Role of cyclooxygenase activation and prostaglandins in antigen-induced excitability changes of bronchial parasympathetic ganglia neurons

Radhika Kajekar,1 Bradley J. Undem,2 and Allen C. Myers2
1Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, Davis, California 95616; and 2Division of Clinical Immunology, Department of Medicine, The Johns Hopkins Asthma and Allergy Center, Baltimore, Maryland 21224

Submitted 3 October 2002; accepted in final form 9 December 2002

Kajekar, Radhika, Bradley J. Undem, and Allen C. Myers. Role of cyclooxygenase activation and prostaglandins in antigen-induced excitability changes of bronchial parasympathetic ganglia neurons. Am J Physiol Lung Cell Mol Physiol 284: L581–L587, 2003. First published January 10, 2003; 10.1152/ajplung.00332.2002.—In vitro antigen challenge has multiple effects on the excitability of guinea pig bronchial parasympathetic ganglion neurons, including depolarization, causing phasic neurons to fire with a repetitive action potential pattern and potentiating synaptic transmission. In the present study, guinea pigs were passively sensitized to the antigen ovalbumin. After sensitization, the bronchi were prepared for in vitro electrophysiological intra-airway parasympathetic ganglia neurons. The methods for animal sensitization and euthanasia were approved by the Johns Hopkins Animal Care and Use Committee, The Johns Hopkins University (Baltimore, MD).

METHODS

The methods for animal sensitization and euthanasia were approved by the Johns Hopkins Animal Care and Use Committee, The Johns Hopkins University (Baltimore, MD).

PREVIOUS STUDIES using the guinea pig bronchus isolated from ovalbumin-sensitized animals demonstrated that mast cells surrounding airway parasympathetic ganglia degranulate on allergen challenge. Concomitant with this response are several changes in the electrophysiological properties of principal neurons within the airway parasympathetic ganglia (14). The electrophysiological changes caused by allergen exposure are consistent with an overall increase in the efficacy of synaptic transmission (19). These changes would likely lead to substantial decrease in the amount of pregan-

Address for reprint requests and other correspondence: A. C. Myers, Division of Clinical Immunology, Dept. of Medicine, The Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle 1A62, Baltimore, MD 21224 (E-mail: amyers@jhmi.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajplung.org 1040-0605/03 $5.00 Copyright © 2003 the American Physiological Society
protocol previously described (18). Briefly, actively sensitized animals received intraperitoneal injections of ovalbumin (OVA; 10 mg/kg) on days 1, 3, and 5 and were killed by carbon dioxide asphyxiation and exsanguination 21–41 days after the last injection, and serum was collected from these animals. Each passively sensitized animal (150–200 g) received an intraperitoneal injection (1 ml/kg) of serum rich in anti-bodies directed against OVA (from actively sensitized animals) 1–2 days before the animal was killed. “Serum control” refers to guinea pigs treated with serum from age-matched, nonallergic guinea pigs. Otherwise, “control” refers to prean-
tigen challenge or vehicle response in tissue from sensitized animals.

**Tissue preparation.** Passively sensitized guinea pigs were killed by a sharp blow to the head and exsanguinated. The thorax was opened, and the lungs, bronchi, and trachea were removed and placed in Krebs bicarbonate buffer (composition in mM: 118 NaCl, 5.4 KCl, 1.0 MgSO4, 1.9 CaCl2, 1.0 NaH2PO4, 25 NaHCO3, and 11.1 dextrose) maintained at room temperature (20–23°C) and equilibrated with 95% O2–5% CO2, pH 7.4.

To allow for tissue preparation and ganglia location, a number of guinea pigs were killed in the presence of OVA (10 μg/ml) to a level >50% of maximal contraction.

