

Endogenous Cannabinoid Receptor Agonists Inhibit Neurogenic Inflammations in Guinea Pig Airways

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Key Words

Cannabinoid · C fibers · Airway · Maxi-K⁺ channels · Guinea pig

Abstract

Background: Although neurogenic inflammation via the activation of C fibers in the airway must have an important role in the pathogenesis of asthma, their regulatory mechanism remains uncertain. **Objective:** The pharmacological profiles of endogenous cannabinoid receptor agonists on the activation of C fibers in airway tissues were investigated and the mechanisms how cannabinoids regulate airway inflammatory reactions were clarified. **Methods:** The effects of endogenous cannabinoid receptor agonists on electrical field stimulation-induced bronchial smooth muscle contraction, capsaicin-induced bronchoconstriction and capsaicin-induced substance P release in guinea pig airway tissues were investigated. The influences of cannabinoid receptor antagonists and K⁺ channel blockers to the effects of cannabinoid receptor agonists on these respiratory reactions were examined. **Results:** Both endogenous cannabinoid receptor agonists, anandamide and palmitoylethanolamide, inhibited electrical field stimulation-induced guinea pig bronchial smooth muscle contraction, but not neurokinin A-induced contraction. A cannabinoid CB2 antago-

nist, SR 144528, reduced the inhibitory effect of endogenous agonists, but not a cannabinoid CB1 antagonist, SR 141716A. Inhibitory effects of agonists were also reduced by the pretreatment of large conductance Ca²⁺-activated K⁺ channel (maxi-K⁺ channel) blockers, iberiotoxin and charybdotoxin, but not by other K⁺ channel blockers, dendrotoxin or glibenclamide. Anandamide and palmitoylethanolamide blocked the capsaicin-induced release of substance P-like immunoreactivity from guinea pig airway tissues. Additionally, intravenous injection of palmitoylethanolamide dose-dependently inhibited capsaicin-induced guinea pig bronchoconstriction, but not neurokinin A-induced reaction. However, anandamide did not reduce capsaicin-induced guinea pig bronchoconstriction. **Conclusions:** These findings suggest that endogenous cannabinoid receptor agonists inhibit the activation of C fibers via cannabinoid CB2 receptors and maxi-K⁺ channels in guinea pig airways.

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Introduction

Several previous studies showed that neurogenic inflammation in the airway must have an important role in the pathogenesis of asthma [1, 2]. By using tachykinin antagonists, Murai et al. [3, 4] directly demonstrated that

airway inflammation is generated by tachykinin released from capsaicin-sensitive afferent sensory nerves (C fibers), which are stimulated by various types of irritants like cigarette smoke [5], cold air [6] and hypertonic saline [7]. These irritants activate C fibers by opening nonselective cation channels with a high Ca^{2+} permeability [8]. Morimoto et al. [9] have shown that ω -conotoxin GVIA blocked the activation of C fibers and the release of substance P from their endings and suggested that the opening of N-type voltage-dependent Ca^{2+} channels might activate C fibers in guinea pig airway tissues.

Cannabinoids are psychoactive compounds which produce a wide array of effects including hypothermia, depressed motor activity, hypotension, inhibition of intestinal motility and antinociception [10]. The biological effects of cannabinoids are mediated by specific cell surface receptors, CB1 and CB2, in the central nervous system and in peripheral tissues [11–13]. Over the last 10 years, much evidence has accumulated favoring the concept that cannabinoids are endogenous modulators of neuronal activity in several tissues [14, 15]. Cannabinoid receptor agonists have been shown to inhibit the function of Ca^{2+} channels [16, 17].

In this report, we examined the effects of endogenous cannabinoid receptor agonists, anandamide and palmitoylethanolamide, on the activation of C fibers in guinea pig airway tissues and suggested that they show the inhibitory effects on airway inflammation induced by the activation of C fibers via the opening of maxi- K^+ channels by the activation of CB2 receptors. These results suggested that cannabinoid CB2 receptors might endogenously and negatively regulate the release of tachykinins from the endings of C fibers in guinea pig airways via the opening of maxi- K^+ channels.

Materials and Methods

Animals

Twelve-week-old male Hartley guinea pigs (280–340 g) were purchased from SLC (Hamamatsu, Japan) at least 1 week before the experiments. The animals were housed in a temperature- and humidity-controlled environment under a 12:12 h light/dark cycle with light on at 7.00 a.m. Animals were allowed free access to food and water ad libitum.

