Viral Infection Induces Dependence of Neuronal M_2 Muscarinic Receptors on Cyclooxygenase in Guinea Pig Lung

Richard M. Kahn,* Olubunmi A. Okanlami,‡ David B. Jacoby,* and Allison D. Fryer§

**Division of Pulmonary and Critical Care Medicine, Department of Medicine, ‡Departments of Anesthesiology and Critical Care Medicine, and §Environmental Health Sciences, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland 21205*

Abstract

Inhibitory M_2 muscarinic receptors on parasympathetic nerve endings in the lungs decrease release of acetylcholine, inhibiting vagally induced bronchoconstriction. Neuronal M_2 receptor function can be studied using selective agonists and antagonists such as pilocarpine and gallamine. In pathogen-free guinea pigs indomethacin (1 mg/kg) did not alter the effect of either gallamine or pilocarpine, thus in pathogen free animals neuronal M_2 muscarinic receptors function independently of cyclooxygenase products. However, in guinea pigs infected with virus, (which causes temporary loss of M_2 receptor function), and then allowed to recover for 8 wk (to allow recovery of M_2 receptors), indomethacin prevented both gallamine's potentiation and pilocarpine's inhibition of vagally induced bronchoconstriction. This new effect of indomethacin was not blocked by the addition of a 5-lipoxygenase inhibitor, AA861. However, the selective COX II inhibitor, L-745,337, had the same effect as indomethacin. Since exposure to ozone also caused neuronal M_2 receptors to become dependent upon cyclooxygenase the effects of viral infection are likely to be due to inflammation. Thus, despite apparent recovery of normal M_2 receptor function after viral infection or ozone, linkage of these receptors is chronically altered such that they become largely dependent on the activity of COX II. (*J. Clin. Invest.* 1996. 98:299–307.)

Key words: parasympathetic nerves • indomethacin • ozone • COX-II inhibitors • 5-lipoxygenase inhibitors

Introduction

Airway smooth muscle tone is largely controlled by the parasympathetic fibers of the vagus nerves (1). Acetylcholine (ACh)[1] is released from parasympathetic nerves and stimulates M_3 muscarinic receptors on airway smooth muscle causing contraction and bronchoconstriction (2, 3). Inhibitory M_2 muscarinic receptors on the postganglionic parasympathetic nerves provide a negative feedback mechanism to control acetylcholine release (4, 5). This effect can be demonstrated pharmacologically, as gallamine, by blocking the M_2 receptor on the nerves, increases ACh release and potentiates bronchoconstriction. Conversely, stimulating the M_2 receptor using pilocarpine decreases ACh release and inhibits vagally mediated bronchoconstriction.

Stimulating muscarinic receptors produces prostaglandins of the E and F series in the brain, heart, and electric organ of the torpedo fish (6–8). While the particular subtype of muscarinic receptor mediating prostaglandin production has not been identified in either the torpedo fish or brain, the heart is known to contain a pure population of M_2 muscarinic receptors (9, 10). Thus, M_2 receptors in the heart are linked to prostaglandin production.

Both endogenous prostaglandins and exogenous PGE_2 inhibit release of acetylcholine from the parasympathetic nerves in the lung (11, 12). Furthermore, blocking cyclooxygenase potentiates vagally induced bronchoconstriction (13). Thus, in the lung, both prostaglandins and neuronal M_2 receptors inhibit acetylcholine release. We have shown that indomethacin blocks neuronal M_2 muscarinic receptor function (14). In the presence of cyclooxygenase inhibitors, gallamine does not potentiate, nor pilocarpine inhibit, vagally induced bronchoconstriction (14). Thus, in nonpathogen-free guinea-pigs, neuronal M_2 muscarinic receptor function requires cyclooxygenase products.

Pathogen-free guinea pigs are bred and maintained in barrier housing, isolated from the microorganisms normally endemic among animal populations. The lungs of pathogen-free animals are different from the lungs of conventional (nonpathogen-free) animals (15). Histologically, the epithelial cells of pathogen-free animals are cuboidal rather than columnar. Neurogenic inflammatory responses are less intense in pathogen free animals.

Asthma attacks are frequently the result of viral airway infections (16). In nonasthmatics, viral infections increase airway responsiveness (17, 18). Much of this virus-induced hyperresponsiveness is vagally mediated as it can be blocked by atropine. Furthermore, the efferent limb of the reflex arc is abnormal, as the bronchoconstriction induced by stimulating the vagi electrically is increased (19). The M_3 receptors on the airway smooth muscle, which are responsible for cholinergic contraction, are normal in virus-infected airways (19–21). In contrast, acute viral infection causes temporary loss of M_2 receptor function (22). Four to six weeks after recovery from viral infection, the ability of gallamine to potentiate vagally induced bronchoconstriction returns, demonstrating recovery of M_2 receptor function (23, 24).

Portions of these data have been presented as abstracts.

Address correspondence to Dr. Allison D. Fryer, Department of Environmental Health Sciences, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland 21205. Phone: 410-955-3612; FAX: 410-955-0299.

Received for publication 22 January 1996 and accepted in revised form 3 May 1996.

1. *Abbreviations used in this paper:* ACh, acetylcholine; COX, cyclooxygenase; Ppi, pulmonary inflation pressure.

J. Clin. Invest.

0021-9738/96/07/0299/09 $2.00
Volume 98, Number 2, July 1996, 299–307

Our previous studies demonstrating that prostaglandins are required for neuronal M_2 muscarinic receptor function were carried out in nonpathogen free guinea pigs (14). These guinea pigs had been exposed to environmental dust, pollutants, or pathogens which may have stimulated an inflammatory response in the lungs. While M_2 receptor function appears normal after recovery from viral infection, it is possible that the coupling of the M_2 receptor to prostaglandins is changed. In the current experiments, we studied whether the M_2 receptor in pathogen-free guinea pigs are cyclooxygenase dependent, and whether viral infections can change the cyclooxygenase dependence of the M_2 receptor. We also determined the role of the inducible form of cyclooxygenase (COX II) and of 5-lipoxygenase in M_2 receptor function. Finally, we tested the effects of another inflammatory condition, that following ozone exposure, on the cyclooxygenase dependence of M_2 receptor function.

Materials and Methods

Animals. Dunkin-Hartley guinea pigs that were bred specific pathogen-free, and maintained in barrier housing (300–350 g; supplied by Hilltop Animal Farms, Scottsdale, PA) were used. All guinea pigs were shipped in filtered crates. Upon arrival, they were housed in wire bottom cages inside laminar flow hoods. Masks and gloves were worn by all personnel entering the room or handling the guinea pigs. Guinea pigs were handled in accordance with the standards established by the U.S.A. Animal Welfare Acts set forth in National Institute of Health guidelines and the Policy and Procedures Manual published by the Johns Hopkins University School of Hygiene and Public Health Animal Care and Use Committee.

Virus infection. Parainfluenza type I (Sendai virus; ATCC VR-105; American Type Culture Collection, Rockville, MD) was grown in embryonated chicken eggs for 2 d at 34°C. Infected fluid was cleared by low-speed centrifugation and stored in aliquots at −70°C. Viral content was determined by titration in Rhesus monkey kidney cell monolayers, as previously described (22). Viral content was determined as the multiple of the amount of stock solution required to produce infection in 50% of the Rhesus monkey kidney cell monolayers, the $TCID_{50}$ (TCID = tissue culture infectious dose).

Guinea pigs were anaesthetized using ketamine (45 mg/kg, i.m.) and xylazine (8 mg/kg, i.m.). Animals in the infected group were inoculated intranasally with 0.5 ml of stock solution that contained 10^6 $TCID_{50}$/ml, obtained by diluting the virus stock in Dulbecco's phosphate-buffered saline. Animals in the uninfected control group were inoculated intranasally with fluids obtained from virus-free eggs that were prepared and diluted in phosphate-buffered saline in the same way as the viral solutions. Control and infected animals were housed separately in pathogen-free environments and allowed to recover for 4–6 wk.