**Electrophysiological methods.** Intracellular micropipettes were fabricated from thick-walled borosilicate capillary stock (0.5-mm inside diameter, 1.0-mm outside diameter; World Precision Instruments, Sarasota, FL) by a Brown-Flaming microelectrode puller (P-87; Sutter Instruments, San Rafael, CA). Electrodes were filled with an electrolyte solution of 3 M KCl (pH 7.4). The micropipettes were connected by an Ag-AgCl wire to an electrometer holder (Axon Instruments, Foster City, CA) to an electrometer (Axoclamp 2A; Axon Instruments), and an Ag-AgCl pellet in the bath was connected to the headstage ground. The electrode delivered constant resistance in Krebs solution was 60 MΩ. Impalement of the neurons was aided by a brief 40–50 ms overcompensation (i.e., buzz) of the capacitance neutralization circuit of the amplifier. The delivery of constant-current pulses through the microelectrode was controlled by a computer (Apple Macintosh; Apple Computer, Cupertino, CA) equipped with an analog-to-digital translation interface. Recorded intracellu-
lar membrane voltage properties were displayed online with a chart recorder and an oscilloscope-simulation/data storage program (AxoData; Axon Instruments) and later analyzed with the AxoGraph program (Axon Instruments).

Baseline control membrane properties were made after the establishment of a stable recording [i.e., <1 mV change in resting potential, no change in input resistance (Ri)]. Once a stable recording membrane potential was observed (usually 2–5 min after impalement), the Ri of the neuron was calculated from the steady-state amplitude of the voltage tran-
sient produced by a hyperpolarizing constant-current step (100 pA; 1–5 s duration). Changes in membrane resistance were also monitored continuously by noting changes in the amplitude of the electrotonic voltage transients produced by hyperpolarizing current steps (100 pA, 100–150 ms, 1 Hz). The duration and amplitude of the action potential and the afterhypolarizing potential (AHP) were monitored for single (2 ms, 2 nA stimulus) and four consecutive (2 ms, 2 nA, 40-Hz stimuli) action potentials. The accommodation charac-
teristics of all neurons were analyzed by noting the pattern of action potentials elicited during a series of incrementing depolarizing steps (500 ms, 1.0–2.0 nA). With the use of this procedure, most neurons exhibit an initial burst of action potentials that terminates within 100 ms of the onset of a depolarizing step (“phasic” neurons) (9, 13), and the remaining cells display a continuous repetitive action potential discharge (“tonic” neurons); neurons with these accommoda-
tive patterns in guinea pig bronchial ganglia are anatomically indistinct (10).

Fast excitatory postsynaptic potentials (fEPSPs) were elicited by stimulation of the vagus nerve. Vagus nerve-evoked fEPSPs were stimulated by 1-Hz square pulses delivered to the rostral end of the vagus nerve 10–30 mm from the bronchi were isolated from passively sensitized guinea pigs 2–4 min in duration (duration and voltage adjusted to obtain sub-
threshold fEPSPs if necessary); in several experiments, the intracellular sodium channel blocker QX-314 (10 mM) was used in the electrode electrolyte to block action potentials (15) and quantify suprathreshold synaptic potential amplitudes in that cell (19). For pharmacological effects of prostaglan-
dins on fEPSPs, 50 consecutive vagus nerve-evoked fEPSPs were averaged for a control value as were 50 more in the presence of OVA or prostaglandins (see Antigen responses).