Materials

Substance P, neurokinin A, iberiotoxin, charybdotoxin and phosphoramidon were purchased from Peptide Institute Inc. (Osaka, Japan). Bacitracin was obtained from Sigma Chemical Company (St. Louis, Mo., USA). Capsaicin, dendrotoxin and glibenclamide were from Nakalai Tesque Chemical Company (Kyoto,

Japan). Anandamide and palmitoylethanolamide were purchased from Funakoshi Chemical Company (Tokyo, Japan). [N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride] (SR 141716A) 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] (SR 144528) were kindly supplied by Sanofi-Synthelabo Recherche (Montpellier, France). [^{125}I]Substance P (74 TBq/mmol) and anti-substance P antiserum for the radioimmunoassay were purchased from Amersham Int., Ltd., UK. All drugs were dissolved in dimethylsulfoxide. In vitro experiments, concentrated solutions of drugs prepared in dimethylsulfoxide were diluted in Tyrode's solution or Krebs-Ringer HEPES buffer. The final bath concentration of dimethylsulfoxide was 0.1%. In vivo experiments, drugs prepared in dimethylsulfoxide were injected intravenously (0.1 ml/kg). The solvent alone or 0.1% dimethylsulfoxide had no effect on the responses in either the in vitro or in vivo studies.

Contractile Response of Isolated Guinea Pig Bronchi in vitro

The procedure of Fox et al. [18] was used with certain modifications. Male Hartley guinea pigs were killed and their bronchi rapidly removed. A ring preparation with 4–5 mm length of the main bronchi (only one bronchi/animal) was mounted in 5-ml organ baths filled with warmed (37°C) and oxygenated (95% O_2 , 5% CO_2) standard Tyrode's solution (pH 7.5) containing 137.0 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl_2 , 1.05 mM MgCl_2 , 0.4 mM NaH_2PO_4 , 5.6 mM dextrose and 11.9 mM NaHCO_3 under a resting tension of 0.5 g. After a 60-min equilibration period, bronchi were stimulated by pre-established submaximal concentrations (data not shown) or stimuli of 1 nM neurokinin A or electrical field stimulation in the presence of 1 μM atropine, propranolol and phosphoramidon, respectively. Electrical field stimulation was delivered using two parallel platinum wires connected to a stimulator, and square-wave pulses of supramaximal voltage (50 V) and 1 ms pulse duration were applied for 30 s every 30 min at a frequency of 10 Hz. After two reproducible responses were obtained (control response) and the tension of the preparation returned to basal levels by washing, the endogenous cannabinoid agonist, anandamide or palmitoylethanolamide, was added, and 10 min later, contraction was induced with the same stimuli of neurokinin A or electrical field stimulation. The contractile response obtained in the presence of the drug was compared with the control response. In separate experiments, K^+ channel blockers, the CB1 receptor antagonist SR 141716A or the CB2 receptor antagonist SR 144528 were added 10 min before application of the agonist. Only one concentration of one agonist and/or antagonist was tested per bronchi preparation. None of the drugs employed in this study did affect the baseline tone.

Substance P Release from Guinea Pig Airway Tissues in vitro

The procedure of Ray et al. [19] was used with certain modifications. Male Hartley guinea pigs were killed and lungs were perfused (6 ml/min, 37°C) with oxygenated (95% O_2 , 5% CO_2) Krebs-Ringer HEPES buffer (pH 7.5) containing 138.0 mM NaCl, 5.6 mM KCl, 1.0 mM CaCl_2 , 1.0 mM MgCl_2 , 1.0 mM NaH_2PO_4 , 11 mM NaHCO_3 , 10 mM dextrose, 20 mM HEPES, 30 μM bacitracin and 1 μM phosphoramidon via a cannula that was inserted into the pulmonary artery through the right ventricle. The left atrium was opened to collect the outflow. Fifteen minutes after the start, perfusates from one period (15 min, 90 ml) were collected on ice in

beakers containing hydrochloric acid to give a final concentration of 0.1 M. Each fraction was desalted on Sep-Pak C₁₈ cartridges (Waters, Milford, Mass., USA) as described for somatostatin [20], and the peptides were concentrated to a final volume of 1 ml. The recovery from the Sep-Pak cartridge was more than 90% for radio-labeled substance P. Chemical irritation of tissues was achieved by perfusion with buffer containing 1 μ M capsaicin for 5 min during the second collection period. Substance P-like immunoreactivity was measured by radioimmunoassay. The amount of substance P released by capsaicin was calculated by subtracting the level detected in the first period perfusate from that in the second period perfusate. Drugs were added in Krebs-Ringer HEPES buffer throughout the experiment. After the experiments were finished, the lungs including trachea and bronchi were dissected out and weighed. The increase in substance P-like immunoreactivity release was calculated in femtomole per gram of tissues.

