ELSEVIER

European Journal of Pharmacology 355 (1998) 57–66

ejp

Pulmonary actions of anandamide, an endogenous cannabinoid receptor agonist, in guinea pigs

Peter W. Stengel [a,*], Marian K. Rippy [a], Sandra L. Cockerham [a], William A. Devane [b], Steven A. Silbaugh [a]

[a] *Eli Lilly, Lilly Research Laboratories, Neuroscience Research, Lilly Corporate Center, Indianapolis, Indiana 46285, USA*
[b] *University of Wisconsin, School of Pharmacy, Madison, WI 53706-1515, USA*

Received 15 January 1998; revised 29 April 1998; accepted 19 June 1998

Abstract

Anandamide (arachidonylethanolamide), 5,8,11,14-eicosatetraenamide, (*N*-2-hydroxyethyl), was tested for bronchodilator and anti-inflammatory activities. Conscious guinea pigs were given cumulative i.v. doses of anandamide (1.0, 3.0, and 10.0 mg/kg) to assess its effect on dynamic compliance (C_{dyn}), total pulmonary resistance (R_L), tidal volume (V_T) and breathing frequency (f). Other guinea pigs were exposed to an aerosol of A23187 (6*S*-[6α(2*S**,3*S**),8β(*R**),9β,11α]-5-(methylamino)-2-[[3,9,11-trimethyl-8-[1-methyl-2-*oxo*-2-(1*H*-pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5.5]undec-2-yl]methyl]-4-benzoxazolecarboxylic acid) until C_{dyn} decreased by 50% (~5 min) and at 20 min, cumulative i.v. doses of anandamide (1.0, 3.0, and 10.0 mg/kg) were administered and reversal of C_{dyn} examined. After the final dose of anandamide, the animals were killed and excised lung gas volumes (ELGV), i.e., pulmonary gas trapping, measured. Other animals were treated i.v. with anandamide (10.0 mg/kg), exposed to an aerosol of A23187 until labored breathing began, and then killed 1 h later. Anandamide did not significantly affect C_{dyn}, R_L, V_T and f. ELGV values of anandamide-treated guinea pigs were not different from those of vehicle-treated animals. Anandamide failed to reverse A23187-induced decreases in C_{dyn} and to reduce A23187-associated ELGV increases. Also, it did not prevent the prolonged airway obstruction caused by A23187. Histological evaluation revealed that anandamide significantly reduced A23187-related airway epithelial injury and pulmonary leukocytosis. However, it did not prevent A23187-induced peribronchiolar granulocytic accumulation. Our results suggest that in vivo anandamide has minimal direct airway smooth muscle-related actions, however it may possess modest anti-inflammatory properties.

Keywords: Airway obstruction; Asthma; Cannabinoid; Pulmonary gas trapping; Inflammation, pulmonary; Δ^9-Tetrahydrocannabinol

1. Introduction

Anandamide (arachidonylethanolamide), 5,8,11,14-eicosatetraenamide (*N*-2-hydroxyethyl), has been isolated from porcine brain and subsequently identified as an endogenous ligand of the cannabinoid receptor (Devane et al., 1992). Although anandamide does not share much structural similarities with the classical cannabinoid agonist Δ^9-tetrahydrocannabinol, recent investigations have demonstrated that anandamide interacts competitively at the cannabinoid receptor (Devane et al., 1992; Felder et al., 1993; Vogel et al., 1993; Childers et al., 1994). In addition, anandamide possesses similar in vitro pharmacological actions such as inhibition of forskolin-stimulated cyclic AMP production (Felder et al., 1993; Vogel et al., 1993) and N-type calcium channel currents (Mackie et al., 1993). Much of the work to date with anandamide has been limited to studying its in vivo pharmacological actions in the central nervous system (Fride and Mechoulam, 1993; Crawley et al., 1993; Smith et al., 1994; Wiley et al., 1995). From these studies, it has been suggested that anandamide possesses similar behavioral and physiological responses associated with psychotropic cannabinoids.

Although much is known about the psychoactive effects of Δ^9-tetrahydrocannabinol, it appears to have other pharmacological properties such as bronchodilator and anti-inflammatory activities (Dewey, 1986; Hollister, 1986). Specific airway conductance of healthy and asthmatic subjects has been shown to increase significantly after either oral or

* Corresponding author. Tel.: +1-317-276-9452; Fax: +1-317-277-0892; E-mail: stengel_peter_w@lilly.com

0014-2999/98/$19.00

aerosol administration of marijuana or its active ingredient Δ^9-tetrahydrocannabinol (Tashkin et al., 1973, 1977; Vachon et al., 1973). In addition, inhaled marijuana (Δ^9-tetrahydrocannabinol) has been found to reverse methacholine- and exercise-induced bronchospasm in asthmatics as rapidly as the β-agonist isoproterenol (Tashkin et al., 1975). The anti-inflammatory effects of Δ^9-tetrahydrocannabinol appear to be more controversial. In one study, Δ^9-tetrahydrocannabinol at the oral dose of 10.0 mg/kg was found to be equally effective as hydrocortisone, but 20 times more potent than aspirin, in inhibiting carrageenan-induced edema in rats (Sofia et al., 1973). However, at even higher oral doses, >25.0 mg/kg, Δ^9-tetrahydrocannabinol had no effect on the paw swelling produced by carrageenan (Kosersky et al., 1973). Thus, it would be important to determine if anandamide also shares the ability to inhibit bronchoconstriction and inflammation as well.

In this study, we evaluated the ability of anandamide to modify pulmonary function and ventilation. In addition, we examined the capability of anandamide to prevent or reverse the prolonged airway constriction and pulmonary inflammation produced by the divalent cationic ionophore A23187 (6*S*-[6α(2*S**,3*S**),8β(*R**),9β,11α]-5-(methylamino)-2-[[3,9,11-trimethyl-8-[1-methyl-2-*oxo*-2-(1*H*-pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5.5]undec-2-yl]methyl]-4-benzoxazolecarboxylic acid). This agent is a non-immunological stimulus which causes the release of bronchoconstrictor and pro-inflammatory substances that are thought to be important in the pathophysiology of asthma (Salari et al., 1985; Sautebin et al., 1985). Inhalation or intravenous delivery of A23187 has been reported to induce bronchospasm in monkeys (Patterson et al., 1979), guinea pigs (Ho and Esterman, 1979; Stengel et al., 1987; Misawa et al., 1989) and cats (Kriseman et al., 1986). These studies and others (Stengel and Silbaugh, 1989; Stengel et al., 1991) have suggested that cyclooxygenase and/or lipoxygenase metabolites of the arachidonic acid cascade, as well as cholinergic and histaminergic mechanisms are involved in the airway obstructive and proinflammatory effects of A23187. To our knowledge, this is the first report describing the in vivo pulmonary responses of anandamide.

