

The Cannabinoid Receptor Agonist WIN 55,212-2 Inhibits Antigen-Induced Plasma Extravasation in Guinea Pig Airways

Hironobu Fukuda Toshio Abe Shigemi Yoshihara

Department of Pediatrics, Dokkyo Medical University, Tochigi, Japan

Key Words

Cannabinoid · WIN 55,212-2 · Plasma extravasation · Antigen challenge · C-fibers · Airway · Guinea pig

Abstract

Background: Although neurogenic inflammation of the airways via activation of C-fibers is thought to be important in the pathogenesis of asthma, the mechanisms regulating C-fiber activity remain uncertain. **Objective:** The influence of a cannabinoid receptor agonist, WIN 55,212-2, on C-fiber activation in guinea pig airways was investigated, as was the mechanism by which cannabinoids regulate antigen-induced airway inflammation. **Methods:** The inhibitory effect of WIN 55,212-2 on antigen-induced plasma extravasation was assessed in guinea pig tracheal tissues by photometric measurement of extravasated Evans blue dye after extraction with formamide. **Results:** Pretreatment with WIN 55,212-2 (0.001, 0.01 or 0.1 mg/kg) significantly and dose-dependently reduced tracheal plasma extravasation induced by inhaling a 5% ovalbumin solution for 2 min after pretreatment with a neutral endopeptidase inhibitor (phosphoramidon at 2.5 mg/kg i.v.). A cannabinoid CB2 receptor antagonist (SR144528) blunted the inhibitory effect of WIN 55,212-2, while a cannabinoid CB1 antagonist (SR141716A) did not. Pretreatment with a neurokinin-1 receptor antago-

nist (FK888) significantly reduced ovalbumin-induced extravasation of Evans blue dye. Pretreatment with the combination of WIN 55,212-2 and FK888 reduced antigen-induced plasma extravasation more markedly than FK888 alone. **Conclusions:** These findings suggest that WIN 55,212-2 inhibits C-fiber activation via the cannabinoid CB2 receptor and thus suppresses antigen-induced inflammation in guinea pig airways.

Copyright © 2010 S. Karger AG, Basel

Introduction

Several previous studies have shown that neurogenic inflammation of the airways plays an important role in the pathogenesis of asthma [1, 2]. In studies using tachykinin antagonists, Murai et al. [3, 4] have directly demonstrated that airway inflammation is generated by tachykinins released from capsaicin-sensitive afferent sensory nerve fibers (C-fibers), which are stimulated by various irritants such as cigarette smoke [5], antigens [6, 7], cold air [7, 8], hypertonic saline [9] and hydrochloric acid [10]. These irritants activate C-fibers by opening nonselective cation channels with a high Ca^{2+} permeability [11]. Morimoto et al. [12] showed that ω -conotoxin GVIA blocks the activation of C-fibers and the release of

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2010 S. Karger AG, Basel
1018–2438/10/1523–0295\$26.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Shigemi Yoshihara
Department of Pediatrics, Dokkyo Medical University
880 Kitakobayashi, Mibu-machi
Tochigi-ken 321-0293 (Japan)
Tel +81 282 86 1111, Fax +81 282 86 7521, E-Mail h-fukuda@dokkyomed.ac.jp

substance P from their endings, and they suggested that opening of N-type voltage-dependent Ca^{2+} channels might activate C-fibers in guinea pig airway tissues.

Cannabinoids are psychoactive compounds with a wide array of effects, including hypothermia, depressed motor activity, hypotension, inhibition of intestinal motility and antinociception [13]. The biological effects of cannabinoids are mediated by two specific cell surface receptors (CB1 and CB2) in the central nervous system and peripheral tissues [14–16]. Over the last 10 years, much evidence has been accumulated favoring the concept that cannabinoids are endogenous modulators of neuronal activity in several tissues [17, 18]. Cannabinoid receptor agonists have been shown to inhibit the activation of Ca^{2+} channels [19, 20].