Ozone exposure. Specific pathogen-free guinea pigs were exposed to 2 ppm ozone for 4 h as previously described (25). Control animals were placed in the same chambers and exposed to filtered air. The ozone exposure chambers are located within rooms where the air supply is high efficiency particulate filtered to remove dust and pathogens. Masks and gloves were worn by all personnel entering the room or handling the guinea pigs. Thus, despite using ozone to induce an inflammatory response in the lungs, the specific pathogen-free guinea pigs were maintained in a pathogen-free environment.

Guinea pigs were exposed to either air or to 2 ppm ozone for 4 h. This dose causes an acute inflammatory response, acute loss of M_2 receptor function and hyperresponsiveness (25). All of these effects are temporary. 2 wk after ozone M_2 receptor function is restored. In addition the numbers of inflammatory cells recovered by bronchoalveolar lavage are back to normal and the response to vagal nerve stimulation as well as to intravenous bronchoconstrictor agents is also normal by 14 d after ozone (25, 26). Animals were studied 8 wk after exposure to ozone, at which time the lungs are histologically normal (27).

Measurement of viral antibodies in ozone exposed pathogen-free guinea pigs. At the end of each experiment, arterial blood was collected from each animal, allowed to clot, and the serum removed. The serum was diluted in 0.9% saline and heated for 30 min at 56°C. Heat-inactivated serum samples were sent to Microbiological Associates Inc. (Rockville, MD) for measurement of viral antibody titers to Sendai virus, reovirus type 3, pneumonia virus of mice, lymphocytic choriomeningitis virus, and simian myxovirus type 5.

Anesthesia and measurement of pulmonary inflation pressure. The guinea pigs were anesthetized with urethane (1.5 g/kg) injected intraperitoneally. This dose produces a deep anesthesia lasting 8–10 h (28), although none of the experiments reported here lasted for longer than 6 h. Heart rate and blood pressure were measured with a Spectromed pressure transducer (DTX; Spectromed, Oxnard, CA) connected to a cannula inserted into the carotid artery. Drugs were administered via cannulas placed in both jugular veins. Both vagus nerves were cut and the distal ends were placed on shielded electrodes immersed in a pool of mineral oil. The animal's body temperature was maintained at 37°C using a heating blanket. The animals were paralyzed with suxamethonium infused at 10 μg/kg/min and artificially ventilated via a tracheal cannula using a positive pressure, constant volume animal ventilator (tidal volume 1 ml/100 g body weight, 100 breaths/min; Harvard Apparatus Co., South Natick, MA). Pulmonary inflation pressure (Ppi) was measured from a sidearm of the tracheal cannula with a Spectromed (DTX) pressure transducer. All signals were displayed on a Grass polygraph (Grass Instruments, Quincy, MA). PO_2 and PCO_2 were measured from carotid arterial blood samples at the beginning and the end of each experiment to ensure that they were within the normal range (28) (Corning 170 pH /blood gas analyzer; Corning Glass, Medfield, MA).

Basal pulmonary inflation pressures of 90–120 mm H_2O were produced by positive pressure ventilation of the specific pathogen-free guinea pigs. Bronchoconstriction was measured as an increase in Ppi over the basal pressure produced by the ventilator (29). The sensitivity of the method was increased by taking the output Ppi signal from the driver to the input of the preamplifier of a second channel on the polygraph. Thus, baseline Ppi was recorded on one channel and increases in Ppi above the baseline were recorded on a second channel at a higher sensitivity. With this method, increases in Ppi as small as 2–3 mm H_2O can be recorded accurately.

Vagal stimulation and M_2 receptor function. Stimulation of both vagi at one minute intervals (2–15 Hz, 0.2 ms pulse duration, 4–10 V, 45 pulses/train) caused bronchoconstriction and a fall in heart rate. Both of these responses were rapidly reversed upon cessation of stimulation. For each series of experiments bronchoconstriction in response to stimulation of the vagus nerves was matched between animals at the beginning of each experiment by adjusting the voltage.

Because the response of the neuronal M_2 receptors to endogenous agonists (acetylcholine released from the nerves) is greater at high stimulus frequencies (4), the effects of antagonists are more readily apparent at the higher frequencies. Conversely, at low frequencies of stimulation exogenous agonists do not have to compete with endogenous acetylcholine, thus the effects of the exogenous agonists are more apparent. Because of this, experiments testing the function of the neuronal M_2 muscarinic receptor with gallamine and pilocarpine in the presence of indomethacin were carried out using a range of frequencies of stimulation (pulses/train were constant). The rest of the experiments, using only the antagonist gallamine, were carried out at 15 Hz.

All animals were pretreated with guanethidine (10 mg/kg, i.v.) to deplete noradrenaline and thus eliminate any involvement of the sympathetic nerves (30). When reproducible baseline responses to vagal stimulation had been obtained, gallamine (0.1–10.0 mg/kg, i.v.) or pilocarpine (1–100 mg/kg, i.v.) were administered in cumulative fashion, and the effects on vagally mediated bronchoconstriction and bradycardia were recorded. Doses of pilocarpine greater than 30 mg/

kg caused transient bronchoconstriction. Therefore the effect of these doses of pilocarpine on vagally induced bronchoconstriction was measured after the Ppi had returned to baseline. Doses of pilocarpine greater than 100 μg/kg were not used because they caused sustained bronchoconstriction via stimulation of the M_3 muscarinic receptors on the airway smooth muscle. At the end of each experiment vagally induced bronchoconstriction and bradycardia were abolished by atropine (1 mg/kg, i.v.) indicating that both of these responses were mediated via stimulation of muscarinic receptors.

M_2 receptor function and indomethacin. In pathogen-free guinea pigs, not exposed to viruses or ozone the effect of indomethacin on M_3 receptors on airway smooth muscle and on the M_2 receptors on the heart, was tested by measuring bronchoconstriction and bradycardia induced by acetylcholine (1 μg/kg, i.v.) before and 1 h after indomethacin. The effects of indomethacin on neuronal M_2 receptor function were determined by testing the effects of gallamine or pilocarpine on the response to vagal stimulation 1 h after indomethacin.

Effect of Indomethacin ±AA-861, or L-745,337 in postvirus and postozone exposed guinea pigs. 1 h after treatment with either indomethacin (1 mg/kg, i.v.), AA-861 (an inhibitor of 5-lipoxygenase activity; 10 mg/kg (31) ±indomethacin (1 mg/kg), or L-745,337 (a selective cyclooxygenase II inhibitor; 2 mg/kg), the function of the neuronal M_2 receptor was determined by testing the effects of gallamine or pilocarpine on the response to vagal stimulation, as described above. To assess the effect of indomethacin on M_3 receptors on airway smooth muscle and the M_2 receptors on the heart, bronchoconstriction and bradycardia induced by methacholine (1–10 μg/kg, i.v.) were measured before and 1 h after indomethacin.

Measurement of inflammatory cells. At the end of each experiment, the lungs were lavaged with phosphate-buffered saline that contained EDTA (0.2 M) and isoproterenol (10^{-4} M). Lavage fluids were centrifuged (400 *g*, 10 min) and the pellets resuspended in phosphate-buffered saline. Cells were counted in a hemocytometer. Slides made of lavaged cells using a cytospin were stained with Diff-Quik (American Scientific Products) to determine differential cell counts. Blood was also obtained via the carotid artery cannula. After eliminating erythrocytes by hypotonic lysis, the remaining leukocytes were counted as above.

In vitro tracheal response to electric field stimulation. Guinea pigs that had recovered from Sendai viral infection, or age matched controls were anesthetized with urethane and their tracheas removed and placed in Krebs-Henseleit solution (37°C) of the following composition: 117.5 mM NaCl, 5.6 mM KCl, 1.18 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.28 mM NaH_2PO_4, 25.0 mM $NaHCO_3$, and 5.55 mM dextrose, bubbled with 5% CO_2 and 95% O_2, pH 7.4. The tracheas were opened along the anterior (cartilaginous) surface, and two strips were mounted under isometric tension in 5 ml water jacketed organ baths (37°C). The tracheal smooth muscle was incubated with propranolol 10^{-6} M, and indomethacin 10^{-5} M in the absence and presence of prostaglandin E2 (10^{-10} and 10^{-8} M) for 1 h. Electrical field stimulation of the muscle (10 Hz, 2.0 ms pulse duration, 100 V, 5 s pulse train, at 1-min intervals) caused brief, contractions of the strips. Pilocarpine (10^{-8}–4×10^{-6} M) was added to the bath in cumulative fashion, and the effects on the response to electrical field stimulation were tested.