2. Methods

2.1. Animals

Barrier-maintained, male outbred Hartley guinea pigs were obtained from Charles River Breeding Laboratories (Portage, MI, USA) and were housed in stainless steel, ventilated rack cages. Animal room temperature was maintained at 22–24°C with a relative humidity of 30–70% and a daily light–dark cycle (0600–1800 h light). Food (Purina Guinea Pig Chow, 5025) and water were provided ad libitum. Guinea pigs weighed 328–492 g. Our experimental procedures were approved by our Animal Care and Use Committee.

2.2. Evaluation of anandamide on pulmonary function and ventilation

Guinea pigs were anesthetized with halothane, a polyethylene catheter (PE-90 tubing) was inserted into the right pleural space (Amdur and Mead, 1958; Silbaugh et al., 1981), and an intravenous (i.v.) catheter (S-54-HL, microbore tubing, 0.76 mm OD) placed in the saphenous vein (2% lidocaine hydrochloride was applied to the leg before catheter placement). Each animal was allowed to regain consciousness in the pressure plethysmograph. Signals from the plethsymograph and the liquid-filled pleural catheter were sensed by differential pressure transducers (Validyne model DP45-14, Northridge, CA and Gould–Statham model P23 ID, Cleveland, OH, USA) coupled to a pulmonary mechanics analyzer (model 6, Buxco, Electronics, Sharon, CT). Calibration of tidal volume and pleural signals indicated proper phase relationships to 10 cps. All signals were recorded on a multichannel CRT visicorder (Honeywell model 1858, Denver, CO, USA).

After baseline measurements of tidal volume (V_T), respiratory rate (f), dynamic compliance (C_{dyn}) and total pulmonary resistance (R_L) were made, anandamide (1.0 mg/kg) or vehicle (0.5% bovine serum albumin in saline) were administered at 0 min as an i.v. bolus. Successively higher doses of anandamide (3.0 and 10.0 mg/kg) or vehicle were given at 5 and 10 min. Total solution volume given to each animal was 1.0 ml/kg. After each injection of drug or vehicle, 0.15 ml of heparinized saline was used to flush the i.v. catheter. At 15 min, the guinea pigs were killed with a 0.2 ml i.v. injection of Euthanasia-5 solution (Veterinary Laboratories, Lenexa, KS, USA) and removed from the plethsymograph. The abdomen of the animal was opened, the diaphragm punctured and the lungs allowed to collapse. The lungs were removed and trimmed of nonpulmonary tissue. The excised lung gas volume (ELGV) measurement, i.e., pulmonary gas trapping, was determined by Archimedes' principle (Stengel and Silbaugh, 1986; Silbaugh et al., 1987). The lungs were attached via the tracheal cannula to a brass anchor, placed in a cup, immersed in saline, and suspended from a hook at the top of a Mettler AE160 balance. Since lung tissue density approximates that of saline, the volume of gas trapped in the lungs could then be determined. The wet weight of the lungs was obtained immediately after the ELGV measurement, and a dry weight determination was made after oven drying for 24 h at 100°C. These values were used to calculate lung wet weight to dry weight ratios (W/D).

2.3. Reversal of A23187-induced airway constriction

Approximately 1 h before A23187 challenge each guinea pig was prepared for pulmonary mechanics evaluations

and then placed in the pressure plethsymograph. After baseline measurements were made, the A23187 aerosol (4.0 mg/ml) was begun at 0 min and continued until C_{dyn} decreased to 50% of baseline. Sham animals were exposed to the A23187 solvent (40% dimethylsulfoxide:42% ethanol:18% water) aerosol for 8 min. At 20 min, anandamide or vehicle was administered as an i.v. bolus. Successively higher doses of anandamide or vehicle were given at 25 and 30 min and a cumulative dose-response relationship (C_{dyn}) for anandamide was established. Total drug or vehicle volume given to each animal was 1.0 ml/kg. After each injection of drug or vehicle, 0.15 ml heparinized saline was used to flush the i.v. catheter. At 35 min, the animals were killed and lungs removed for ELGV measurements and W/D determinations as described above.

2.4. Effect of anandamide on A23187-induced pulmonary gas trapping and inflammation

Guinea pigs were briefly anesthetized with halothane and then injected i.v. with anandamide (10.0 mg/kg) or vehicle (1.0 ml/kg). The animals were then placed in plastic restraining tubes (Research and Consulting, Basel, Switzerland). The tubes were attached via the nose port to a 7.6-l polyvinyl chloride inhalation exposure chamber (Silbaugh et al., 1987). After 20 min, the guinea pigs were exposed to an aerosol of A23187 (2.0 mg/ml) until labored breathing, i.e., dyspnea, began. Chamber airflow was 20 l/min and relative humidity was under 40%. The time of onset of dyspnea was determined by an impartial observer. Sham guinea pigs were dosed with vehicle and exposed to the A23187 solvent for 15 min. All animals were killed with a 2 ml intraperitoneal injection of Euthanasia-5 solution at 1 h after challenge. ELGV values were then determined.

Following the ELGV measurements, the lungs were prepared for histological evaluation. Briefly, the lungs were infused via the trachea with 7 ml of 10% phosphate-buffered formalin. The trachea was tied and the lungs were placed in jars containing additional formalin. Four sections (two from anterior and two from diaphragmatic lobes) from each animal were processed for histology, embedded

Table 1
Effect of anandamide or vehicle (0.5% bovine serum albumin) on excised lung gas volumes (ELGV) and lung wet weight to dry weight ratios (W/D) following ventilatory and pulmonary function measurements

Group	Vehicle	Anandamide	*P*-value
ELGV (ml/kg)	1.32 ± 0.10	2.51 ± 0.61	0.0854
W/D	5.10 ± 0.03	5.24 ± 0.08	0.1395

Values are mean ± S.E.M.
$n = 6$ per group.