Bronchoconstriction in Guinea Pigs in vivo

Male Hartley guinea pigs were anesthetized by intraperitoneal injection of α -chloralose (100 mg/kg) and urethane (500 mg/kg). The jugular vein and trachea were cannulated and the animals were artificially ventilated (5 ml of air, 60 strokes/min). The pressure in the respirator system, i.e. the insufflation pressure, was constantly measured using a transducer (TP 200T, Nihon Kohden, Tokyo, Japan). Each stimulant was injected intravenously every 15 min through the jugular vein cannula to induce bronchoconstriction. Stimulants were repeatedly administered (approximately 6 times) until reproducible constriction (control response) was obtained. Cannabinoid agonists were dissolved in dimethylsulfoxide and administered intravenously (0.1 ml/kg) 2 min before the further challenge was repeated. Administration of the vehicle had no effect. The resulting constriction was compared with the control constriction. The following stimulants were used at the doses indicated: neurokinin A (1 nmol/kg) and capsaicin (10 nmol/kg). The values of control constriction after administration of dimethylsulfoxide were not changed until the final injection of neurokinin A or capsaicin for 120 min, meaning no influence by repeated challenges of agonists.

Statistical Analysis

Results are given as the means $\pm$ SEM of five experiments. Statistical analyses were performed by either analysis of variance followed by Dunnett's multicomparison test (contractile response of isolated guinea pig bronchi and bronchoconstriction) or by means of the unpaired Student's *t* test (others). $p < 0.05$ was considered statistically significant.

Results

Effects of Endogenous Cannabinoid Receptor Agonists on Isolated Guinea Pig Bronchial Smooth Muscle Contraction

The effects of endogenous cannabinoid receptor agonists on isolated guinea pig bronchial smooth muscle contraction were examined. After electrical field stimulation in the presence of atropine and propranolol, isolated

Table 1. Effect of endogenous cannabinoid receptor agonists, anandamide and palmitoylethanolamide, on guinea pig isolated bronchial smooth muscle contraction induced by electrical field stimulation and neurokinin A

Dose, μ M	Inhibition of control contraction, %	
	electrical field stimulation	neurokinin A
<i>Anandamide</i>		
0.0288	20.0 $\pm$ 14.3	ND
0.288	32.4 $\pm$ 18.9*	ND
2.88	61.0 $\pm$ 12.5*	ND
28.8	85.4 $\pm$ 3.1*	3.7 $\pm$ 2.8
<i>Palmitoylethanolamide</i>		
0.003	30.8 $\pm$ 7.8	ND
0.033	65.0 $\pm$ 5.5*	ND
0.33	79.0 $\pm$ 6.2*	ND
3.3	97.7 $\pm$ 2.3*	5.4 $\pm$ 3.0

Values are given as the mean $\pm$ SEM of five experiments.

ND = Not done.

* Significantly different from control contraction, $p < 0.05$.

guinea pig bronchial smooth muscles evoked tachykinin-dependent prolonged contraction [21]. Electrical field stimulation and 1 nM neurokinin A elicited the guinea pig isolated bronchial smooth muscle contraction of 0.28 ± 0.02 and 0.23 ± 0.03 g, respectively. Endogenous cannabinoid receptor agonists, anandamide (0.029–29.0 μ M) and palmitoylethanolamide (0.0033–3.3 μ M), dose-dependently inhibited electrical field stimulation-induced tachykinin-dependent contraction (table 1). On the other hand, neurokinin A-induced guinea pig bronchial smooth muscle contraction was not affected by these cannabinoid agonists at their maximal doses. The IC₅₀ values of anandamide and palmitoylethanolamide were 0.926 and 0.015 μ M, respectively. The rank order of inhibitory effects of cannabinoid receptor agonists was palmitoylethanolamide > anandamide. To identify the cannabinoid receptor subtype that was involved in these effects of cannabinoid receptor agonists, we examined the influences of selective cannabinoid CB1 (SR 141716A) and CB2 (SR 144528) receptor antagonists. SR 144528 (10 nM), but not SR 141716A (10 nM), reduced the inhibitory effects of anandamide and palmitoylethanolamide on the guinea pig bronchial smooth muscle contraction induced by electrical field stimulation (fig. 1).

