl instillation in guinea-pigs is mediated through cannabinoid CB_2 receptor activation.

Keywords: Cannabinoids; Microvascular leakage; Bronchoconstriction; (Guinea-pig)

1. Introduction

Cannabinoid receptor agonists are a class of compounds that produce a wide array of biological effects of potential therapeutic interest, such as analgesia, neuroprotection, appetite control, immunosuppression, inhibition of inflammation and antineoplasic effects (Porter and Felder, 2001; Ravinet-Trillou et al., 2003). Their biological effects are mainly mediated through two types of receptors named CB_1 and CB_2 (Howlett et al., 2002). The cannabinoid CB_1 receptor subtype was initially characterised in the central nervous system (Devane et al., 1998; Matsuda et al., 1993) and designated as the central cannabinoid receptor. However cannabinoid CB_1 receptors are also expressed in peripheral tissues (Croci et al., 1998; Ralevic, 2003). In contrast, cannabinoid CB_2 receptors have been found

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0014-2999/$ - see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2007.06.040

predominantly in peripheral tissues and especially in tissues involved in immune functions (Munro et al., 1993; Berdyshev et al., 1997; Berdyshev, 2000; Germain et al., 2002; Howlett et al., 2002; Walter and Stella, 2004). Cannabinoid CB_2 receptors are also involved in sensory nerve transmission and their activation attenuates nociceptive responses (Elmes et al., 2004). On airway functions, cannabinoids have been shown to suppress, *in vivo*, the cough reflex induced in the guinea-pig by inhalation of citric acid or capsaicin (Calignano et al., 2000; Patel et al., 2003,) and the bronchospasm evoked by the latter (Calignano et al., 2000). *In vitro*, they also inhibit constriction of the guinea-pig bronchial smooth muscle evoked by electrical field stimulation (Yoshihara et al., 2004) or the hyperresponsiveness induced by Nerve Growth Factor (NGF) (de Vries et al., 2001). Finally, cannabinoids inhibit capsaicin evoked sensory nerve depolarisation in the human or guinea-pig isolated vagus nerve (Patel et al., 2003). All these protective effects appear to involve a down-regulation of sensory nerve activation (Calignano et al., 2000; de Vries et al., 2001; Patel et al., 2003; Yoshihara et al., 2004).

Gastroesophageal reflux is a common clinical disorder associated with a variety of respiratory symptoms, including bronchoconstriction and chronic cough. It has been shown to be more frequent in asthmatic patients (Harding and Sontag, 2000; Leggett et al., 2005). Experimental studies have shown that oesophageal hydrochloric acid (HCl) perfusion induces a slight bronchoconstriction in dogs (Mansfield et al., 1981), cats (Tuchman et al., 1984), and rabbits (Gallelli et al., 2003) as well as in asthmatic patients (Hervé et al., 1986). Oesophageal HCl perfusion also induces airway microvascular leakage in guinea-pigs (Hamamoto et al., 1997; Daoui et al., 2002; Advenier et al., 2002). The mechanism by which gastroesophageal reflux can enhance asthmatic symptoms remains unclear (Ricciardolo, 2001). A reflex theory involving both the sensory and vagus nerves has been suggested (Hervé et al., 1986; Hamamoto et al., 1997; Ricciardolo, 2001; Daoui et al., 2002). Sensory nerves and tachykinins are involved in airway effects of HCl infusion as demonstrated by the inhibitory effects of pretreatments with capsaicin to cause depletion of tachykinins (Hamamoto et al., 1997; Daoui et al., 2002; Gallelli et al., 2003), with neurokinin receptor antagonists (Hamamoto et al., 1997, Daoui et al., 2002, Gallelli et al., 2003) or with nociceptin (Rouget et al., 2004; D'Agostino et al., 2005).

The purpose of the present study was to assess the inhibitory effect of cannabinoid receptor agonists on the sensory nerve pathways involved in bronchoconstriction and airway microvascular leakage induced by intraoesophageal HCl instillation in the guinea-pig. We used the cannabinoid CB_1/CB_2 receptor agonist WIN 55,212-2 (Howlett et al., 2002), the cannabinoid CB_2 receptor selective agonist JWH 133 (Huffman et al., 1999), the cannabinoid CB_1 receptor antagonist SR 141716 (Rinaldi-Carmona et al., 1994) and the cannabinoid CB_2 receptor antagonist SR 144528 (Rinaldi-Carmona et al., 1998) as pharmacological tools to characterise the cannabinoid receptor subtype involved in the response and to evaluate the potential therapeutic interest of this class of compounds.

2. Materials and methods

2.1. Animal preparation

The study was approved by the University Animal Ethics Committee. Male Hartley guinea-pigs weighing 250–350 g were used throughout this study. The animals were housed in the animal unit for at least 24 h before experimentation and given free access to food and water. On the day of experimentation, guinea-pigs were anaesthetised intraperitoneally with urethane (1.75 g/kg) and then a jugular vein was cannulated for drug injection. Forty-five minutes before HCl exposition, the animals were pre-treated with atropine and propranolol (both at 1 mg/kg i.p.) to block muscarinic and β-adrenergic receptors, and phosphoramidon (1 mg/kg i.p.) to inhibit tachykinin metabolism. The oesophageal wall was partly sectioned at the level of the 3rd–5th tracheal cartilage ring and a catheter was placed in the mid-oesophagus. The oesophagus was ligated at the upper portion to avoid HCl leakage. The lower end of the oesophagus was then exposed from the abdomen and ligated to block communication between the oesophagus and the stomach. Thus, there was no leakage of fluid from the oesophagus.

2.2. Measurement of airway microvascular leakage

The vascular permeability was quantified by the extravasation of Evans blue dye (Rogers et al., 1988, Rouget et al., 2004). Evans blue dye (30 mg/kg) was injected into the jugular vein, followed 1-min later by intraoesophageal HCl (1 N, 0.45 ml) or saline (0.9%) infusion. Ten minutes after completion of the intraoesophageal HCl infusion, the lungs were removed and the connective tissues, vasculature and parenchyma were gently scraped away. The airways were divided into two components, trachea and main bronchi (Rogers et al., 1988). Tissues were blotted dry, weighed and their dye content was extracted in formamide at 37 °C for 18 h. Dye concentration was quantified by light absorbance at 620 nm (DCP spectrophotometer; Vital, Dieren, Netherlands), and tissue content (ng dye/mg wet weight tissue) was calculated from a standard curve of dye concentrations.

2.3. Measurement of lung function

Lung function was measured in spontaneous breathing guinea-pigs anaesthetised using the same protocol as for the assessment of microvascular leakage. A cannula was then inserted in the trachea and connected to a pneumotachograph with a pressure transducer (±2 cm H_2O, model MP-45-14-871, Validyne Engineering).

The flow signal was integrated to give a measure of tidal volume. An intrathoracic cannula was inserted between the third and fifth intercostal spaces and connected to the negative side of the pressure transducer (±20 cm H_2O, Validyne Engineering). The positive side of the transducer was connected to the side of the pneumotachograph proximal to the animal. The difference between mouth (trachea) and thoracic pressure was used as a measure of transpulmonary pressure (D'Agostino et al., 2005).