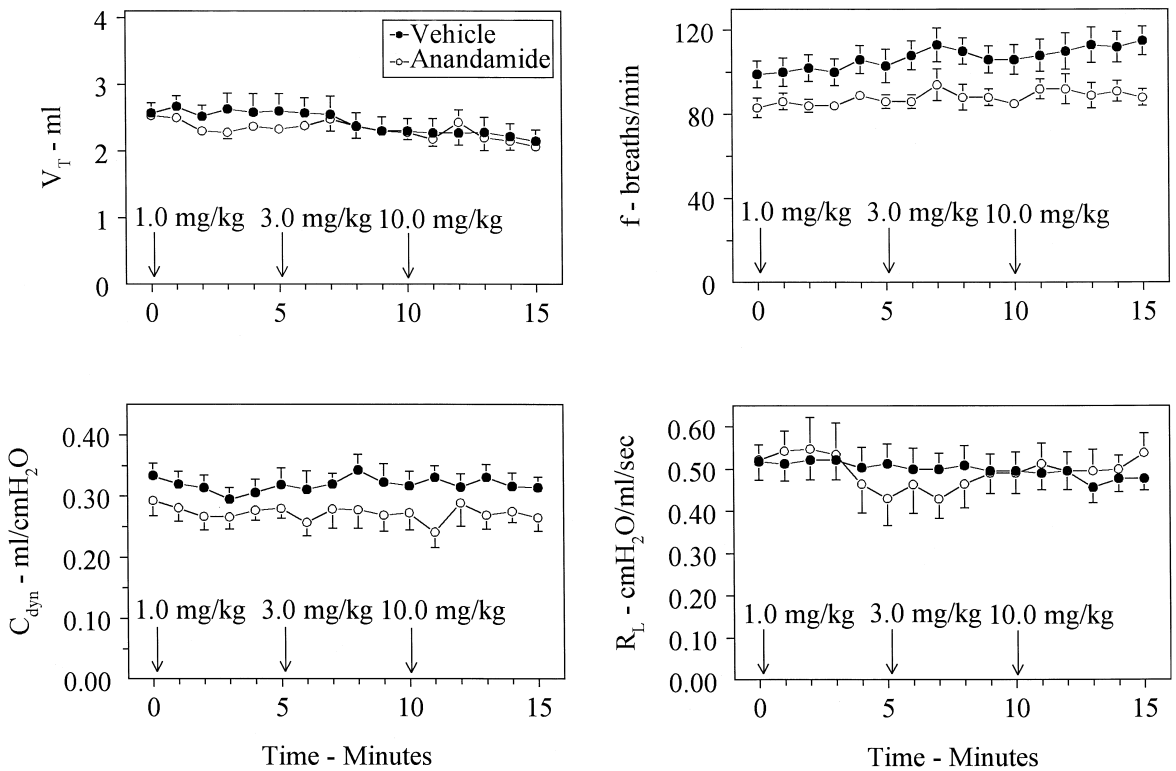


Fig. 1. Time-course of changes in tidal volume (V_T), respiratory rate (f), dynamic compliance (C_{dyn}), and total pulmonary resistance (R_L) of guinea pigs after increasing cumulative doses of anandamide. Doses of anandamide or its vehicle (0.5% bovine serum albumin in saline) were given i.v. at 0, 5, and 10 min. Values are the mean ± S.E.M. ($n = 6$ guinea pigs/group). Absence of error bars indicates that the magnitude of error was less than the symbol size.

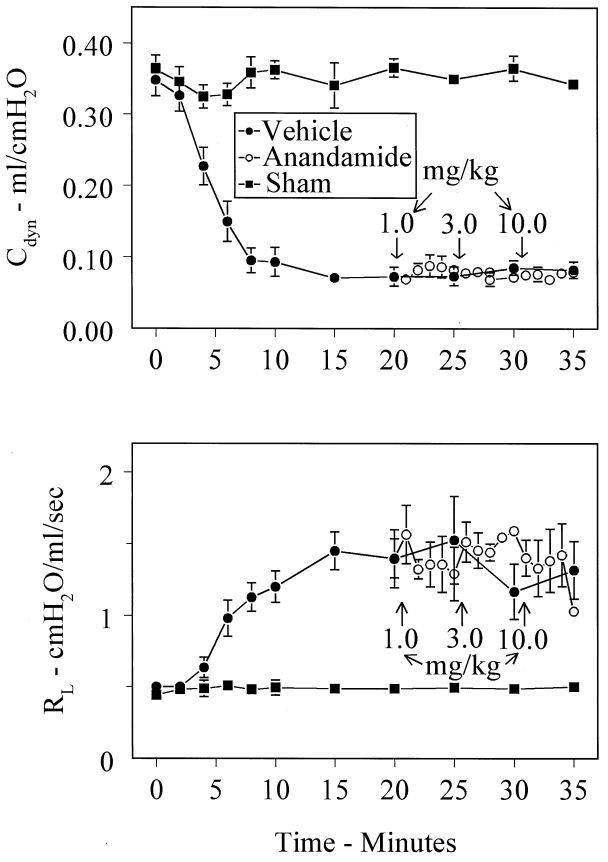


Fig. 2. Lack of effect on A23187-induced C_{dyn} and R_L changes after increasing cumulative doses of anandamide. Doses of anandamide or its vehicle were given i.v. at 20, 25 and 30 min. Ionophore aerosol was started at 0 min and stopped when C_{dyn} decreased to 50% of baseline. Values are means ± S.E.M. ($n = 4$ guinea pigs/group). Absence of error bars indicates that the magnitude of error was less than the symbol size.

in paraffin, sectioned, and stained with Mayer's hematoxylin–eosin. These sections were evaluated blindly and assigned numerical scores ranging from 0.0 to 3.0 (0.0 = normal morphology), to indicate the severity of inflammation, i.e., airway epithelial injury, peribronchiolar granulocytic accumulation and pulmonary leukocytosis. Each parameter was scored individually for each lung section, and a sum of the four sections was calculated.