In our previous studies, we have demonstrated that a synthetic cannabinoid receptor agonist (WIN 55,212-2) inhibits the activation of C-fibers and the release of neuropeptides from their endings via the opening of maxi- K^+ channels by activation of CB2 receptors in vitro [21] and in vivo [22–24]. Immunohistochemistry has shown that another cannabinoid receptor agonist (CP55, 940) prevents antigen-induced inflammation by the activation of inflammatory cells, such as mast cells and eosinophils, in guinea pig lung tissues [25]. Therefore, WIN 55,212-2 may be useful for the treatment of airway inflammatory diseases such as asthma. In the present study, we investigated whether WIN 55,212-2 could inhibit antigen-induced airway inflammation in guinea pigs.

Materials and Methods

Experimental Animals

Male Hartley guinea pigs (SLC, Shizuoka, Japan) weighing 300–500 g at the time of purchase were used in this study. They were housed in a temperature- and humidity-controlled environment with free access to standard laboratory food and water.

Sensitization

Animals were sensitized according to a previously reported protocol [26–28] that involved the injection of 70 mg of ovalbumin (grade V) in 1.5 ml of 0.9% NaCl intraperitoneally twice, with a 1-week interval between injections. The animals were studied 2 weeks after the second injection.

Experimental Design

On the day of the experiment, the guinea pigs (300–500 g) were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). Then a midline cervical incision was made to expose the larynx and upper trachea. The trachea was incised immediately below the larynx, and a cannula was inserted into the trachea to a depth of 4 mm. Each animal was ventilated at a fre-

quency of 70 breaths/min with a tidal volume of 6 ml [29] using a constant volume ventilator (Rodent Ventilator Model 683; Harvard Apparatus, Millis, Mass., USA). The neutral endopeptidase inhibitor phosphoramidon (2.5 mg/kg i.v.) dissolved in 0.9% NaCl [30] was injected into the jugular vein over a 10-second period at 5 min before the inhalation of ovalbumin. Evans blue dye (a 3% solution in 0.9% NaCl) was used to measure the plasma extravasation. Immediately after injection of the dye (30 mg/kg i.v. over 5 s) into the jugular vein, ovalbumin was delivered to the animals for 2 min as a 5% aerosol via the tracheal cannula using an ultrasonic nebulizer (Pulmo-sonic model 25; DeVilbiss Co., Somerset, Pa., USA; aerosol rate: 0.2 ml/min). The vehicle for ovalbumin was 0.9% NaCl. WIN 55,212-2 (0.001, 0.01 or 0.1 mg/kg i.v.), the neurokinin-1 (NK-1) receptor antagonist FK888 (1.0 mg/kg i.v.) or the vehicle was injected at 5 min before exposure to ovalbumin. A combination of WIN 55,212-2 and a cannabinoid receptor antagonist (SR141716A at 0.1 mg/kg i.v. or SR144528 at 0.1 mg/kg i.v.), FK888 (1.0 mg/kg i.v.) or vehicle was also given 10 min before exposure. WIN 55,212-2, SR141716A, SR144528 and FK888 were dissolved in dimethyl sulfoxide (DMSO). The chest was opened at 10 min after injection of the dye, a cannula was inserted into the ascending aorta through the left ventricle and the animal was perfused for 3 min with 10 mM phosphate buffer (pH 5) at a pressure of 120 mm Hg. The trachea was then removed, opened longitudinally along the ventral midline, blotted on absorbent paper and weighed. Tracheal tissue was incubated in 3 ml of formamide at 50°C for 18 h to extract the extravasated Evans blue dye [31]. Results were compared with those obtained from animals without WIN 55,212-2 treatment.

Measurement of Plasma Extravasation

Extravasation of the dye-labeled macromolecules was assessed by measuring the optical density of the formamide extracts at a wavelength of 620 nm with a spectrophotometer (model 220A; Hitachi, Japan). The amount of dye was expressed as nanogram per milligram of tissue wet weight, and was derived from a standard curve of Evans blue dye concentrations.

Drugs

(R)-(+)-[2,3-dihydro-5-methyl-3-[(4-merpholino)methyl]pyrrolo-[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphthyl)methanone (WIN 55,212-2), ovalbumin, Evans blue dye and DMSO were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride] (SR141716A) and {N-[(1S)-endo-1,3,3-trimethylbicyclo[2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide} (SR144528) were kindly supplied by Sanofi-Synthelabo Recherche (Montpellier, France). (N²-[(4R)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-prolyl]-N-methyl-N-phenylmethyl-3-(2-naphthyl)-L-alaninamide) (FK888) was kindly supplied by Astellas Pharma Inc. (Tokyo, Japan).