Drugs. The drugs used in these experiments were: urethane, guanethidine, suxamethonium, gallamine, pilocarpine, propranolol, atropine, and methacholine, all purchased from Sigma Chemical Co., (St. Louis, MO.) Indocin (indomethacin sodium trihydrate) was purchased from Merck, Sharpe & Dohme (West Point, PA) AA861 was purchased from Biomol. L-745,337 was a generous gift from Dr. Ian Rodger of Merck-Frosst (Montreal, Canada). All drugs were dissolved and diluted in 0.9% NaCl except L-745,337 which was dissolved in 5% dextrose in 10% DMSO and diluted in saline. The volume of DMSO used in these experiments was tested and found to have no effect on either baseline Ppi, vagally induced bronchoconstriction, or M_2 receptor function.

Statistics. All data are expressed as mean±SEM. The responses to vagal stimulation or to intravenous acetylcholine before and after indomethacin were compared using Student's paired *t*-test. Dose–response curves to intravenous methacholine before and after indomethacin, AA-861, or L-745,337 were compared using a two-way analysis of variance. Control responses to vagal stimulation were compared between groups of guinea pigs using Student's *t*-test for unpaired samples. The effects of pilocarpine and gallamine on vagally induced bronchoconstriction in the absence and presence of indomethacin, AA-861, or L-745,337 were compared using a two-way analysis of variance for repeated measures. A two-way analysis of variance was also used to test whether the effect of prior exposure to ozone or viral infection altered the response to gallamine. A *P* value of less than 0.05 was considered statistically significant.

Results

In pathogen-free guinea pigs, baseline heart rate was 280–320 beats/min and baseline Ppi was 90–120 mm H_2O. Electrical

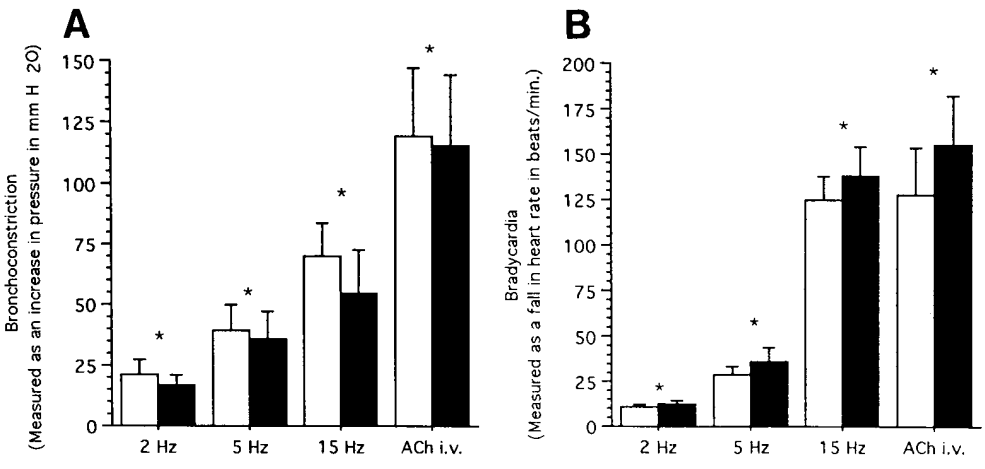

Figure 1. Indomethacin (1 mg/kg) causes a small but significant decrease in vagally and acetylcholine-induced bronchoconstriction in pathogen-free guinea pigs (*A*). In the heart, both vagally and acetylcholine-induced bradycardia were potentiated by indomethacin (*B*). Bronchoconstriction and bradycardia were induced either by electrical stimulation of the vagus nerves (2, 5, or 15 Hz, 0.2 ms, 5–15 V, 45 pulses/train) or by intravenously administered acetylcholine (1 μg/kg). Vagally and acetylcholine-induced bronchoconstriction and bradycardia are shown before (*open columns*) and 1 h after indomethacin (*shaded columns*). Each column is the mean of 10 animals±SEM shown as vertical bars. $*P < 0.05$ using Student's paired *t*-test).

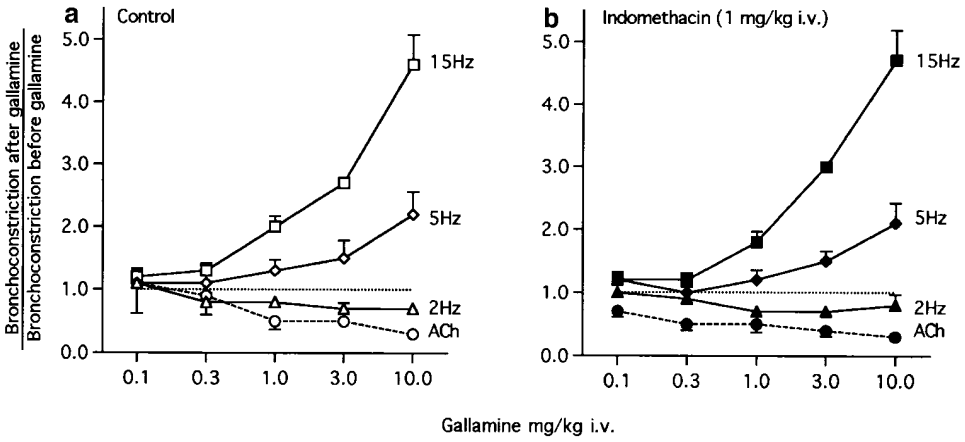

Figure 2. Indomethacin does not alter the potentiation of vagally induced bronchoconstriction by gallamine in pathogen-free guinea pigs. In the absence of indomethacin, gallamine dose-dependently potentiates vagally induced bronchoconstriction (0.2 ms, 14.5±4.4 V, 45 pulses/train) at 2 Hz (*triangles*), 5 Hz (*diamonds*) and 15 Hz (*squares*) but not bronchoconstriction induced by intravenous acetylcholine (1 μg/kg, *circles*) (*a*). The effect of gallamine on vagally and acetylcholine-induced bronchoconstriction is not altered by 1 mg/kg indomethacin (*b*). Vagally induced bronchoconstriction before gallamine at each frequency for control and indomethacin treated were: at 2 Hz, 6.9±1.2 and 7.2±1.0; at 5 Hz, 10.7±1.8 and 11.9±1.9; and at 15 Hz, 26.7±4.0 and 29.9±6.2; mm H_20. Acetylcholine-induced bronchoconstriction before gallamine was for control 43.6±9.5 and in indomethacin treated 60.8±15.2 mm H_20. Each point is the mean of five animals±SEM.

stimulation of the vagus nerves caused frequency related bronchoconstriction (measured as an increase in Ppi, Fig. 1 *A*) and bradycardia (Fig. 1 *B*). Intravenous acetylcholine (1 μg/kg) also caused bronchoconstriction and bradycardia. In these animals, gallamine (0.1–10.0 mg/kg, i.v.) potentiated, and pilocarpine (1–300 μg/kg, i.v.) inhibited vagally induced bronchoconstriction (Fig. 2 and 3, *left*). The effect of gallamine was greatest at high frequencies of stimulation.

In pathogen-free guinea pigs, indomethacin did not alter baseline Ppi or heart rate. However, 1 mg/kg indomethacin caused a small but statistically significant decrease in the bronchoconstriction response to both vagal stimulation and intravenous acetylcholine when measured 60 min after administration (Fig. 1 *A*). A small increase in bradycardic responses to both electrical stimulation of the vagus and intravenous acetylcholine was also seen after indomethacin in these animals (Fig. 1 *B*).

In these pathogen-free animals, gallamine potentiated, and pilocarpine inhibited, the bronchoconstriction response to vagal stimulation equally in the presence and absence of indomethacin (Fig. 2 and 3). Likewise, in these animals the response to intravenous acetylcholine was also inhibited by gallamine (due to the effect of gallamine on M_3 receptors) equally in the presence and absence of indomethacin (Fig. 2, *circles*).