2.5. Aerosol exposure conditions and characterization

Aerosols of A23187 and its solvent were generated with Lovelace nebulizers (Mercer et al., 1968). Aerosol particle size was determined by gravimetric analysis of cascade impactor samples (Mercer et al., 1970). Mass median aerodynamic diameters of A23187 ranged from 0.5 to 6.3 μm with geometric standard deviations ranging from 1.4 to 2.8.

Table 2
ELGV and lung W/D values for A23187-exposed, anandamide- and vehicle-treated guinea pigs and A23187-solvent, vehicle-treated animals (sham)

Group	Vehicle	Anandamide	Sham
ELGV (ml/kg)	10.13 ± 0.83	10.64 ± 0.56	0.94 ± 0.05[a]
W/D	5.16 ± 0.09	5.10 ± 0.12	5.01 ± 0.08

[a] $P < 0.05$ vs. A23187-exposed, vehicle- and anandamide-treated guinea pigs.
Values are mean ± S.E.M.
$n = 4$ per group.

2.6. Statistical analyses

The results are expressed as the mean ± the standard error of mean (S.E.M.) of 4–10 animals per group. The effectiveness of drug treatment was evaluated for each animal by subtracting the pre-drug C_{dyn} value at 20 min from the value of C_{dyn} at the end of each dose (25, 30 and 35 min) and then dividing by the difference between C_{dyn} at 20 min and baseline. ELGV measurements were normalized on a body weight basis (ml/kg). One-way analysis of variance was used to compare values of C_{dyn}, excised lung gas volume and inflammatory indices and Student Newman–Keuls method for all pairwise comparisons was performed when appropriate. Analyses were run using SigmaStat for Windows (version 1.0, Jandel Scientific Software, San Rafael, CA, USA) on a Compac (Deskpro 386/20e Compac Computer, Houston, TX, USA) personal computer. Comparisons were considered significant for P values of 0.05 or less.

2.7. Drug sources

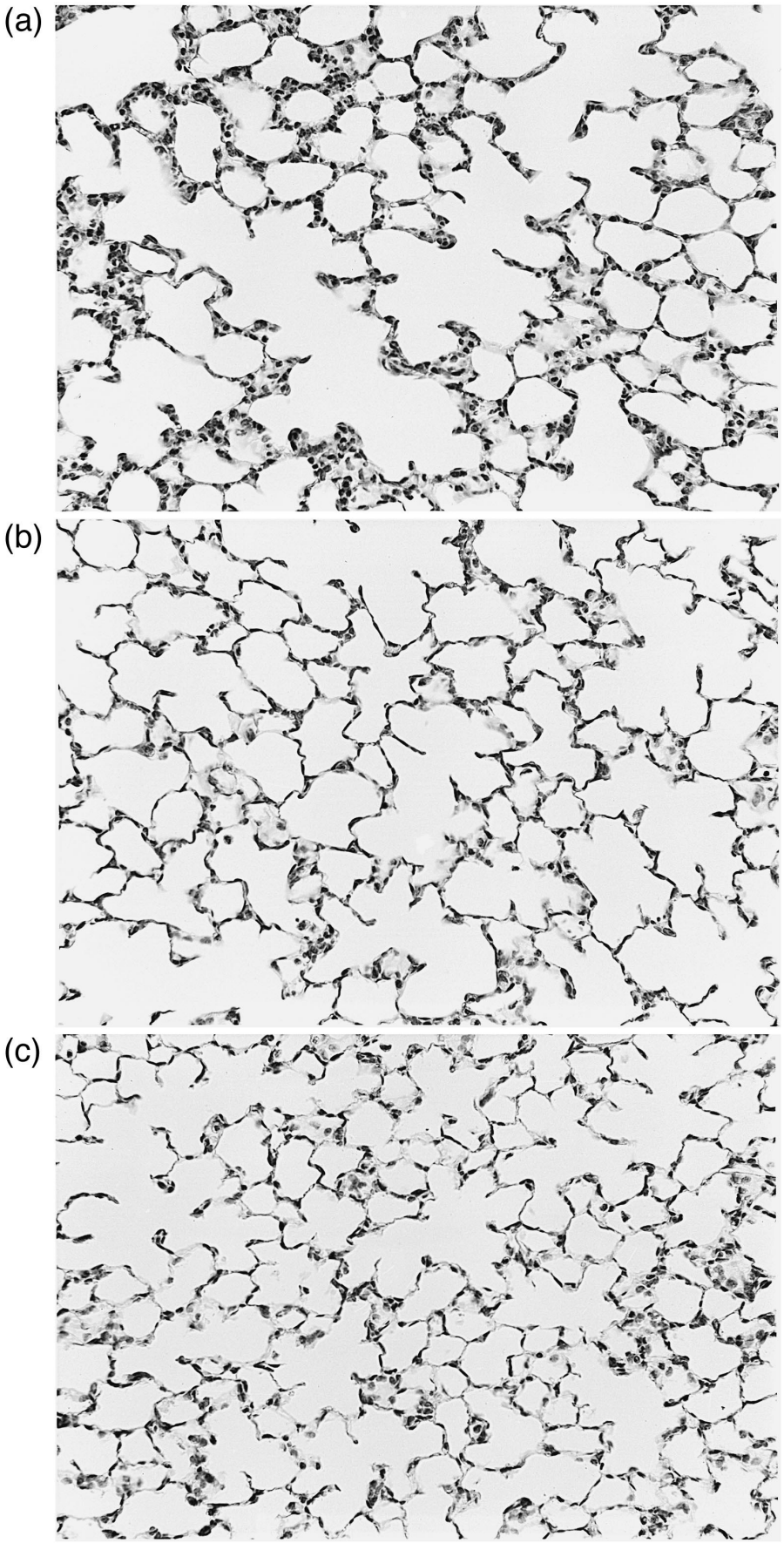
A23187 was produced within Lilly Research Laboratories (Eli Lilly, Indianapolis, IN, USA). Bovine serum albumin was purchased from Sigma (St. Louis, MO, USA). Anandamide was provided by Dr. W.A. Devane and was prepared by evaporating a chloroform/methanol stock solution under nitrogen and reconstituting with 0.5% bovine serum albumin in saline for i.v. administration.