We examined the influence of K⁺ channel blockers on inhibitory effects of cannabinoid receptor agonists on C fibers. A maxi-K⁺ channel antagonist, iberiotoxin

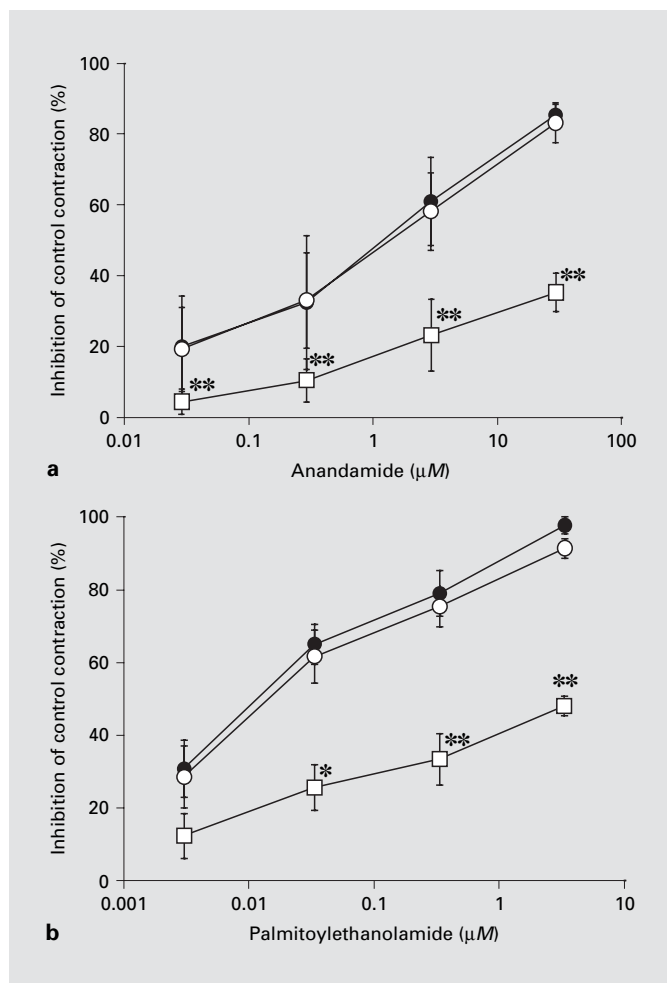


Fig. 1. Effects of endogenous cannabinoid receptor agonists, anandamide (**a**) and palmitoylethanolamide (**b**), on isolated guinea pig bronchial smooth muscle contraction induced by electrical field stimulation with cannabinoid receptor antagonists, 10 nM SR 141716A (○), 10 nM SR 144528 (□) or vehicle (●). * Significantly different from vehicle, $p < 0.05$. ** Significantly different from vehicle, $p < 0.01$. Values are given as percent inhibition of control contraction and are the mean $\pm$ SEM of five experiments.

(0.1 μ M) and charybdotoxin (0.01 μ M) greatly reduced the inhibitory effect of palmitoylethanolamide on electrical field stimulation-induced guinea pig bronchial smooth muscle contraction (fig. 2), while a voltage-sensitive A-type K^+ channel blocker, dendrotoxin (1 μ M), or an ATP-sensitive K^+ channel blocker, glibenclamide (1 μ M), had no effect. Data were not shown, but the inhibitory effect of anandamide on electrical field stimulation-induced contraction was affected by these K^+ channel blockers, the same as palmitoylethanolamide.

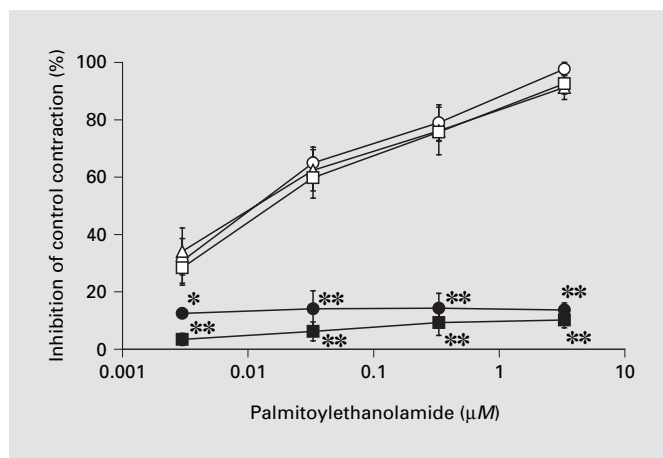


Fig. 2. Effect of palmitoylethanolamide on isolated guinea pig bronchial smooth muscle contraction induced by electrical field stimulation with K^+ channel blockers, 1 μ M dendrotoxin (□), 1 μ M glibenclamide (△), 0.01 μ M charybdotoxin (●), 0.1 μ M iberiotoxin (■) or vehicle (○). * Significantly different from vehicle, $p < 0.05$. ** Significantly different from vehicle, $p < 0.01$. Values are given as percent inhibition of control contraction and are the mean $\pm$ SEM of five experiments.