Statistical Analysis

Results are expressed as the mean $\pm$ SEM. Statistical analysis was performed by using the Tukey-Kramer multiple comparison test. In all analyses, a p value of less than 0.05 was considered significant.

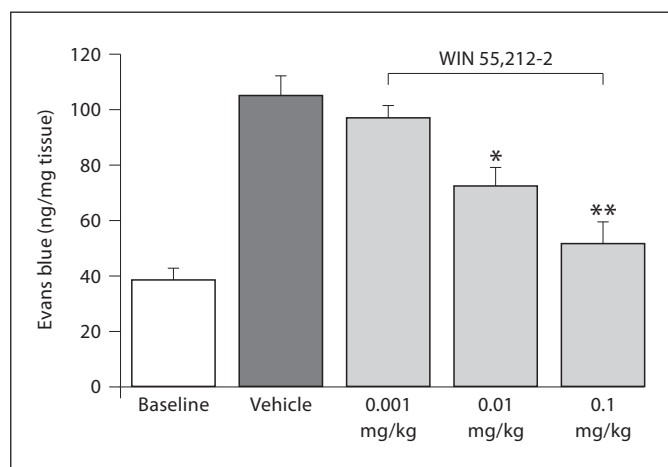


Fig. 1. Effects of WIN 55,212-2 (0.001, 0.01 or 0.1 mg/kg) on Evans blue dye extravasation into guinea pig tracheal tissues induced by inhalation of ovalbumin (5% solution for 2 min) in the presence of phosphoramidon (2.5 mg/kg i.v. at 5 min before exposure). The plasma extravasation was evaluated by measuring the amount of Evans blue dye in the tracheal tissues after 10 min. Values are the mean $\pm$ SEM (n = 5 per group). * p < 0.05 vs. vehicle; ** p < 0.01 vs. vehicle.

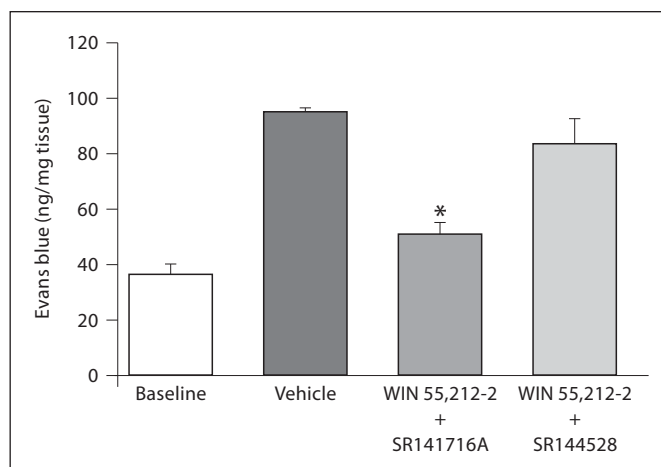


Fig. 2. Effects of the combination of WIN 55,212-2 (0.1 mg/kg) and a cannabinoid receptor antagonist (SR141716A or SR144528; 0.1 mg/kg i.v.) on Evans blue dye extravasation into guinea pig tracheal tissues induced by inhalation of ovalbumin (5% solution for 2 min) in the presence of phosphoramidon (2.5 mg/kg i.v. at 5 min before exposure). The plasma extravasation was evaluated by measuring the amount of Evans blue dye in the tracheal tissues after 10 min. Values are the mean $\pm$ SEM (n = 5 per group). * p < 0.05 vs. vehicle.