3. Results

3.1. Effect of anandamide on pulmonary function and ventilation

During the 15-min period when increasing doses of anandamide were administered to conscious guinea pigs,

Fig. 3. Photomicrographs of lung sections depicting pulmonary leukocytosis (panels A–C) and airway epithelial necrosis (panels D–F) from guinea pigs pretreated i.v. with vehicle (panels A and D) or anandamide (panels B and E), 1 h after A23187. Lung sections of shams are shown in panels C and F. Anandamide reduced the pulmonary leukocytosis and airway epithelial injury caused by A23187. Hematoxylin–eosin stain; 50×.


(a)
(b)
(c)

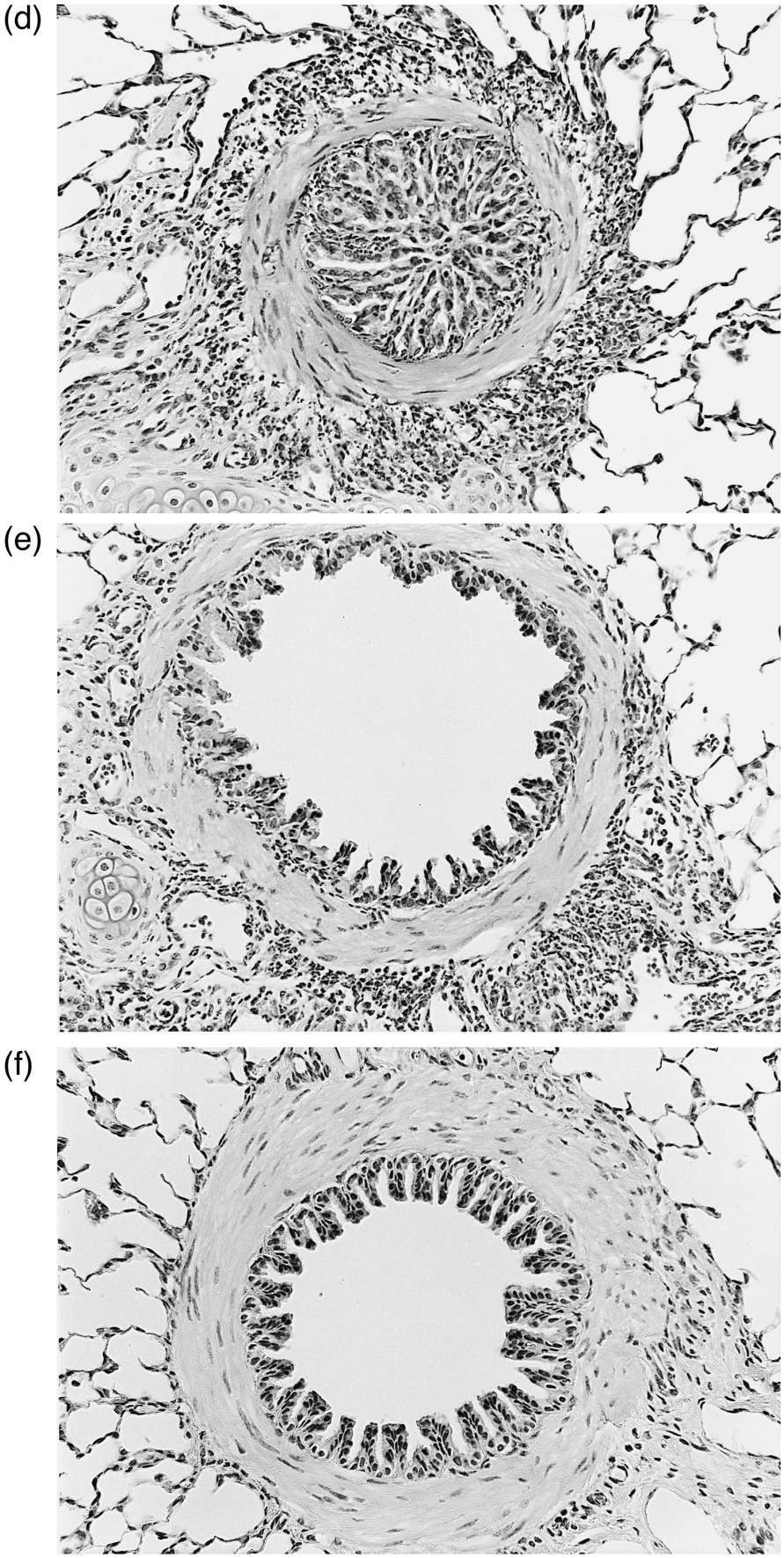


Fig. 3 (continued).

no changes were observed in V_T, f, C_{dyn} and R_L of animals which received anandamide versus those which received vehicle (Fig. 1). A similar lack of a difference was found comparing ELGV and W/D values of anandamide-treated and vehicle-treated animals (Table 1). Anandamide by itself did not produce any changes on the pulmonary responses measured.

3.2. Reversal of A23187-induced airway constriction

The duration of aerosol exposure required to decrease C_{dyn} to 50% of baseline was 5.5 ± 0.5 min (Fig. 2). Anandamide had no effect on A23187-induced C_{dyn} changes at any dose tested. Also, anandamide was ineffective in reversing the increases in R_L produced by A23187. The ELGV values of A23187-exposed, anandamide- and vehicle-treated guinea pigs were similarly elevated compared to sham-treated animals (Table 2). No differences were found in W/D among anandamide-, vehicle- and sham-treated guinea pigs.

3.3. Effect of anandamide on A23187-induced airway responses

All A23187-exposed guinea pigs appeared dyspneic within 15 min from the start of the aerosol exposure. Time of onset of dyspnea of anandamide-treated animals, 9.3 ± 1.0 min, was not different from that of vehicle-treated guinea pigs, 10.0 ± 0.9 min. None of the sham-treated animals became dyspneic during the 15-min solvent aerosol exposure.