Table 2. Effect of endogenous cannabinoid receptor agonists on substance P-like immunoreactivity release from guinea pig airway tissues induced by capsaicin

Treatment	Increase of substance P-like immunoreactivity release, fmol/g tissue	Inhibition %
Control	48.8 $\pm$ 4.9	–
Anandamide 0.43 μ M	39.6 $\pm$ 2.5	18.8
Anandamide 4.3 μ M	30.0 $\pm$ 2.6*	38.6
Palmitoylethanolamide 0.05 μ M	30.3 $\pm$ 4.3*	38.0
Palmitoylethanolamide 0.5 μ M	7.1 $\pm$ 1.9*	85.4

Values are given as the mean $\pm$ SEM of five experiments. Substance P-like immunoreactivity release was induced by capsaicin (1 μ M). Anandamide and palmitoylethanolamide were added in Krebs-Ringer HEPES buffer throughout the experiment.

* Significantly different from control release, $p < 0.01$.

Effects of Cannabinoid Receptor Agonists on Substance P-Like Immunoreactivity Release from Guinea Pig Airway Tissues

To clarify the mechanisms involved in cannabinoid receptor agonist inhibition of electrical field stimulation-induced guinea pig bronchial smooth muscle contraction, their effects on capsaicin-induced substance P-like immunoreactivity release from guinea pig lungs were exam-

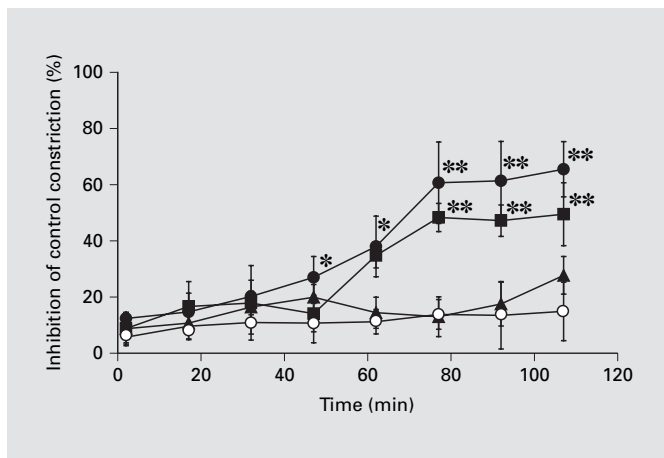


Fig. 3. Effects of 0.32 mg/kg (▲), 1 mg/kg (■) and 3.2 mg/kg (●) palmitoylethanolamide or 3.2 mg/kg anandamide (○) on capsaicin-induced guinea pig bronchoconstriction. * Significantly different from control bronchoconstriction, $p < 0.05$. ** Significantly different from control bronchoconstriction, $p < 0.01$. Values are given as percent inhibition of control bronchoconstriction and are the mean $\pm$ SEM of five experiments.

ined (table 2). Anandamide (0.43 and 4.3 μM) and palmitoylethanolamide (0.05 and 0.5 μM) significantly inhibited the capsaicin-induced release of substance P-like immunoreactivity from guinea pig airway tissues. The rank order of inhibitory effects of cannabinoid receptor agonists was palmitoylethanolamide > anandamide. In the presence of SR 144528 (10 nM) and anandamide (4.3 μM) or palmitoylethanolamide (0.5 μM), the capsaicin-induced release of substance P-like immunoreactivity from guinea pig airway tissues was 47.6 ± 1.3 or 45.4 ± 2.7 fmol/g tissue, respectively (not significantly different from control, 48.2 ± 1.4). In contrast, SR 141617A (10 nM) did not reduce the inhibitory effects of anandamide and palmitoylethanolamide (data not shown).

Effects of Cannabinoid Receptor Agonists on Bronchoconstriction in Guinea Pigs

We examined the effects of cannabinoid receptor agonists on capsaicin-induced bronchoconstriction in guinea pig airway in vivo. Intravenous administration of palmitoylethanolamide (0.32–3.2 mg/kg) dose-dependently inhibited capsaicin-induced constriction (fig. 3). However, neurokinin A-induced guinea pig bronchoconstriction was unaffected by palmitoylethanolamide at a dose of 3.2 mg/kg (data not shown). In contrast, anandamide (3.2 mg/kg, intravenous) had no effect on capsaicin-in-

duced constriction. None of the cannabinoid receptor agonists tested altered baseline airway resistance (percent inhibition of baseline: 3.2 mg/kg palmitoylethanolamide, 0.5 ± 0.3 ; 3.2 mg/kg anandamide, 1.8 ± 1.2 , $n = 5$).