In guinea pigs that had recovered from viral infections for 6 to 8 wk, the bronchoconstriction and bradycardic responses to vagal stimulation and to intravenous methacholine were similar to those in pathogen-free guinea pigs (data not shown). Furthermore, M_2 receptor function appeared to be normal in these animals, as shown by the dose-dependent potentiation of vagally mediated bronchoconstriction at 15 Hz by gallamine (Fig. 4, *a* and *b*, *open circles*). However, in guinea pigs that had recovered from viral infections, the function of the M_2 receptor was substantially decreased by indomethacin (Fig. 4 *b*, *closed triangles*). In the absence of indomethacin gallamine potentiated vagally mediated bronchoconstriction 5-fold in these animals, whereas after indomethacin treatment, the maximal potentiation by gallamine was only 2.5-fold.

When M_2 receptor function in the heart was tested using intravenous methacholine (1–10 μg/kg), indomethacin did not affect methacholine-induced bradycardia in either pathogen-free or postvirus guinea pigs (data not shown). When M_3 re-

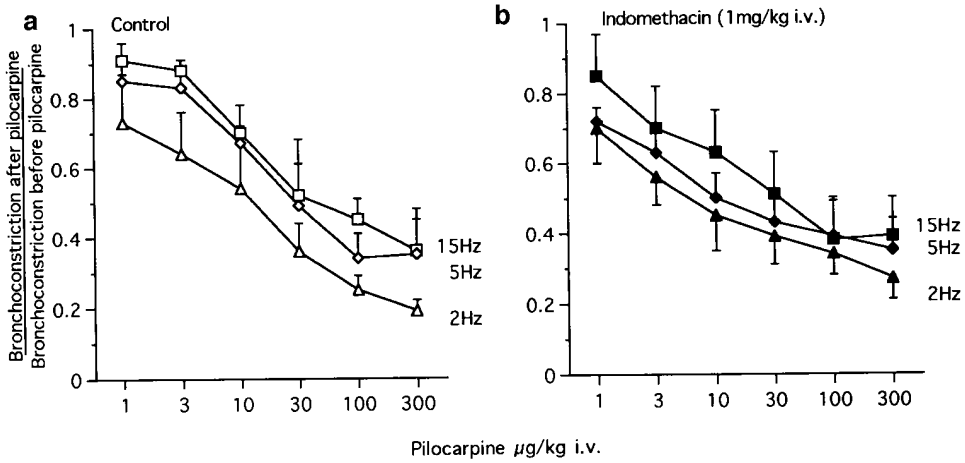

Figure 3. Indomethacin does not alter the inhibition of vagally induced bronchoconstriction by pilocarpine in pathogen-free guinea pigs. In the absence of indomethacin, pilocarpine dose-dependently inhibits vagally induced bronchoconstriction (0.2 ms, 22.4±7.4 V, 45 pulses/train) at 2 Hz (*triangles*), 5 Hz (*diamonds*), and 15 Hz (*squares*), (*a*). The effect of pilocarpine on vagally induced bronchoconstriction is not altered by 1 mg/kg indomethacin (*b*). Vagally induced bronchoconstriction before pilocarpine at each frequency for control and indomethacin treated were: at 2 Hz, 34.4±6.25 and 35.7±15.9; at 5 Hz, 70.0±9.9 and 73.8±34.5; and at 15 Hz; 133.5±20.1 and 192.5±79.9 mm H20. Each point is the mean of four animals±SEM.

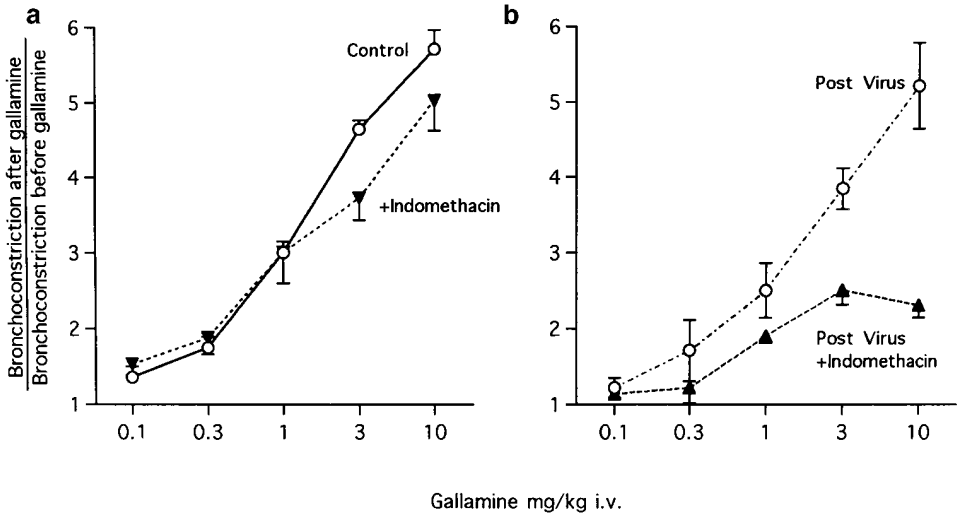

Figure 4. The effect of indomethacin on potentiation of vagally induced bronchoconstriction by gallamine is altered after virus infection. In guinea pigs acutely infected with parainfluenza virus, and then allowed to recover for 8 wk, gallamine potentiated vagally induced bronchoconstriction (all at 15 Hz, 0.2 ms, 13.5±2.8 V, 45 pulses/train) in the absence of indomethacin (*postvirus: open circles* and *dashed line*, *b*). This potentiation by gallamine was not significantly different from the effect of gallamine on guinea pigs exposed to control medium and then allowed to recover for 8 wk (*control*: *open circles* and *solid line*, *a*). Treatment with 1 mg/kg indomethacin did not alter the potentiation of vagally induced bronchoconstriction by gallamine in control animals (*indomethacin*: *solid triangles* and *dashed line*, *a*) but significantly attenuated the potentiation by gallamine in postvirus guinea pigs (*virus + indomethacin: solid triangles* and *dashed line*, *b*). Vagally induced bronchoconstriction before gallamine was not different between groups (control, 20.0±2.8; indomethacin, 15.5±1.8; postvirus, 13.9±1.7; postvirus and indomethacin, 13.5±0.9 mm H_2O). Each point is the mean of four to six animals±SEM.

ceptor function on the airway smooth muscle was tested using intravenous methacholine (1–10 μg/kg), a very small, but statistically significant, increase in bronchoconstriction was observed after indomethacin treatment in postvirus guinea pigs (data not shown).

To determine whether the development of cyclooxygenase dependence of M_2 receptor function after viral infection was specific to viral infection or a general phenomenon associated with pulmonary inflammation, pathogen-free guinea pigs were exposed to 2 ppm ozone or filtered air for 4 h. 8 wk later both air and ozone exposed guinea pigs had normal baseline Ppi, baseline heart rates, and identical yields of inflammatory cells from lung lavage (data not shown). Gallamine potentiated vagally induced bronchoconstriction equally in both groups (Fig. 5, *a* and *b*, *open circles*). In the age and weight-matched air-exposed guinea pigs, indomethacin did not significantly alter the response to gallamine (Fig. 5 *a*, *closed triangles*). However, in those guinea pigs previously exposed to ozone, gallamine did not potentiate vagally induced bronchoconstriction in the presence of indomethacin (Fig. 5 *b*, *closed triangles*). There were no detectable viral antibodies in the sera of any of the ozone exposed animals indicating that they were still pathogen free.