Results

Effect of WIN 55,212-2 on Plasma Extravasation

The extravasation of Evans blue dye into the tracheal tissues of guinea pigs after exposure to the aerosolized vehicle (0.9% NaCl) for ovalbumin in the presence of phosphoramidon (2.5 mg/kg i.v.) amounted to 38.1 ± 4.4 ng/mg (n = 5; fig. 1). Injection of the vehicle for WIN 55,212-2 (DMSO) at 10 min before inhalation of 5% ovalbumin for 2 min in the presence of phosphoramidon (2.5 mg/kg i.v.) significantly increased the extravasation of Evans blue dye into the tracheal tissues (105.1 ± 7.2 ng/mg, n = 5; fig. 1). Pretreatment with WIN 55,212-2 (0.001, 0.01 or 0.1 mg/kg) at 10 min before aerosol ovalbumin challenge led to a significant and dose-dependent decrease in ovalbumin-induced plasma extravasation of dye into the tracheal tissue (97.0 ± 4.4 ng/mg and 72.0 ± 7.2 ng/mg p < 0.05 vs. vehicle; 51.4 ± 8.0 ng/mg p < 0.01 vs. vehicle, n = 5 each; fig. 1).

Effect of WIN 55,212-2 and a Cannabinoid Receptor Antagonist (SR141716A or SR144528) on Plasma Extravasation

The extravasation of Evans blue dye into the tracheal tissues of guinea pigs after exposure to the aerosolized

vehicle (0.9% NaCl) for ovalbumin in the presence of phosphoramidon (2.5 mg/kg i.v.) amounted to 36.8 ± 3.8 ng/mg (n = 5; fig. 2). Injection of the vehicle (DMSO) for WIN 55,212-2, SR141716A and SR144528 at 10 min before inhalation of 5% ovalbumin in the presence of phosphoramidon (2.5 mg/kg i.v.) significantly increased the extravasation of Evans blue dye into the tracheal tissues (95.2 ± 1.6 ng/mg, n = 5; fig. 2). The combination of WIN 55,212-2 (0.1 mg/kg i.v.) and SR141716A (0.1 mg/kg i.v.) significantly reduced ovalbumin-induced plasma extravasation (51.1 ± 4.5 ng/mg; 75.5% inhibition of the plasma extravasation after inhalation of the vehicle, p < 0.01 vs. vehicle, n = 5; fig. 2). In contrast, the combination of WIN 55,212-2 (0.1 mg/kg i.v.) and SR144528 (0.1 mg/kg i.v.) did not significantly influence ovalbumin-induced plasma extravasation (83.9 ± 9.0 ng/mg; 19.4% inhibition of the plasma extravasation after inhalation of the vehicle, n = 5; fig. 2).

Effect of WIN 55,212-2 and the NK-1 Receptor Antagonist FK888 on Plasma Extravasation

The extravasation of Evans blue dye into the tracheal tissues of guinea pigs after exposure to the aerosolized vehicle (0.9% NaCl) for ovalbumin in the presence of phosphoramidon (2.5 mg/kg i.v.) amounted to 36.3 ± 3.5

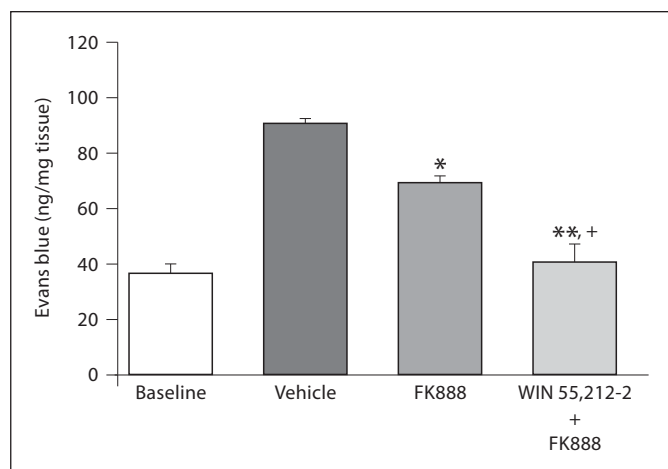


Fig. 3. Effect of the combination of WIN 55,212-2 (0.1 mg/kg) and FK888 (1.0 mg/kg) on Evans blue dye extravasation into guinea pig tracheal tissues induced by inhalation of ovalbumin (5% solution for 2 min) in the presence of phosphoramidon (2.5 mg/kg i.v. at 5 min before exposure). The plasma extravasation was evaluated by measuring the amount of Evans blue dye in the tracheal tissues after 10 min. Values are the mean $\pm$ SEM ($n = 4-5$ per group). * $p < 0.05$ vs. vehicle; ** $p < 0.01$ vs. vehicle; + $p < 0.05$ vs. FK888.