Discussion

It has been suggested that various stimuli act on irritant receptors in respiratory mucosa and produce various respiratory reactions (e.g. bronchoconstriction, tracheal plasma extravasation and mucus hypersecretion) via the release of tachykinins from the endings of C fibers [22]. The influx of Ca^{2+} into presynaptic nerve endings through voltage-dependent Ca^{2+} channels is essential for the release of neurotransmitters within the nervous system. Ca^{2+} influx is a key step in excitation-release coupling in C fibers [8, 23]. For asthma research, it is important to examine how Ca^{2+} influx into C fibers is modulated. Morimoto et al. [9] suggested that N-type voltage-dependent Ca^{2+} channels are involved in the activation of C fibers and that an agent which blocks N-type voltage-dependent Ca^{2+} channels could be a valuable tool for asthma research.

The biological effects of cannabinoids are mediated by endogenous receptor agonists, such as anandamide and palmitoylethanolamide, physiologically metabolized from arachidonic acid. Anandamide, isolated from porcine brain, has been shown to bind to cannabinoid receptors, suggesting that it might behave as endogenous ligand for the cannabinoid receptor, the molecular target of Δ^9 -tetrahydrocannabinol in marijuana [24]. Anandamide behaves as a partial agonist at CB1 cannabinoid receptors and exhibits less reactive intrinsic activity at CB2 cannabinoid receptors than at CB1 receptors [25–28]. By activating CB1 cannabinoid receptors, anandamide, through reducing adenylate cyclase activity, inhibiting the activity of voltage-gated Ca^{2+} currents and activating inwardly rectifying K^+ currents [15, 17, 29, 30], reduces neuronal excitability and transmitter release. However, anandamide has been reported to bind to CB2 cannabinoid receptors while remaining functionally inactive [31] and activate a nonselective cation channel, TRPV1 receptors, which stimulates sensory nerves in airway tissues [32]. Palmitoylethanolamide inhibits mast cell activation and reduces inflammatory responses [33, 34] by a mechanism that may involve binding to CB2 cannabinoid receptors [31, 35]. However, palmitoylethanolamide binds to both cannabinoid receptor subtypes [24, 27] and potentiates the action of anandamide by minimizing its deg-

radation by fatty acid amide hydrolase [36]. Although there is no doubt about the anti-inflammatory effect of these endogenous cannabinoid receptor agonists in rodents, the molecular mechanisms underlying their actions have been puzzles. In this study, we found that both anandamide and palmitoylethanolamide inhibited electrical field stimulation-induced isolated guinea pig bronchial smooth muscle contraction and capsaicin-induced guinea pig bronchoconstriction. These reactions are dependent on tachykinins because they were inhibited by tachykinin receptor antagonists [4, 5]. In contrast, these cannabinoid agonists did not influence neurokinin A-induced bronchial smooth muscle contraction or bronchoconstriction. These results suggested that endogenous cannabinoid receptor agonists might block the release of tachykinins from C fibers, but do not antagonize the interaction of tachykinins on their receptors. Indeed, anandamide and palmitoylethanolamide did significantly reduce the capsaicin-induced release of substance P-like immunoreactivity from guinea pig airway tissues. Next, the inhibitory effects of these agonists on electrical field stimulation-induced isolated guinea pig bronchial smooth muscle contraction and capsaicin-induced release of substance P from guinea pig airway tissues were reduced by a specific CB2 cannabinoid receptor antagonist, SR 144528, but not by a CB1 antagonist, SR 141716A. Some reports showed that pharmacological effects of palmitoylethanolamide were reversed by SR 144528 [37, 38] and thus supported our results. It has been reported that the activation of cannabinoid receptors caused the inhibition of neurotransmitters in the central nervous systems and could affect the activities of peripheral tissues [10]. However, in the *in vivo* examinations of this study, because animals were anesthetized by α -chloralose and urethane, the influence of the endogenous cannabinoid agonists in the central nervous systems may be blocked to affect capsaicin-induced bronchoconstriction via the exclusion of efferent nervous transmissions. According to these facts, in both the *in vitro* and the *in vivo* studies, it is possible that CB2 receptors, which were not only interacted by palmitoylethanolamide but also by anandamide, exist on the C fibers in guinea pig airway and down-regulate the activation of C fibers and tachykinin release as peripheral and physiological roles.

In the second part of this study, we examined which mechanism is involved in the inhibitory effect of endogenous cannabinoid agonists on the activation of C fibers. The function of C fibers may be inhibited at a presumed prejunctional level via the activation of several receptor types. Agonists at μ -opioid, α_2 -adrenergic, GABA_B, his-