To test whether diversion of arachidonic acid into the lipoxygenase pathway, with production of leukotrienes, was responsible for the loss of M_2 receptor function after indomethacin, the effect of indomethacin on M_2 receptor function was studied in postvirus-infected guinea pigs after pretreatment

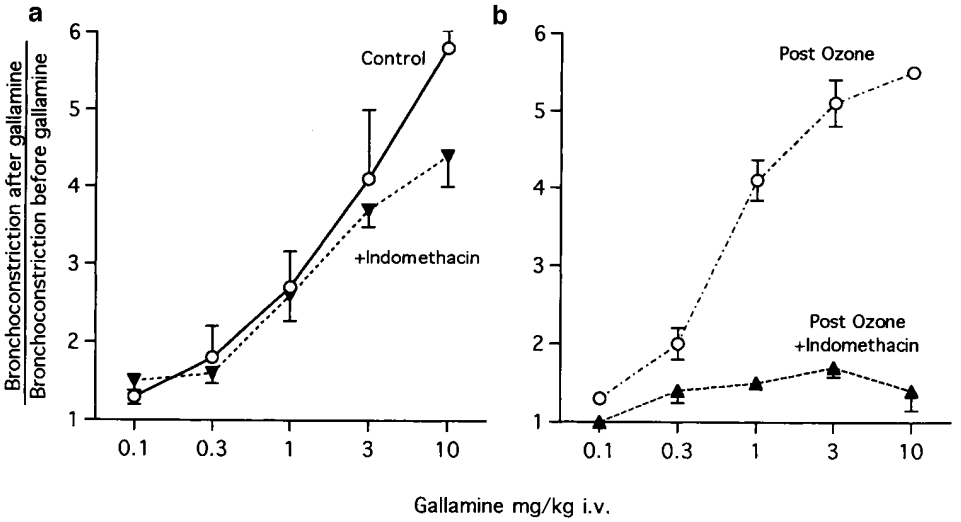

Figure 5. The effect of indomethacin on potentiation of vagally induced bronchoconstriction by gallamine is altered following exposure to ozone. In guinea pigs acutely exposed to ozone, and then allowed to recover for 8 wk, gallamine potentiated vagally induced bronchoconstriction (all at 15 Hz, 0.2 ms, 10.3±2.7 V, 45 pulses/train) in the absence of indomethacin (*post-ozone*: *open circles* and *dashed line*, *b*). This potentiation by gallamine was not significantly different from the effect of gallamine on guinea pigs exposed to air and then allowed to recover for 8 wk (*control*: *open circles* and *solid line*, *a*). Treatment with 1 mg/kg indomethacin did not alter the potentiation of vagally induced bronchoconstriction by gallamine in air exposed animals (*indomethacin*: *solid triangles* and *dashed line*, *a*) but significantly attenuated the potentiation by gallamine in ozone-exposed guinea pigs (*ozone and indomethacin*: *solid triangles* and *dashed line*, *b*). Vagally induced bronchoconstriction before gallamine was not different between groups (*control*, 18.3±2.4; *indomethacin*, 18.3±1.0; *postozone*, 22.0±1.2; *ozone and indomethacin*, 18.7±1.8 mm H_2O). Each point is the mean of four animals±SEM.

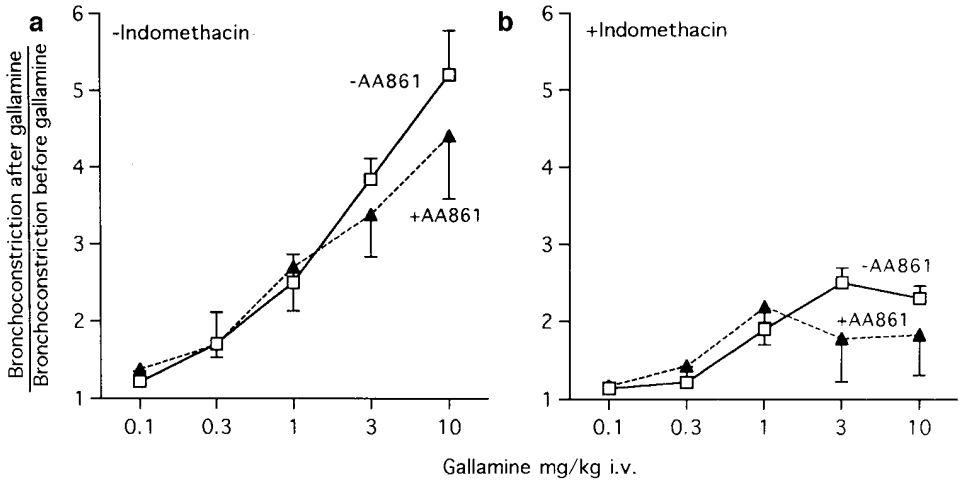

Figure 6. The effect of indomethacin on potentiation of vagally induced bronchoconstriction by gallamine is not affected by the 5-lipoxygenase inhibitor AA861. In guinea pigs infected with parainfluenza virus, and then allowed to recover for 8 wk, gallamine potentiated vagally induced bronchoconstriction equally in the absence (*squares*) or presence (*triangles*) of AA-861 (10 mg/kg, i.v.; *a*). The inhibitory effect of indomethacin was not affected by pretreatment with AA861 (*b*). Vagally induced bronchoconstriction before gallamine was not different between groups (*control*, 13.9±1.7; AA861, 16.0±0.9; *indomethacin*, 13.5±0.9; AA861 and indomethacin, 21.5±4.8 mm H_2O). Each point is the mean of three to six animals±SEM.

with the 5-lipoxygenase inhibitor AA861 (10 mg/kg, i.v.). Treatment with AA861 did not in itself affect the potentiation of vagally mediated bronchoconstriction by gallamine in postvirus guinea pigs (Fig. 6 *a*). In these animals, potentiation of vagally mediated bronchoconstriction by gallamine was blocked by indomethacin equally in the presence or absence of AA861 (Fig. 6 *b*). Treatment with AA861 did not affect the bradycardic response to intravenous methacholine (1–10 μg/kg), and caused a very small potentiation of methacholine-induced bronchoconstriction (data not shown).

To determine whether inhibition of the constitutive (COX I) or the inducible (COX II) form of cyclooxygenase was responsible for the effects of indomethacin, a selective COX II inhibitor L-745,337 (2 mg/kg, i.v.) was used in place of indomethacin. In postvirus guinea pigs, L-745,337 blocked potentiation of vagally mediated bronchoconstriction by gallamine to a degree similar to that seen after indomethacin (Fig. 7). Treatment with L-745,337 did not affect the bradycardic response to intravenous methacholine (1–10 μg/kg), and caused a very small potentiation of methacholine-induced bronchoconstriction (data not shown).

In tracheal tissues from postvirus infected guinea pigs, pilocarpine inhibited the contractile response to electrical field stimulation (Fig. 8). Pilocarpine's effect was decreased by indomethacin (10^{-5} M). Even though PGE_2 10^{-8} M decreased the contractile response to electrical field stimulation by 50%, the ability of pilocarpine to further inhibit electrical field stim-

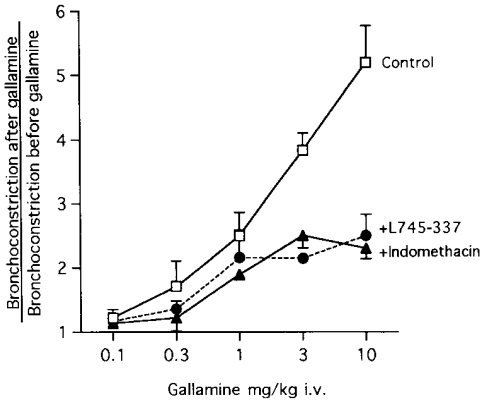

Figure 7. The effect of the cyclooxygenase II inhibitor L-745,337 on potentiation of vagally induced bronchoconstriction by gallamine was identical to the effect of indomethacin. In guinea pigs infected with parainfluenza virus, and then allowed to recover for 8 wk, gallamine potentiated vagally induced bronchoconstriction (*square*). Both L-745,337 (2 mg/kg, i.v.; *circles*) and indomethacin (1 mg/kg, i.v.; *triangles*) inhibited the ability of gallamine to potentiate vagally induced bronchoconstriction. Vagally induced bronchoconstriction before gallamine was not different between groups (*control*, 13.9±1.7; L-745,337, 16.3±1.2; *indomethacin*, 13.5±0.9 mm H_2O). Each point is the mean of five to six animals±SEM.