**Antigen responses.** The effect of bath-applied OVA (10 μg/ml) on active and synaptic membrane properties of bron-
chial parasympathetic ganglion neurons from serum control and sensitized animals was studied. The concentration of OVA chosen has previously been shown to cause optimal mast cell degranulation in guinea pig bronchi (18) and to be the lowest concentration that consistently depolarizes bron-
chial parasympathetic ganglion neurons in actively and pas-
sively sensitized guinea pigs (12, 14). OVA was perfused directly over the parasympathetic ganglion preparation for 2–4 min, a period of time previously reported to produce peak responses after drug application (11); if histamine receptor antagonists were not used, the neurons depolarized, and then current clamped to the pre-OVA resting membrane potential before measurement of action and synaptic potentials. The effect of antigen application on active membrane properties, such as action potential AHP amplitude and duration, were monitored. During antigen or prostaglandin application, the amplitudes of 50 consecutive fEPSPs were recorded and averaged. In time control studies, fEPSP amplitudes did not vary significantly over the periods used at these frequencies. For fEPSP experiments, the histamine H1 receptor antago-
nist pyrilamine (1 μM), histamine H2 and H3 receptor antago-
nists burimamide (30 μM) and atropine (0.1 μM) were added to the Krebs buffer at least 1 h before experimentation. A single neuron from each animal was used for antigen challenge. The effects of OVA on sensitized tissue was studied in the presence of COX inhibitors piroxicam (0.05 μM) or indomethacin (3.0 μM).

**Prostaglandin release measurements.** The right and left bronchial parasympathetic neurons were passively sensitized guinea pigs and placed in Krebs buffer solution. The buffer solution was maintained at 37°C and gassed with 95% O2 and 5% CO2. The left and right bronchi were divided into four equal rings and placed in tubes containing Krebs buffer solution (2.5 ml); thus each tube contained one section from each bronchus.
The buffer bathing the tissue was replaced with fresh solution at 15-min intervals for 90 min. After this period of equilibration, the tissue was incubated in 2.5 ml of either Krebs buffer or Krebs buffer containing piroxicam (0.05 M) or indomethacin (3.0 M) for an additional 30 min, after which a 100-μl sample was taken to determine the quantities of prostaglandins released spontaneously before OVA challenge. The tissues were then treated with OVA (10 μg/ml) in the presence or absence of COX inhibitors for 15 min, at the end of which a 100-μl sample was collected for analysis.

Prostaglandin release was assayed using combined gas chromatography-electron capture mass spectrophotometry (GC/MS) as previously described (3). Briefly, a 100-μl aliquot of sample was added to 300 μl of acetone in a silanized vial.

Table 1. Effects of indomethacin or piroxicam on prostaglandin release from the guinea pig

<table>
<thead>
<tr>
<th></th>
<th>PGD₂</th>
<th>PGE₂</th>
<th>PGF₂α</th>
<th>TxB₂</th>
<th>6-Keto-PGF₁α</th>
<th>9α11β-PGF₁₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>3.9 ± 0.6</td>
<td>2.8 ± 0.5</td>
<td>3.2 ± 0.5</td>
<td>1.7 ± 0.7</td>
<td>4.2 ± 1.7</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>+ OVA</td>
<td>55.6 ± 17.2</td>
<td>8.3 ± 1.5</td>
<td>4.4 ± 1.4</td>
<td>32.4 ± 16.3</td>
<td>11.9 ± 6.1</td>
<td>3.5 ± 0.7</td>
</tr>
<tr>
<td>Control (piroxicam)</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.3</td>
<td>0.7 ± 0.4</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.3</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>OVA with piroxicam</td>
<td>0.9 ± 0.2*</td>
<td>0.8 ± 0.5*</td>
<td>0.8 ± 0.5*</td>
<td>0.5 ± 0.4*</td>
<td>0.2 ± 0.1*</td>
<td>1.1 ± 0.6*</td>
</tr>
<tr>
<td>Control (indomethacin)</td>
<td>1.7 ± 0.5</td>
<td>1.0 ± 1.0</td>
<td>1.4 ± 0.6</td>
<td>0.5 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>OVA with indomethacin</td>
<td>11.8 ± 4.6*</td>
<td>1.0 ± 0.5*</td>
<td>1.1 ± 0.2*</td>
<td>2.3 ± 1.7*</td>
<td>1.0 ± 0.6*</td>
<td>1.1 ± 0.3*</td>
</tr>
</tbody>
</table>