tamine H₃, neuropeptide Y₂ and 5-HT₁-like receptors have all been shown to inhibit excitatory nonadrenergic noncholinergic bronchoconstriction in the guinea pig, implying an action on airway C fibers [18]. Miura et al. [39, 40] also reported that nonadrenergic noncholinergic inhibitory nerves depressed the allergic reaction in cat airways via the release of protease-sensitive neurotransmitters, such as vasoactive intestinal peptide. Vasoactive intestinal peptide has been suggested to inhibit mediator release by elevating the level of intracellular cyclic AMP [41]. In the *in vivo* studies, it may be possible that nonadrenergic noncholinergic inhibitory nerves were stimulated by the injection of capsaicin. Still, we could not decide on the influence of nonadrenergic noncholinergic inhibitory nerves on capsaicin-induced guinea pig bronchoconstriction in this study; however, it has been proposed that the inhibition evoked by a number of these receptors is mediated via a common mechanism blocking the influx of Ca²⁺ into the endings of C fibers. Mackie and Hille [17] suggested that the inhibitory effect of cannabinoids on N-type voltage-dependent Ca²⁺ channels might be mediated by their interaction with other ion channels coupled either directly or indirectly to G proteins, e.g. potassium channels. In this study, we discovered maxi-K⁺ channel blockers, iberiotoxin and charybdotoxin, reduced the inhibitory effect of endogenous cannabinoid agonists on electrical field stimulation-induced guinea pig bronchial smooth muscle contraction, but not an A channel blocker, dendrotoxin, or an ATP-sensitive K⁺ channel blocker, glibenclamide. This evidence suggests that endogenous cannabinoid agonists may prevent the activation of C fibers via the opening of maxi-K⁺ channels. Cook et al. [42] have reported that cannabinoid-induced antinociception is mediated by the effector systems such as calcium or potassium channels. Our results support their conclusion and suggest that cannabinoid-induced antinociception is caused by the inhibition of N-type voltage-dependent Ca²⁺ channels and the release of substance P from sensory nerves via the opening of maxi-K⁺ channels.

When excitatory C fibers are stimulated, not only substance P but other neuropeptides, e.g. neurokinin A and calcitonin gene-related peptide, are also released from their nerve endings and exert various respiratory reactions [43]. The inflammatory effects of these neuropeptides on airway tissues may be of pathological relevance in human bronchial hyperreactivity, and we showed that antiasthmatic compounds, disodium cromoglycate and nedocromil sodium, inhibited hypertonic saline-induced plasma extravasation in guinea pig airways by the inacti-

vation of C fibers [7]. We conclude that endogenous cannabinoid receptor agonists, anandamide and palmitoylethanolamide, which inhibit the activation of C fibers and the release of these neuropeptides from their endings via the opening of maxi-K⁺ channels by the activation of CB2 receptors, might be involved in the clinical effects of antiasthmatic compounds described above.

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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* 1992a;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* 1992b;262:403–408.
- ▶ 5 Morimoto H, Yamashita M, Matsuda A, Miyake H, Fujii T: Effects of FR 113680 and FK 224, novel tachykinin antagonists, on cigarette smoke-induced rat tracheal plasma extravasation. *Eur J Pharmacol* 1992;224:1–5.
- ▶ 6 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.
- ▶ 7 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.
- ▶ 8 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.
- ▶ 9 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.
- ▶ 10 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.
- ▶ 11 Munro S, Thomas KL, Abu-Shaar M: Molecular characterization of peripheral receptor for cannabinoids. *Nature* 1993;365:61–65.
- ▶ 12 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.
- ▶ 13 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.
- ▶ 14 Howlett AC, Johnson MR, Melvin LS, Mine GM: Nonclassical cannabinoid analgesics inhibit adenylyl cyclase: development of a cannabinoid receptor model. *Mol Pharmacol* 1988;33:297–302.
- ▶ 15 Mackie KY, Westenbroek LR, Mitchell R: Cannabinoid activate an inwardly rectifying potassium conductance and inhibit Q-type cannabinoid receptor. *J Neurosci* 1995;15: 6552–6561.
- ▶ 16 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.
- ▶ 17 Mackie K, Hille B: Cannabinoid inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci USA* 1992;89: 3825–3829.
- ▶ 18 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.
- ▶ 19 Ray NJ, Jones AJ, Keen P: Morphine, but not sodium cromoglycate, modulates the release of substance P from capsaicin-sensitive neurones in the rat trachea in vitro. *Br J Pharmacol* 1991;102:797–800.
- ▶ 20 Wu P, Penman E, Coy DH, Rees LH: Evidence for direct production of somatostatin-14 from a larger precursor than somatostatin-28 in a pheochromocytoma. *Regul Pept* 1983;5:219–233.
- ▶ 21 Mulkey RM, Zucker RS: Action potentials must admit calcium to evoke transmitter release. *Nature* 1991;350:153–155.
- ▶ 22 Saria A, Martling CR, Yan Z, Theodorsson-Norheim E, Gamse R, Lundberg JM: Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenyl piperazinium, and vagal nerve stimulation. *Am Rev Respir Dis* 1988;137:1330–1335.
- ▶ 23 Franco-Cereceda A, Saria A, Lundberg JM: Differential release of calcitonin-gene related peptide and neuropeptide Y from the isolated heart by capsaicin, ischemia, nicotine, bradykinin and ouabain. *Acta Physiol Scand* 1989; 135:173–187.
- ▶ 24 Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R: Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992;258: 1946–1949.
- ▶ 25 Bayewitch M, Avidor-Reiss T, Levy R, Barg J, Mechoulam R, Vogel Z: The peripheral cannabinoid receptor: adenylyl cyclase inhibition and G protein coupling. *FEBS Lett* 1995; 375: 143–147.
- ▶ 26 Rinaldi-Carmona M, Calandra B, Shire D, Bouaboula M, Oustric D, Barth F, Casellas P, Ferrara P, Le Fur G: Characterization of two cloned human CB1 cannabinoid receptor isoforms. *J Pharmacol Exp Ther* 1996;278:871–878.
- ▶ 27 Griffin G, Atkinson PJ, Showalter VM, Martin BR, Abood ME: Evaluation of cannabinoid receptor agonists and antagonists using the guanosine-5'-O-(3-[³⁵S]thio)-triphosphate binding assay in rat cerebellar membranes. *J Pharmacol Exp Ther* 1998;285:553–560.
- ▶ 28 Partwee RG: Pharmacology of cannabinoid receptor ligands. *Curr Med Chem* 1999;6:635–664.
- ▶ 29 Felder CC, Briley EM, Axelrod J, Simpson JT, Mackie K, Devane WA: Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. *Proc Natl Acad Sci USA* 1993;90:7656–7660.
- ▶ 30 Vogel Z, Barg J, Levy R, Saria D, Heldman E, Mechoulam R: Anandamide, a brain endogenous compound, interacts specifically with cannabinoid receptors and inhibits adenylyl cyclase. *J Neurochem* 1993;61:352–355.