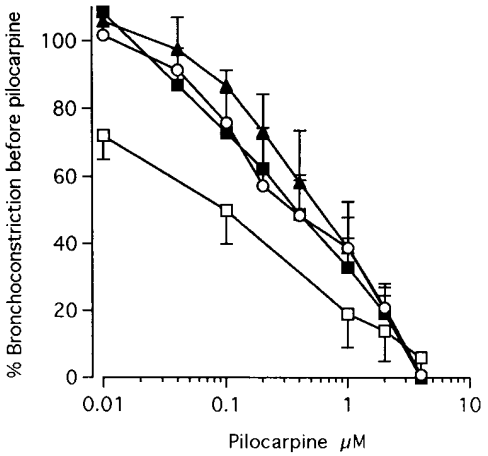

Figure 8. Indomethacin decreases the inhibitory effect of pilocarpine on the contractile response to electrical field stimulation (100V, 15Hz, 0.4 ms pulse duration, 5 s pulse train); the effect of pilocarpine is not restored by adding prostaglandin E_2 to the bathing medium. Tracheal tissues were taken from guinea pigs that had recovered from parainfluenza virus infection for 8 wk. Cumulative dose response curves for pilocarpine were generated in untreated tissues (*open squares*), tissues that had been exposed to indomethacin (10^{-5} M, *circles*), or indomethacin + prostaglandin E_2 at concentrations of either 10^{-10} M (*closed squares*) or 10^{-8} M (*triangles*). Each doseresponse curve represents data from five to eight tissues, each from a separate guinea pig.

ulation induced contractile responses was not affected by exogenous prostaglandin E_2 (10^{-10}–10^{-8} M; Fig. 8).

Discussion

Eight weeks after viral infection or exposure to ozone the function of the M_3 muscarinic receptors on airway smooth muscle was not altered since the contractile responses to i.v. acetylcholine and methacholine were not different (data not shown). Since the function of the postjunctional M_3 receptors is not altered during acute viral infection (22) or immediately following exposure to ozone when the inflammatory response is greatest (25) it is not surprising that there are no long term effects of inflammation on M_3 function.

The function of inhibitory neuronal M_2 muscarinic receptors in the airways, which is lost acutely after viral infections (22) or exposure to ozone (25), recovers after 2–4 wk (23). Thus the ability of gallamine to potentiate vagally mediated bronchoconstriction, and of pilocarpine to inhibit vagally mediated bronchoconstriction, by blocking and stimulating M_2 receptors, respectively, appears normal in guinea pigs that have recovered from viral infection or exposure to ozone (see Figs. 2 and 3).

Despite the apparent recovery of neuronal M_2 muscarinic receptor function in the lungs of animals that have recovered from a viral infection, the mechanism by which the M_2 receptor functions is not in fact the same as in pathogen-free animals. In pathogen-free guinea pigs the function of inhibitory neuronal M_2 receptors in the airways is largely independent of the generation of cyclooxygenase products since gallamine potentiated and pilocarpine inhibited vagally induced bronchoconstriction in the presence of indomethacin in these animals (Figs. 2 and 3). In contrast, after recovery from acute viral infection, M_2 receptor function becomes nearly totally dependent on cyclooxygenase activity and is largely blocked by treatment with indomethacin (Fig. 4). This was not due to age or weight, since in the age and weight matched, sham-infected animals, indomethacin had no effect on the potentiation of vagally induced bronchoconstriction by gallamine (Fig. 4). This effect on M_2 receptor function is clearly the result of the cyclooxygenase-inhibiting properties of indomethacin, as the same result can be obtained by using the cyclooxygenase inhibitor naproxen in nonpathogen-free guinea pigs (but not with the inactive enantiomer of naproxen; 32).

The lack of effect of indomethacin on M_2 receptor function in pathogen free guinea pigs is reflected in the failure of indomethacin to potentiate vagally induced bronchoconstriction in these animals (Fig. 1). In nonpathogen-free guinea pigs, indomethacin increases vagally induced bronchoconstriction by blocking M_2 receptor function (32).

It has been suggested that some of the deleterious effects of indomethacin and other cyclooxygenase inhibitors on airway function in patients with asthma are due to redirection of arachidonic acid into the 5-lipoxygenase pathway, generating bronchoconstricting leukotrienes (33, 34). This mechanism does not appear to be responsible for the effects of cyclooxygenase inhibitors on M_2 receptor function, as the 5-lipoxygenase inhibitor AA-861 does not prevent loss of M_2 receptor function after indomethacin treatment of postvirus animals (Fig. 6).

In postvirus animals M_2 muscarinic receptor function is blocked by treatment with L-745,337 (Fig. 7), which inhibits COX II activity at less than one-five hundredth the concentration required to inhibit COX I activity (35). Thus, it appears to be the inducible form of cyclooxygenase (COX II) that is required for M_2 receptor function in the postvirus animals.

Muscarinic receptor stimulation causes release of prostaglandins in the brain (7), endothelium (8), and electoplax (6). We postulate that, in postvirus infected guinea pigs, stimulation of M_2 muscarinic receptors causes release of prostaglandins that then decrease the release of acetylcholine from the nerve endings. Such effects of prostaglandin E_2 have been demonstrated both in functional studies (13) and by direct measurement of acetylcholine release (36). An alternative explanation of the effects of cyclooxygenase inhibitors is that prostaglandins, produced independently of M_2 receptor stimulation, are required for M_2 receptor function. We tested this possibility in vitro, adding prostaglandin E_2 (the principal cyclooxygenase product in the airway; 37) to the bathing medium in indomethacin-treated airway segments. No effect of exogenous prostaglandin on M_2 muscarinic receptor function was found in these experiments, despite the concentration of prostaglandin being great enough to significantly inhibit vagally mediated bronchoconstriction (Fig. 8). Thus it does not appear that prostaglandins have an effect on M_2 receptor function. It appears more likely that stimulating the M_2 receptor causes production of prostaglandins that, in turn, inhibit acetylcholine release.

Virus-induced inflammation might be expected to participate in making the M_2 muscarinic receptor dependent on COX II, as expression of this enzyme is induced by some inflammatory mediators (see reference 38). To determine whether inflammation caused by a nonviral stimulus had similar effects on M_2 receptor function, we tested the effects of ozone inhalation. Ozone exposure converted the neuronal M_2 receptors in pathogen-free guinea pig lungs to prostaglandin-dependent receptors like those in the non pathogen-free guinea pigs (Fig. 5). Acute exposure to 2 ppm ozone for 4 h results in an immediate influx of inflammatory cells into the lungs and acute loss of function of the neuronal M_2 receptors (25, 26). This loss of function is temporary; it appears immediately following ozone exposure and resolves by two weeks following exposure (25). At eight weeks after ozone, the response of the M_2 receptors to gallamine was identical to the air-exposed guinea-pigs (Fig. 5, *open circles*) indicating that the immediate effects of ozone exposure on the M_2 receptors had resolved. However, in the guinea-pigs that were previously exposed to ozone, indomethacin blocked the potentiation of vagally induced bronchoconstriction by gallamine (Fig. 5). Thus it appears that it is inflammation, rather than another effect specific to viruses, that causes long-term changes in M_2 receptor dependence on cyclooxygenase.

We have previously demonstrated that acute viral infection does not impair M_2 muscarinic receptor function in the heart (22, 25). This is not surprising in that the virus, and its inflammatory response, are largely localized to the lungs. In the present study, we have shown a similar limitation of the chronic effects of viral infection and ozone exposure to the M_2 receptors of the lungs. Thus bradycardia, induced by stimulating M_2 receptors in the heart, in response to exogenous agonists or vagal stimulation was not altered by indomethacin in these guinea pigs.

Acute loss of M_2 muscarinic receptor function after infection with virus, or exposure to antigen or ozone, depends on lung inflammation (39, 40). The similar chronic effects of both viral infection and ozone exposure on the cyclooxygenase de-

pendence of M_2 receptor function suggest that inflammation is also important in causing these changes. However, persistent inflammation is not required to maintain cyclooxygenase dependence, as lung lavage in the postvirus-infected animals did not show an increase in leukocytes. Similarly, the change in M_2 receptor dependence on cyclooxygenase after recovery from ozone exposure cannot be explained by the continued presence of inflammatory cells since the inflammatory cell populations, measured in bronchoalveolar lavage and by histological examination, are back to normal by four weeks post ozone (25, 27).

Our previous study demonstrating cyclooxygenase dependence of M_2 receptor function in nonpathogen-free guinea pigs (32) suggested that this change may be permanent. However, in a single guinea pig tested one year after viral infection, indomethacin did not affect the ability of pilocarpine to inhibit vagally induced bronchoconstriction compared to an age-matched control (data not shown). Thus virus-induced cyclooxygenase dependence may not be permanent.