ng/mg ($n = 5$; fig. 3). Injection of the vehicle (DMSO) for WIN 55,212-2 and FK888 at 10 min before inhalation of 5% ovalbumin in the presence of phosphoramidon (2.5 mg/kg i.v.) significantly increased the extravasation of Evans blue (90.7 ± 1.9 ng/mg, $n = 5$; fig. 3). Pretreatment with FK888 (1.0 mg/kg) at 10 min before aerosol challenge reduced ovalbumin-induced plasma extravasation (69.6 ± 2.6 ng/mg; 38.3% inhibition of the plasma extravasation after inhalation of the vehicle, $p < 0.05$, $n = 4$; fig. 3). The combination of WIN 55,212-2 (0.1 mg/kg i.v.) and FK888 (1.0 mg/kg) reduced ovalbumin-induced plasma extravasation further compared with the effect of FK888 alone (40.8 ± 6.6 ng/mg; 91.7% inhibition of the plasma extravasation after inhalation of the vehicle, $p < 0.01$ vs. vehicle and $p < 0.05$ vs. FK888 alone, $n = 5$; fig. 3).

Discussion

Cannabinoids are known to show cannabis-mimetic activity and can block voltage-dependent Ca^{2+} channels [19, 20]. The biological effects of cannabinoids can be exhibited by both endogenous and synthetic cannabinoid receptor agonists. Endogenous cannabinoid receptor ag-

onists (anandamide and palmitoylethanolamide) dose-dependently inhibit electrical field stimulation-induced contraction of guinea pig bronchial smooth muscle [24]. The synthetic cannabinoid receptor agonist WIN 55,212-2 dose-dependently inhibits both electrical field stimulation- and capsaicin-induced contraction of isolated guinea pig bronchi [22]. WIN 55,212-2 also dose-dependently inhibits capsaicin-induced plasma extravasation into the trachea [23]. These reports have suggested that WIN 55,212-2 may be a potential treatment for inflammatory diseases of the airways such as asthma. Antigen-induced plasma extravasation in the pig airway was reported to occur in response to neurogenic inflammation by the release of tachykinins from C-fiber endings [6]. Therefore, we investigated the influence of WIN 55,212-2 on antigen-induced plasma extravasation in the guinea pig trachea. Our results showed that pretreatment with WIN 55,212-2 significantly and dose-dependently reduced the extent of antigen-induced extravasation in sensitized guinea pigs.

In the second part of this study, we investigated the cannabinoid receptor involved in the inhibitory effect of cannabinoid receptor agonists on C-fiber activation. The biological effects of cannabinoids are mediated by two specific receptors, CB1 and CB2, in the central nervous system and peripheral tissues [14–16]. WIN 55,212-2 can bind with and activate both CB1 and CB2 receptors [32]. A specific CB2 cannabinoid receptor agonist, JWH133, was reported to dose-dependently inhibit electrical field stimulation-induced bronchial contraction and capsaicin-induced substance P release, with its effects being reversed by a specific CB2 cannabinoid receptor antagonist (SR144528) but not by a CB1 antagonist (SR141716A) [33]. In our previous study, we found that CB2 agonists inhibited cigarette smoke-induced plasma extravasation into the rat trachea and cigarette smoke-induced substance P release from rat airway tissues by the activation of maxi- K^{+} channels [23]. The present study showed that pretreatment with SR144528 abolished the inhibitory effect of WIN 55,212-2 on the release of neuropeptides in guinea pig airways after antigen challenge. Accordingly, WIN 55,212-2 inhibited antigen-induced airway inflammation by interacting with CB2 cannabinoid receptors.