The results of anandamide on airway inflammation and pulmonary gas trapping caused by inhaled A23187 are shown in Figs. 3 and 4, respectively. Fig. 3, panels A and D, illustrates typical histological changes found in lungs of vehicle-treated, A23187-exposed guinea pigs, while panels B and E are representative of anandamide-treated,

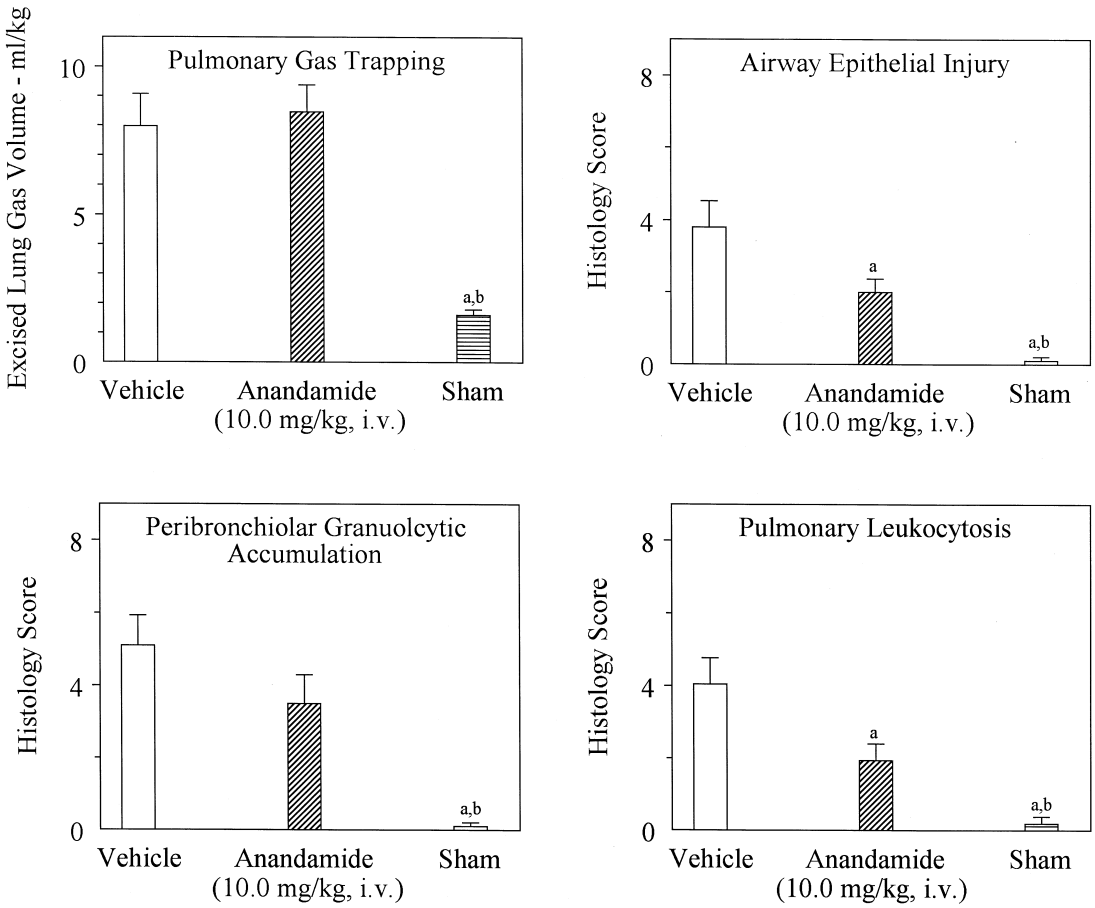


Fig. 4. Effect of anandamide (10.0 mg/kg) pretreatment on A23187-induced airway responses. Guinea pigs were dosed i.v. with anandamide (diagonally hatched bar) or vehicle (open bar) and then 20 min later challenged with A23187 until dyspneic. The effect of anandamide on airway obstruction, airway epithelial injury, peribronchiolar granulocytic accumulation and pulmonary leukocytosis are shown in the four panels 1 h after A23187 challenge. Sham (horizontally hatched bars) animals were dosed with vehicle, exposed to A23187 solvent aerosol and then killed 1 h post exposure. Each bar represents the mean ± S.E.M. ($n = 8–10$ guinea pigs/group). (a) $P < 0.05$ between anandamide- or sham-treated guinea pigs vs. vehicle-treated animals. (b) $P < 0.05$ between sham- and anandamide-treated guinea pigs.

A23187-exposed animals. For comparison, lung sections of shams are shown in panels C and F. The histological changes produced by A23187 included diffuse airway epithelial injury, peribronchiolar granulocytic infiltration, peribronchial and perivascular edema and pulmonary leukocytosis. The cellular infiltrate consisted of primarily neutrophils admixed with few eosinophils. One hour after A23187 aerosol challenge, ELGV values and inflammatory scores of vehicle-treated guinea pigs were 5.0 and 21.5 times those of sham animals, respectively. Guinea pigs dosed i.v. with anandamide at 10.0 mg/kg, 20 min before a A23187 challenge were not significantly protected against the airway obstructive response 1 h after exposure to A23187. Also, anandamide failed to alter the peribronchiolar granulocytic accumulation caused by A23187. The level of inhibition was $31.4 \pm 15.7\%$ ($P = 0.11$). In contrast, anandamide inhibited the diffuse airway epithelial injury, $47.8 \pm 9.4\%$ ($P = 0.0156$), and the pulmonary leukocytosis, $55.7 \pm 12.0\%$ ($P = 0.0089$), 1 h after A23187.

4. Discussion

Our results clearly show that anandamide did not produce any adverse effects on pulmonary mechanical and ventilatory parameters in conscious guinea pigs. The observation that anandamide did not alter basal airway responses or reverse an ongoing airway obstruction when administered intravenously suggests that this agent is not acting directly to relax guinea pig airway smooth muscle. However, anandamide appears to possess modest anti-inflammatory properties in A23187-challenged animals. Additional investigation is needed to determine if anti-inflammatory effects of anandamide will occur with other pro-inflammatory stimuli.