- ▶ 31 Facci L, Dal Toso R, Romanello S, Buriani A, Skaper SD, Leon A: Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc Natl Acad Sci USA* 1995;92:3376–3380.
- ▶ 32 Tucker RC, Kagaya M, Page CP, Spina D: The endogenous cannabinoid agonist, anandamide stimulates sensory nerves in guinea-pig airways. *Br J Pharmacol* 2001;132:1127–1135.
- ▶ 33 Aloe L, Leon A, Levi-Montalcini R: A proposed autacoid mechanism controlling mastocyte behaviour. *Agents Actions* 1993;39:C145–C147.
- ▶ 34 Mazzari S, Canella R, Petrelli L, Marcolongo G, Leon A: N-(2-hydroxyethyl)hexadecanamide is orally active in reducing edema formation and inflammatory hyperalgesia by down-modulating mast cell activation. *Eur J Pharmacol* 1996;300:227–236.
- ▶ 35 Abood ME, Martin BR: Molecular neurobiology of the cannabinoid receptor. *Int Rev Neurobiol* 1996;39:197–221.
- ▶ 36 Lambert DM, Di Marzo V: The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimimetic? *Curr Med Chem* 1999;6:757–773.
- ▶ 37 Calignano A, La Rana G, Piomelli D: Antinociceptive activity of the endogenous fatty acid amide, palmitoylethanolamide. *Eur J Pharmacol* 2001;419:191–198.
- ▶ 38 Conti S, Costa B, Colleoni M, Parolaro D, Giagnoni G: Antiinflammatory action of endocannabinoid palmitoylethanolamide and the synthetic cannabinoid nabilone in a model of acute inflammation in the rat. *Br J Pharmacol* 2002;135:181–187.
- ▶ 39 Miura M, Inoue H, Ichinose M, Kimura K, Katsumata U, Takishima T: Effect of nonadrenergic noncholinergic inhibitory nerve stimulation on the allergic reaction in cat airways. *Am Rev Respir Dis* 1990;141:29–32.
- ▶ 40 Miura M, Ichinose M, Kimura K, Katsumata U, Takahashi T, Inoue H, Takishima T: Dysfunction of nonadrenergic noncholinergic inhibitory system after antigen inhalation in actively sensitized cat airways. *Am Rev Respir Dis* 1992;145:70–74.
- ▶ 41 Friedman MM, Kaliner MA: Human mast cells and asthma. *Am Rev Respir Dis* 1987;135:1157–1164.
- ▶ 42 Cook SA, Welch SP, Lichtman H, Martin BR: Evaluation of cAMP involvement in cannabinoid-induced antinociception. *Life Sci* 1995;56:2049–2056.
- ▶ 43 Holzer P: Local effector function of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience* 1988;24:739–768.