In the third part of this study, we examined the effect of a combination of WIN 55,212-2 and an NK-1 receptor antagonist (FK888). The tachykinins include substance P, neurokinin A (NKA), neurokinin B (NKB), and their precursors. Substance P and NKA are believed to be present in C-fiber sensory neurons. Immunohistochemically, substance P and NKA are found in vessel walls, glands, near basement membranes and in the epithelium of the

nasal mucosa [34]. Three tachykinin receptor subtypes (NK-1, NK-2 and NK-3) have been reported, and it has been shown that substance P, NKA and NKB are the most potent natural ligands for NK-1, NK-2 and NK-3 receptors, respectively [19]. Edema and vasodilation in the airway mucosa is induced more markedly by substance P than by NKA [35], while NKA is more potent at causing bronchoconstriction [36]. Administration of NK-1 receptors antagonists FK888 [27] and CP-99,994 [37] has been shown to inhibit antigen-induced tracheal plasma extravasation in guinea pigs. In addition, C-fibers have been demonstrated to release tachykinins such as substance P, NKA and calcitonin gene-related peptide (CGRP) in response to capsaicin [38], while an immunohistochemical study showed that CGRP coexists with substance P in guinea pig airways [39, 40]. Furthermore, cannabinoids reduce plasma extravasation by inhibiting the release of substance P and CGRP, which are closely related to edema formation and plasma leakage in a rat model of neuropathic pain [41].

In the present study, pretreatment with the combination of WIN 55,212-2 and FK888 reduced antigen-induced plasma extravasation more markedly than treatment with FK888 alone. These results suggest that WIN 55,212-2 inhibits the release of substance P from C-fibers and that it also inhibits the release of other peptides such as CGRP.

When C-fibers are stimulated, not only substance P but also other neuropeptides (like NKA and CGRP) are

released and exert various effects on the respiratory system [37]. The inflammatory effect of such neuropeptides on the airways may contribute to bronchial hyperreactivity in humans, and we have previously demonstrated that antiasthma drugs (disodium cromoglycate, DSCG, and nedocromil sodium) inhibit antigen-induced plasma extravasation related to the activation of C-fibers in guinea pig airways [42]. Therefore, we compared the inhibitory effects of WIN 55,212-2 and DSCG on plasma extravasation. Pretreatment with DSCG reduced ovalbumin-induced plasma extravasation (90.0 ± 6.2 ng/mg; 80.0% of the vehicle level, $n = 5$). Pretreatment with WIN 55,212-2 caused a further decrease of ovalbumin-induced plasma extravasation compared with the effect of DSCG (44.8 ± 6.7 ng/mg; 39.8% of the vehicle level, $n = 5$). WIN 55,212-2 was more effective at blocking antigen-induced plasma extravasation in guinea pig airways.

In conclusion, the cannabinoid receptor agonist WIN 55,212-2 inhibits the activation of C-fibers and the release of various neuropeptides that might be related to the clinical effects of known antiasthma drugs.

Acknowledgments

This research was supported by Sanofi-Synthelabo Recherche and Astellas Pharma Inc., who kindly provided several drugs. We also thank Dr. Hiroshi Morimoto (Towa Pharmaceutical Co. Ltd.) for his help in preparing this manuscript.