While the continued presence of inflammatory cells is not necessary for dependence of M_2 receptor function on prostaglandins it is possible that the inflammatory process may have triggered other events within the lung. A variety of pro-inflammatory substances, including interleukin-1, lipopolysaccharide, and platelet-activating factor, may induce expression of COX II, which appears to be responsible for the cyclooxygenase dependence of M_2 receptor function in these studies. Cyclooxygenase-II has been demonstrated in neurons in the central nervous system, where it appears to be increased by synaptic activity as in seizures (41). It is therefore possible that the increase in acetylcholine release and synaptic activity caused by acute loss of neuronal M_2 muscarinic receptor function during the viral infection contributes to the chronic induction of COX-II.

While inflammation can induce expression of COX II, there is no good explanation of how a receptor can alter coupling to second messengers as suggested by the data in this manuscript. This change in receptor linkage seen after inflammation is, to our knowledge, the first example of such a change. Stimulation of M_2 muscarinic receptors can have multiple effects within the same cell (such as inhibition of adenylate cyclase and stimulation of phospholipase A2 and phospholipase C), all achieved via the same Gi protein (42). After stimulation of the receptor, the heterotrimeric G protein dissociates and the α subunit inhibits adenylate cyclase. The product of this enzyme, cAMP, increases acetylcholine release in airway parasympathetic nerves (43). At the same time the β subunit of the Gi protein can activate phospholipase A2 (44), the rate limiting step in prostaglandin synthesis. Thus the M_2 receptor has the potential to simultaneously decrease cAMP and increase prostaglandin production, both of which inhibit acetylcholine release.

Alternatively, a new pathway may be activated by inflammatory mediators, which the muscarinic receptors can influence. For example, when phospholipase A2 activity is stimulated via purinergic receptors, stimulation of M_2 receptors substantially potentiates this stimulation by a mechanism thought to involve protein kinase C (45). Thus, it is possible in vivo that phospholipase A2 activity is stimulated via another receptor after inflammation, and that this stimulation is then potentiated via M_2 muscarinic receptors.

Cyclooxygenase inhibitors such as indomethacin and aspirin can induce asthma attacks in a subgroup of patients with asthma. Although the role of vagal reflexes in aspirin-sensitive asthma has not been tested, several animal models suggest that inhibiting cyclooxygenase can increase release of acetylcholine in the airways. Ito showed that dogs wheeze after receiving indomethacin, and that this was due to increased vagally mediated bronchoconstriction (12). Similarly, in nonpathogen-free guinea pigs the bronchoconstriction response to vagal stimulation is increased by indomethacin (32).

In nonasthmatic humans it has been demonstrated that the neuronal M_2 muscarinic receptor is not dependent upon prostaglandins (46). However, it is tempting to speculate that in aspirin-sensitive asthma previous exposure to inflammatory mediators at some critical period has altered coupling of the neuronal M_2 muscarinic receptor to cyclooxygenase. Loss of neuronal M_2 receptor function has been linked to asthma (47, 48), and it may be that in some people with asthma the M_2 receptor requires cyclooxygenase to function.

Thus, viral infections and exposure to ozone, probably by causing inflammation, cause a chronic change in M_2 receptors on the airway parasympathetic nerves, resulting in their dependence on the inducible form of cyclooxygenase, COX II. Thus in nonpathogen-free animals, and in those that have been exposed to ozone (another proinflammatory stimulus), M_2 muscarinic receptor function can be largely blocked by both indomethacin and L-745,337, an inhibitor of COX II. This effect does not involve diversion of arachidonic acid into the 5-lipoxygenase pathway, nor is it the result of chronic inflammation.

Acknowledgments

The authors wish to thank Dr. George Jakab for the use of his inhalation facilities supported by grant ES-03819, and Amanda Kim for technical support.

This work was supported by grants ES-03819 and HL-44727 (A.D. Fryer), HL-47120 and AI-37163 (D.B. Jacoby), HL-09116 (R.M. Kahn) and HL-10342 (O.A. Okanlami) from the National Institutes of Health, and by grants from the Council for Tobacco Research, American Lung Association, and American Heart Association.

References

1. Boushey, H.A. 1985. Role of the vagus nerves in bronchoconstriction in humans. *Chest.* 5:197S–201S.

2. Barnes, P.J., P. Minette, and J. Maclagan. 1988. Muscarinic receptor subtypes in the airways. *Trends in Pharmacol. Sci.* 9:412–416.

3. Roffel, A.F., C.R.S. Elzinga, and J. Zaagsma. 1990. Muscarinic M3 receptors mediate contraction of human central and peripheral airway smooth muscle. *Pulmon. Pharmacol.* 3:47–51.

4. Fryer, A.D., and J. Maclagan. 1984. Muscarinic inhibitory receptors in pulmonary parasympathetic nerves in the guinea-pig. *Br. J. Pharmacol.* 83:973–978.

5. Fryer, A.D., and D.B. Jacoby. 1993. Effect of inflammatory cell mediators on M2 muscarinic receptors in the lungs. *Life Sci.* 52:529–536.

6. Pinchasi, I., M. Burstein, and D. Michaelson. 1984. Metabolism of arachadonic acid and prostaglandins in the torpedo electric organ: modulation by the presynaptic muscarinic acetylcholine receptor. *Neurosci.* 13:1359–1364.

7. Reichman, M., W. Nen, and L. Hokin. 1987. Highly sensitive muscarinic receptors in the cerebellum are coupled to prostaglandin formation. *Biochem. Biophys. Res. Commun.* 146:1256–1261.

8. Jaiswal, N., and K.U. Malik. 1988. Prostaglandin synthesis elicited by cholinergic stimuli is mediated by activation of M2 muscarinic receptors in rabbit heart. *J. Pharmacol. Exp. Thera.* 245:59–66.

9. Kubo, T., K. Sukuda, A. Mikami, A. Maeda, H. Takahashi, M. Mishina, T. Haga, K. Haga, A. Ichiyama, K. Kangawa, M. Kojima, H. Matsuo, T. Hirose, and S. Numa. 1988. Cloning, sequencing, and expression of complementary DNA encoding the muscarinic acetylcholine receptor. *Nature (Lond.).* 323:411–416.

10. Peralta, E.G., A. Ashkenazi, J.W. Winslow, D.H. Smith, J. Ramachandran, and D.J. Capon. 1987. Distinct primary structures, ligand-binding proper-

ties and tissue-specific expression of four human muscarinic acetylcholine receptors. *EMBO J.* 6:3923–3929.

11. Daniel, E., C. Davis, and V. Sharma. 1987. Effects of endogenous and exogenous prostaglandin in neurotransmission in canine trachea. *Can. J. Physiol. Pharmacol.* 65:1433–1441.

12. Ito, Y., and K. Tajima. 1981. Actions of indomethacin and prostaglandins on neuro-effector transmission in the dog trachea. *J. Physiol.* 319:379–392.

13. Walters, E.H., P.M. O'Byrne, L.M. Fabbri, P.D. Graf, M.J. Holtzman, and J.A. Nadel. 1984. Control of neurotransmission by prostaglandins in canine trachealis smooth muscle. *J. Appl. Physiol.* 57:129–134.

14. Fryer, A., and O. Okanlami. 1993. Neuronal M2 muscarinic receptor function in guinea-pig lungs is inhibited by indomethacin. *Am. Rev. Respir. Dis.* 147:559–564.

15. McDonald, D.M. 1988. Respiratory tract infections increase susceptibility to neurogenic inflammation in rat trachea. *Am. Rev. Respir. Dis.* 137:1432–1440.

16. Johnston, S.L., P.K. Pattemore, G. Sanderson, S. Smith, F. Lampe, L. Josephs, P. Symington, S. Otoole, S.H. Myint, D. Tyrrell, and S. T. Holgate. 1995. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *Brit. Med. J.* 310:1225–1229.

17. Empey, D.W., L.A. Laitinen, L. Jacobs, W.M. Gold, and J.A. Nadel. 1976. Mechanisms of bronchial hyperreactivity in normal subjects following upper respiratory tract infection. *Am. Rev. Respir. Dis.* 113:523–527.