At the i.v. doses used in this study, anandamide did not produce any changes in C_{dyn}, R_L, V_T and f, nor did anandamide alter A23187-induced decreases in C_{dyn} and increases in R_L. The inability of anandamide to alter changes in airway smooth muscle tone or to reverse existing bronchoconstriction suggests that this agent is devoid of airway smooth muscle contractile and relaxant properties. If anandamide possessed even mild bronchodilator actions, the doses used in this study should have been sufficient to produce a reduction in an ongoing airway constriction since the prolonged airway obstructive response caused by a brief aerosol exposure to A23187 is very sensitive to reversal (Stengel and Silbaugh, 1989). For instance, at doses similar or lower than those used for anandamide, we have observed significant reversal of obstruction for a variety of agents acting by different mechanisms—including the bronchodilators, aminophylline and salbutamol, the thromboxane synthetase inhibitor, dazoxiben, the 5-lipoxygenase inhibitors, phenidone and REV-6866 (*N*-methyl-4-benzyl-oxyphenyl-acetohydroxamic acid), the muscarinic receptor antagonist, atropine, and the 5-hydroxytryptamine$_2$ receptor antagonist, LY53857 ((6-methyl-1-(1-methylethyl) ergoline-8-carboxylic acid 2-hydroxy-1-methylpropylester (*Z*)-2-butenedioate[1:1]).

That this reversal of A23187-induced decrease in C_{dyn} by the known bronchodilators aminophylline and salbutamol occurred rapidly, argued that contraction of airway smooth muscle is the primary mechanism producing airway obstruction. This lack of an effect of anandamide on pulmonary smooth muscle function is similar to what was found following i.v. administration of Δ^9-tetrahydrocannabinol in anesthetized spontaneously breathing guinea pigs (Ackerman, 1977). In that study, Δ^9-tetrahydrocannabinol did not alter histamine- and acetylcholine-induced bronchoconstriction indicating that Δ^9-tetrahydrocannabinol did not have pulmonary smooth muscle relaxant effects. Similarly, our results suggest that anandamide does not have direct airway smooth muscle actions in vivo.

One possible explanation for this apparent lack of an observable airway smooth muscle effect with anandamide may be due to its short duration of action (Smith et al., 1994). Anandamide is readily degraded to arachidonic acid and ethanolamine (Deutsch and Chin, 1993; Omeir et al., 1995). However, if hydrolysis of anandamide to arachidonic acid occurred after i.v. dosing, then airway obstruction should have been evident since bolus i.v. administration of arachidonic acid leads to bronchoconstriction in guinea pigs (Lefort and Vargaftig, 1978). That no bronchospasm was observed following i.v. delivery of anandamide suggests that either insufficient amounts of arachidonic acid were generated or that the compound was stable for the duration of our experiments.

The airway obstruction and pulmonary inflammatory changes (i.e., diffuse epithelial injury, peribronchiolar granulocytic infiltration, and edema) observed 1 h after a brief aerosol A23187 exposure are in agreement with our previous findings (Stengel et al., 1991). In addition, we found inhaled A23187 in this study to be associated with the development of a marked pulmonary leukocytosis, comprised predominantly of neutrophils admixed with few eosinophils. That anandamide significantly reduced A23187-related pulmonary leukocytosis and airway epithelial injury indicates that anandamide has anti-inflammatory actions. Although the mechanisms by which anandamide exerts these effects are unclear, inhibition of leukocyte adhesion and other anti-inflammatory actions have been described for Δ^9-tetrahydrocannabinol and other synthetic cannabinoids (Audette and Burstein, 1990; Burstein et al., 1992). In those investigations, Δ^9-tetrahydrocannabinol and other synthetic cannabinoids, at doses comparable to the dose of anandamide used in this study, are believed to have produced their anti-inflammatory effects by inhibiting formation of pro-inflammatory metabolites of arachidonic acid, such as leukotriene B_4. Since we have previously found the leukotriene B_4 receptor antagonist SC-41930 (7-[3-(4-acetyl-3-methoxy-2-propylphenoxy)-propoxy]-

3,4-dihydro-8-propyl-2*H*-1-benzopyran-2-carboxylic acid) to inhibit A23187-induced pulmonary leukocytic inflammatory response (Stengel et al., 1991), it might be possible that anandamide could be working through a similar anti-inflammatory mechanism.

In conclusion, our results demonstrate that anandamide, at the doses used in this study, had no direct effect on airway smooth muscle responses since basal pulmonary mechanical and ventilatory measurements remained unchanged and an ongoing airway constriction was unaffected. Although its mechanism of action is unknown, anandamide substantially reduced some of the inflammatory changes caused by A23187.

Acknowledgements

This study was presented in part at the Experimental Biology 95™ meeting in Atlanta, GA, from April 9–13, 1995.

References

Ackerman, N.R., 1977. The lack of an effect of Δ^9-THC on pulmonary smooth muscle function in the guinea pig. Toxicol. Appl. Pharmacol. 41, 321–328.

Amdur, M.O., Mead, J., 1958. Mechanics of respiration in unanesthetized guinea pigs. J. Appl. Physiol. 192, 364–368.

Audette, C.A., Burstein, S., 1990. Inhibition of leukocyte adhesion by the in vivo and the in vitro administration of cannabinoids. Life Sci. 47, 753–759.

Burstein, S.H., Audette, C.A., Breuer, A., Devane, W.A., Colodner, S., Doyle, S.A., Mechoulam, R., 1992. Synthetic nonpsychotropic cannabinoids with potent anti-inflammatory, analgesic and leukocyte antiadhesion activities. J. Med. Chem. 35, 3135–3141.

Childers, S.R., Sexton, T., Roy, M.B., 1994. Effects of anandamide on cannabinoid receptors in rat brain membranes. Biochem. Pharmacol. 47, 711–715.

Crawley, J.N., Corwin, R.L., Robinson, J.K., Felder, C.C., Devane, W.A., Axelrod, J., 1993. Anadamide, an endogenous ligand of the cannabinoid receptor, induces hypomotility and hypothermia in vivo in rodents. Pharmacol. Biochem. Behav. 46, 967–972.

Deutsch, D.G., Chin, S.A., 1993. Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. Biochem. Pharmacol. 46, 791–796.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Dewey, W.L., 1986. Cannabinoid pharmacology. Pharmacol. Rev. 38, 151–178.

Felder, C.C., Briley, E.M., Axelrod, J., Simpson, J.T., Mackie, K., Devane, W.A., 1993. Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. Proc. Natl. Acad. Sci. 90, 7656–7660.