References

- 1 Barnes PJ: Neuroeffector mechanisms: the interface between inflammation and neuronal responses. *J Allergy Clin Immunol* 1996; 98:S73–S83.
- 2 Joos GF, Pauwels RA: Tachykinin receptor antagonists: potential in airways diseases. *Curr Opin Pharmacol* 2001;1:235–241.
- 3 Murai M, Morimoto H, Maeda Y, Fujii T: Effects of the tripeptide substance P antagonist, FR 113680, on airway constriction and airway edema induced by neurokinins in guinea-pigs. *Eur J Pharmacol* 1992;217:23–27.
- 4 Murai M, Morimoto H, Maeda Y, Kiyotoh S, Nishikawa M, Fujii T: Effects of FK 224, a novel compound NK1 and NK2 receptor antagonist, on airway constriction and airway edema induced by neurokinins and sensory nerve stimulation in guinea-pigs. *J Pharmacol Exp Ther* 1992;262:403–408.
- 5 Morimoto H, Yamashita M, Matsuda A, Miyake H, Fujii T: Effects of FR 113680 and FK224, novel tachykinin antagonists, on cigarette smoke-induced rat tracheal plasma extravasation. *Eur J Pharmacol* 1992;224:1–5.
- 6 Bertrand C, Geppetti P, Baker J, Yamawaki I, Nadel JA: Role of neurogenic inflammation in antigen-induced vascular extravasation in guinea pig trachea. *J Immunol* 1993;150: 1497–1485.
- 7 Yoshihara S, Geppetti P, Linden A, Hara M, Chan B, Nadel JA: Tachykinins mediate the potentiation of antigen-induced bronchoconstriction by cold air in guinea pigs. *J Allergy Clin Immunol* 1996;97:756–760.
- 8 Yoshihara S, Chan B, Yamawaki I, Pierangelo G, Ricciardolo FLM, Massion PP, Nadel JA: Plasma extravasation in the rat trachea induced by cold air is mediated by tachykinin release from sensory nerves. *Am J Respir Crit Care Med* 1995;151:1011–1017.
- 9 Ono M, Abe T, Watanabe M, Yamada Y, Yoshihara S: Nedocromil sodium inhibits hypertonic saline-induced plasma extravasation in guinea-pig airways. *Biomed Res* 1999; 20:233–238.
- 10 Cui Y, D'Agostino B, Risse PA, Marrocco G, Naline E, Zhang Y, Chen HZ, Finance O, Rinaldi-Carmona M, Rossi F, Advenier C: Cannabinoid CB2 receptor activation prevents bronchoconstriction and airway oedema in a model of gastro-oesophageal reflux. *Eur J Pharmacol* 2007;573:206–213.
- 11 Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D: The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997;389:816–824.
- 12 Morimoto H, Matsuda A, Ohori M, Fujii T: Effects of ω -conotoxin GVIA on the activation of capsaicin-sensitive afferent sensory nerves in guinea pig airway tissues. *Jpn J Pharmacol* 1996;71:161–166.