18. Aquilina, A.T., W.J. Hall, R.G. Douglas, and M.J. Utell. 1980. Airway reactivity in subjects with viral upper respiratory tract infections: the effects of exercise and cold air. *Am. Rev. Respir. Dis.* 122:3–10.

19. Buckner, C.K., V. Songsiridej, E.C. Dick, and W.W. Busse. 1985. In vivo and in vitro studies of the use of the guinea pig as a model for virus-provoked airway hyperreactivity. *Am. Rev. Respir. Dis.* 132:305–310.

20. Jacoby, D.B., J. Tamaoki, D.B. Borson, and J.A. Nadel. 1988. Influenza infection causes airway hyperresponsiveness by decreasing enkephalinase. *J. Appl. Physiol.* 64:2653–2658.

21. Dusser, D.J., D.B. Jacoby, T.D. Djokic, I. Rubenstein, D.B. Borson, and J.A. Nadel. 1989. Virus induces airway hyperresonsiveness to tachykinins: role of neutral endopeptidase. *J. Appl. Physiol.* 67:1504–1511.

22. Fryer, A.D., and D.B. Jacoby. 1991. Parainfluenza virus infection damages inhibitory M2 muscarinic receptors on pulmonary parasympathetic nerves in the guinea-pig. *Br. J. Pharmacol.* 102:267–271.

23. Sorkness, R., J.J. Clough, W.L. Castleman, and R.J. Lemanske. 1994. Virus-induced airway obstruction and parasympathetic hyperresponsiveness in adult rats. *Am. J. Respir. Crit. Care Med.* 150:28–34.

24. Kahn, R., A. Fryer, and D.B. Jacoby. 1996. Effect of indomethacin on inhibitory M2 muscarinic receptor function in guinea-pig airways: Influence of previous viral infections. *Am. Rev. Respir. Crit. Care Med.* 149:899a.

25. Schultheis, A., D. Bassett, and A. Fryer. 1994. Ozone-induced airway hyperresponsiveness and loss of neuronal M2 muscarinic receptor function. *J. Appl. Physiol.* 76:1088–1097.

26. Schultheis, A.H., and D.J.P. Bassett. 1991. Inflammatory cell influx into ozone exposed guinea-pig lung interstitial and airway spaces. *Agents and Actions.* 34:270–273.

27. Bassett, D.J.P., E. Bowen-Kelly, E.L. Brewster, C.L. Elbon, S.S. Reichenbaugh, T. Bunton, and J.S. Kerr. 1988. A reversible model of acute lung injury based on ozone exposure. *Lung.* 166:355–369.

28. Green, C.J. 1982. Animal Anaesthesia. *In* Laboratory Animal Handbooks. Laboratory Animals Ltd. London. 8:81–82.

29. Dixon, W.E., and T.G. Brody. 1903. Contributions to the physiology of the lungs. Part 1, the bronchial muscles and their innervation and the action of drugs upon them. *J. Physiol.* 29:97–173.

30. Blaber, L.C., and A.D. Fryer. 1985. The response of the cat airways to histamine in vivo and in vitro. *Br. J. Pharmacol.* 84:309–316.

31. Sakuma, Y., H. Tsunoda, S. Katayama, S. Abe, I. Yamatsu, and K. Katayama. 1991. Determination of plasma leukotrienes in antigen-induced bronchoconstrictive guinea pigs. *Prostaglandins.* 41:315–329.

32. Okanlami, O.A., C.A. Hirshman, and A.D. Fryer. 1992. Pulmonary M2 receptor function depends on cyclooxygenase products in normal but not in specific pathogen free guinea-pigs. *Am. Rev. Respir. Dis.* 145:A615.

33. Christie, P.E., C.M. Smith, and T.H. Lee. 1991. The potent and selective sulfidopeptide leukotriene antagonist, SK&F 104353, inhibits aspirin-induced asthma. *Am. Rev. Respir. Dis.* 144:957–958.

34. Israel, E., A.R. Fischer, M.A. Rosenberg, C.M. Lilly, J.C. Callery, J. Shapiro, J. Cohn, P. Rubin, and J.M. Drazen. 1993. The pivotal role of 5-lipoxygenase products in the reaction of aspirin-sensitive asthmatics to aspirin. *Am. Rev. Respir. Dis.* 148:1447–1451.

35. Chan, C.C., S. Boyce, C. Brideau, A.W. Ford-Hutchinson, R. Gordon, D. Guay, R.G. Hill, C.S. Li, J. Mancini, M. Penneton, et al. 1995. Pharmacology of a selective cyclooxygenase-2 inhibitor, l-745,337— a novel nonsteroidal anti-inflammatory agent with an ulcerogenic sparing effect in rat and nonhuman primate stomach. *J. Pharmacol. Exp. Ther.* 274:1531–1537.

36. Shore, S., B. Collier, and J.G. Martin. 1987. Effect of endogenous prostaglandins on acetylcholine release from dog trachealis muscle. *J. Appl. Physiol.* 62:1837–1844.

37. Widdicombe, J.H., I.F. Ueki, D.L. Emery, D. Margolskee, J. Yergey, and J. Nadel. 1989. Release of cyclooxygenase products from primary cultures of tracheal epithelia of dog and human. *Am. J. Physiol.* 257:L361–L365.

38. Vane, J.R., J.A. Mitchell, I. Appleton, A. Tomlinson, B.D. Bishop, J. Croxtall, and D.A. Willoughby. 1994. Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. *Proc. Natl. Acad. Sci. USA.* 91:2046–2050.

39. Gambone, L.M., and A.D. Fryer. 1992. Inflammatory cell depletion preserves neuronal M2 receptor function in guinea-pigs exposed to ozone. *Am. Rev. Respir. Dis.* 145:A614.

40. Fryer, A.D., K.A. Yarkony, and D.B. Jacoby. 1994. The effect of leukocyte depletion on pulmonary M2 muscarinic receptor function in parainfluenza virus-infected guinea-pigs. *Br. J. Pharmacol.* 112:588–594.

41. Yamagata, K., K.I. Andreasson, W.E. Kaufmann, C.A. Barnes, and P.F. Worley. 1993. Expression of a mitogen-inducible cyclooxygenase in brain neurons: Regulation by synaptic activity and glucocorticoids. *Neuron.* 11:371–386.

42. Hunt, T.W., R.C. Carroll, and E.G. Peralta. 1994. Heterotrimeric G proteins containing G alpha i3 regulate multiple effector enzymes in the same cell. Activation of phospholipases C and A2 and inhibition of adenylyl cyclase. *J. Biol. Chem.* 269:29565–29570.

43. Zhang, X.Y., N.E. Robinson, and F.X. Zhu. 1995. Increasing cAMP augments acetylcholine release from tracheal parasympathetic nerves. *Am. J. Respir. Crit. Care Med.* 151:112a (Abstr.).

44. Tsunoda, Y., and C. Owyang. 1995. The regulatory site of functional GTP binding protein coupled to the high affinity cholecystokinin receptor and phospholipase A2 pathway is on the G beta subunit of Gq protein in pancreatic acini. *Biochem. Biophys. Res. Commun.* 211:648–655.

45. Felder, C.C., H.L. Williams, and J. Axelrod. 1991. A transduction pathway associated with receptors coupled to the inhibitory guanine nucleotide binding protein Gi that amplifies ATP-mediated arachidonic acid release. *Proc. Natl. Acad. Sci. USA.* 88:6477–6480.

46. Wessler, I., H. Bender, P. Harle, K.D. Hohle, G. Kirdorf, H. Klapproth, T. Reinheimer, J. Ricny, K.E. Schnieppmendelssohn, and K. Racke. 1995. Release of [H-3]acetylcholine in human isolated bronchi—effect of indomethacin on muscarinic autoinhibition. *Am. J. Respir. Crit. Care Med.* 151:1040–1046.

47. Ayala, L.E., and T. Ahmed. 1989. Is there loss of a protective muscarinic receptor in asthma? *Chest.* 96:1285–1291.

48. Minette, P.J., J.W.J. Lammers, C.M.S. Dixon, M.T. McCusker, and P.J. Barnes. 1989. A muscarinic agonist inhibits reflex bronchoconstriction in normal but not asthmatic subjects. *J. Appl. Physiol.* 67:2461–2465.