Fride, E., Mechoulam, R., 1993. Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. Eur. J. Pharmacol. 231, 313–314.

Ho, P.P.K., Esterman, M., 1979. Calcium ionophore A23187 induced anaphylaxis in guinea pig. Pharmacologist 21, 154.

Hollister, L.E., 1986. Health aspects of cannabis. Pharmacol. Rev. 38, 1–20.

Kosersky, D.S., Dewey, W.L., Harris, L.S., 1973. Antipyretic, analgesic and anti-inflammatory effects of Δ^9-tetrhydrocannabinol in the rat. Eur. J. Pharmacol. 24, 1–7.

Kriseman, T., Underwood, D.C., Hyman, A.L., Kadowitz, P.J., 1986. Analysis of airway responses to A23187 in the cat. J. Pharmacol. Exp. Ther. 239, 395–399.

Lefort, J., Vargaftig, B.B., 1978. Role of platelets in aspirin-sensitive bronchoconstriction in the guinea pig: interactions with salicylic acid. Br. J. Pharmacol. 63, 35–42.

Mackie, K., Devane, W.A., Hille, B., 1993. Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. Mol. Pharmacol. 44, 498–503.

Mercer, T.T., Tillery, M.I., Chow, H.Y., 1968. Operating characteristics of some compressed air nebulizers. Am. Ind. Hyg. Assoc. 29, 66–78.

Mercer, T.T., Tillery, M.I., Newton, G.J., 1970. A multi-stage, low flow rate cascade impactor. J. Aerosol Sci. 1, 9–15.

Misawa, M., Kawamura, T., Takenouchi, K., 1989. A new bronchial asthma model using calcium ionophore A23187 in guinea pigs. Jpn. J. Pharmacol. 51, 446–449.

Omeir, R.L., Chin, S., Hong, Y., Ahern, D.G., Deutsch, D.G., 1995. Arachidonoyl ethanolamide-[1,2-^{14}C] as a substrate for anandamide amidase. Life Sci. 56, 1999–2005.

Patterson, R., Harris, K.E., Greenberger, P.A., 1979. Ionophore and arachidonic acid stimulation of airway responses in Rhesus monkeys. J. Clin. Invest. 67, 67–72.

Salari, H., Borgeat, P., Fournier, M., Hebert, J., Pelletier, G., 1985. Studies on the release of leukotrienes and histamine by human lung fragments upon immunologic and nonimmunologic stimulation: effects of nordihydroguaiaretic acid, aspirin, and sodium cromoglycate. J. Exp. Med. 162, 1904–1915.

Sautebin, L., Vigano, T., Grassi, E., Crivellari, M.T., Galli, G., Berti, F., Mezzetti, M., Folco, G., 1985. Release of leukotriene, induced by the Ca++ ionophore A23187, from human lung parenchyma in vitro. J. Pharmacol. Exp. Ther. 234, 217–221.

Silbaugh, S.A., Wolff, R.K., Johnson, W.K., Mauderly, J.L., Macken, C.A., 1981. Effect of sulfuric acid aerosols on pulmonary function of guinea pigs. J. Toxicol. Environ. Health 7, 339–352.

Silbaugh, S.A., Stengel, P.W., Dillard, R.D., Bemis, K.G., 1987. Pulmonary gas trapping in the guinea pig and its application in pharmacological testing. J. Pharmacol. Methods 18, 295–303.

Smith, P.B., Compton, D.R., Welch, S.P., Razdan, R.K., Mechoulam, R., Martin, B.R., 1994. The pharmacological activity of anandamide, a putative endogenous cannabinoid, in mice. J. Pharmacol. Exp. Ther. 270, 219–227.

Sofia, R.D., Nalepa, S.D., Harakal, J.J., Vassar, H.B., 1973. Anti-edema and analgesic properties of Δ^9-tetrahydrocannabinol (THC). J. Pharmacol. Exp. Ther. 186, 646–655.

Stengel, P.W., Silbaugh, S.A., 1986. Mechanisms of gas loss from normal and hyperinflated excised guinea pig lungs. Respir. Physiol. 63, 129–138.

Stengel, P.W., Silbaugh, S.A., 1989. Reversal of A23187-induced airway constriction in the guinea pig. J. Pharmacol. Exp. Ther. 248, 1084–1090.

Stengel, P.W., Pechous, P.A., Silbaugh, S.A., 1987. Mechanism of A23187-induced airway obstruction in the guinea pig. Prostaglandins 33, 567–577.

Stengel, P.W., Williams, G.D., Silbaugh, S.A., 1991. A23187-induced pulmonary gas trapping and inflammation in the guinea pig. Agents Actions 32, 270–276.

Tashkin, D.P., Shapiro, B.J., Frank, I.M., 1973. Acute pulmonary physiologic effects of smoked marijuana and oral Δ^9-tetrahydrocannabinol in healthy young men. N. Eng. J. Med. 289, 336–341.

Tashkin, D.P., Shapiro, B.J., Lee, Y.E., Harper, C.E., 1975. Effects of smoked marijuana in experimentally induced asthma. Am. Rev. Respir. Dis. 112, 377–386.

Tashkin, D.P., Reiss, S., Shapiro, B.J., Calvarese, B., Olsen, J.L., Lodge, J.W., 1977. Bronchial effects of aerosolized Δ^9-tetrahydrocannabinol in healthy and asthmatic subjects. Am. Rev. Respir. Dis. 115, 57–65.

Vachon, L., Fitzgerald, M.X., Solliday, N.H., Gould, I.A., Gaensler, E.A., 1973. Single-dose effect of marihuana smoke: bronchial dynamics and respiratory-center sensitivity in normal subjects. N. Eng. J. Med. 288, 985–989.

Vogel, Z., Barg, J., Levy, R., Saya, D., Heldman, E., Mechoulam, R., 1993. Anandamide, a brain endogenous compound, interacts specifically with cannabinoid receptors and inhibits adenylate cyclase. J. Neurochem. 61, 352–355.

Wiley, J., Balster, R., Martin, B., 1995. Discriminative stimulus effects of anandamide in rats. Eur. J. Pharmacol. 276, 49–54.