- 13 Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG: International Union of Pharmacology. 27. Classification of cannabinoid receptors. *Pharmacol Rev* 2002;54:161–202.
- 14 Munro S, Thomas KL, Abu-Shaar M: Molecular characterization of peripheral receptor for cannabinoids. *Nature* 1993;365:61–65.
- 15 Glass M, Dragunow M, Faull RL: Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* 1997;77:299–318.
- 16 Griffin G, Fernando SR, Ross RA, McKay NG, Ashford ML, Shire D, Huffman JW, Yu S, Lainton JA, Pertwee RG: Evidence for the presence of CB2-like cannabinoid receptors on peripheral nerve terminals. *Eur J Pharmacol* 1997;339:53–61.
- 17 Howlett AC, Johnson MR, Melvin LS, Mine GM: Nonclassical cannabinoid analgesics inhibit adenylate cyclase: development of a cannabinoid receptor model. *Mol Pharmacol* 1988;33:297–302.
- 18 Mackie KY, Westenbroek LR, Mitchell R: Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J Neurosci* 1995;15:6552–6561.
- 19 Caulfield MP, Brown DA: Cannabinoid receptor agonists inhibit Ca current in NG108–15 neuroblastoma cells via a pertussis toxin-sensitive mechanism. *Br J Pharmacol* 1992;106:231–232.
- 20 Mackie K, Hille B: Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci USA* 1992;89:3825–3829.
- 21 Fox AJ, Barnes PJ, Venkatesan P, Belvisi MG: Activation of large conductance potassium channels inhibits the afferent and efferent function of airway sensory nerves in the guinea pig. *J Clin Invest* 1997;99:513–519.
- 22 Yoshihara S, Morimoto H, Yamada Y, Abe T, Arisaka O: Cannabinoid receptor agonists inhibit sensory nerve activation in guinea pig airways. *Am J Respir Crit Care Med* 2004;170:941–946.
- 23 Yoshihara S, Morimoto H, Ohori M, Yamada Y, Abe T, Arisaka O: The cannabinoid receptor agonist WIN 55212-2 inhibits neurogenic inflammations in airway tissues. *J Pharmacol Sci* 2005;98:77–82.
- 24 Yoshihara S, Morimoto H, Ohori M, Yamada Y, Abe T, Arisaka O: Endogenous cannabinoid receptor agonists inhibit neurogenic inflammations in guinea pig airways. *Int Arch Allergy Immunol* 2005;138:80–87.
- 25 Giannini L, Nistri S, Mastroianni R, Cinci L, Vannacci A, Mariottini C, Passani MB, Mannaioni PF, Bani D, Masini E: Activation of cannabinoid receptors prevents antigen-induced asthma-like reaction in guinea pigs. *J Cell Mol Med* 2008;12:2381–2394.
- 26 Dunn CJ, Elliott GA, Oostveen JA, Richards IM: Development of a prolonged eosinophil-rich inflammatory leukocyte infiltration in the guinea pig asthmatic response to ovalbumin inhalation. *Am Rev Respir Dis* 1988;137:541–547.
- 27 Yoshihara S, Riccardolo FL, Geppetti P, Linden A, Hara M, Chan B, Nadel JA: Corticotropin-releasing factor inhibits antigen-induced plasma extravasation in airways. *Eur J Pharmacol* 1995;280:113–118.
- 28 Watanabe M, Yoshihara S, Abe T, Oyama M, Arisaka O: Effects of orally active non-peptide bradykinin B2 receptor antagonist, FR173657, on plasma extravasation in guinea pig airways. *Eur J Pharmacol* 1999;367:373–378.
- 29 Bertrand C, Geppetti P, Baker J, Petersson G, Piedimonte G, Nadel JA: Role of peptidases and NK1 receptors in vascular extravasation induced by bradykinin in rat nasal mucosa. *J Appl Physiol* 1993;74:2456–2461.
- 30 Piedimonte G, Bertrand C, Geppetti P, Snider RM, Desai MC, Nadel JA: A new NK1 receptor antagonist (CP-99,994) prevents the increase in tracheal vascular permeability produced by hypotonic saline. *J Pharmacol Exp Ther* 1993;266:270–273.
- 31 Lundberg JM, Saria A: Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature* 1983;302:251–253.
- 32 Pertwee RG, Ross RA: Cannabinoid receptors and their ligands. *Prostaglandins Leukot Essent Fatty Acids* 2002;66:101–121.
- 33 Huffman JW, Liddle J, Yu S, Aung MM, Abood ME, Wiley JL, Martin BR: 3-(1',1'-Dimethylbutyl)-1-deoxy-D8-THC and related compounds: synthesis of selective ligands for the CB2 receptor. *Bioorg Med Chem* 1999;7:2905–2914.
- 34 Regoli D, Drapeau G, Dion S, D'Orleans-Juste P: Pharmacological receptors for substance P and neurokinins. *Life Sci* 1987;40:109–117.
- 35 Regoli D, Drapeau G, Dion S, Counter R: New selective agonists for neurokinin receptors: pharmacological tools for receptor characterization. *Trends Pharmacol Sci* 1988;9:290–295.
- 36 Joos G, Pauwels R, Van Der Straeten: Effect of inhaled substance P and neurokinin A on the airways of normal and asthmatic subjects. *Thorax* 1987;42:779–783.
- 37 Holzer P: Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience* 1988;24:739–768.
- 38 Stretton D: Non-adrenergic, non-cholinergic neural control of the airways. *Clin Exp Pharmacol Physiol* 1991;18:675–84.
- 39 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 Ther* 2005;18:187–197.
- 40 Watanabe N, Horie S, Michael GJ, Keir S, Spina D, Page CP, Priestley JV: Immunohistochemical co-localization of transient receptor potential vanilloid (TRPV1) and sensory neuropeptides in the guinea-pig respiratory system. *Neuroscience* 2006;141:1533–1543.
- 41 La Rana G, Russo R, D'Agostino G, Sasso O, Raso GM, Iacono A, Meli R, Piomelli D, Calignano A: AM404, an anandamide transport inhibitor, reduces plasma extravasation in a model of neuropathic pain in rat: role for cannabinoid receptors. *Neuropharmacology* 2008;54:521–529.
- 42 Yoshihara S, Ono M, Yamada Y, Fukuda N, Abe T, Arisaka O: Nedocromil sodium inhibits bradykinin- and antigen-induced plasma extravasation in guinea pig airways. *Biomed Res* 2001;22:19–24.