Data represent the means ± SE (μg/ml) concentration of prostanoid in the superfusate surrounding the sensitized guinea pig bronchi in vitro (n = 4). Each bronchus was incubated in Krebs buffer with either vehicle or cyclooxygenase (COX) inhibitor (Control), and levels of prostaglandins were measured in the supernatant (see METHODS) before and after the tissue was challenged with ovalbumin (OVA; 10 μg/ml). Similar experiments were performed in the presence of piroxicam (0.05 μM) or indomethacin (30 μM). 9α,11β-PGF₁₂ and 6-keto-PGF₁α are metabolites of prostacyclin. Compared with control (Vehicle), all levels of prostaglandins were reduced in the presence of both COX inhibitors; other than PGF₂α, all levels were increased with OVA without COX inhibitor. *P < 0.05 when compared with OVA without COX inhibitor.

The buffer bathing the tissue was replaced with fresh solution at 15-min intervals for 90 min. After this period of equilibration, the tissue was incubated in 2.5 ml of either Krebs buffer or Krebs buffer containing piroxicam (0.05 μM) or indomethacin (3.0 μM) for an additional 30 min, after which a 100-μl sample was taken to determine the quantities of prostaglandins released spontaneously before OVA challenge. The tissues were then treated with OVA (10 μg/ml) in the presence or absence of COX inhibitors for 15 min, at the end of which a 100-μl sample was collected for analysis.

Prostaglandin release was assayed using combined gas chromatography-electron capture mass spectrophotometry (GC/MS) as previously described (3). Briefly, a 100-μl aliquot of sample was added to 300 μl of acetone in a silanized vial.
Samples were dried under a stream of nitrogen gas, and the residue was treated with 2% methoxylamine hydrochloride dissolved in pyridine. Excess pyridine was evaporated under nitrogen gas, and the residue was subjected to sequential procedures for the synthesis of pentafluorobenzyl ester and trimethylsilyl ether derivatives as previously described (3). GC/MS analysis of the derivatized samples (1-ml vol) was performed with a Varian model 3400 gas chromatograph interfaced with a Finnigan SSQ 710 mass spectrophotometer supplied with an ICIS data system. The sensitivity of the technique is <0.01 fmol/injection for each of the six prostanoids assayed.

Materials. QX-314 was purchased from Alomone Labs (Jerusalem, Israel). Reagents used to prepare the Krebs solution were purchased from J. T. Baker Chemicals (Phillipsburg, NJ). All remaining reagents were purchased from Sigma (St. Louis, MO). At the dilutions used (≥1:10,000) in these studies, dimethyl sulfoxide (piroxicam), ethanol (prostaglandins, indomethacin), or distilled water (atropine, pyrilamine, burimamide, OVA) had no effect on the active or passive properties of parasympathetic ganglion neurons. Final dilutions of all drugs were made in Krebs buffer solution.

Data analysis. All data are expressed as the arithmetic means ± SE. Control values for resting membrane potential, membrane Rₚ, cumulative AHP duration, and amplitude were noted before each drug application. These values were compared with peak changes evoked by antigen challenge or drug application using one-way ANOVA, followed by Student’s t-test. Peak changes evoked by antigen with and without COX inhibitors were compared using unpaired analysis. Statistical tests were performed using Statview statistics program (Abacus Concepts, Lafayette, CA). Statistical significance was accepted at the 0.05 level of probability (P).

RESULTS

Exposing bronchi isolated from passively sensitized guinea pigs to OVA resulted in the production and release of each of all prostaglandins measured with the exception of PGF₂α (Table 1). OVA caused a 10- to 20-fold increase in the production of PGD₂ and thromboxanes and an approximate threefold increase in PGE₂ and PGI₂ (measured as the metabolite, 6-keto-PGF₉α). As expected, indomethacin significantly inhibited the antigen-induced increase in prostaglandin release from the bronchus (Table 1).

OVA inhibited action potential accommodation in bronchial ganglion neurons, and this antigen-induced action was reduced by indomethacin. PGD₂ inhibited antigen-induced changes in fEPSPs (Table 1). OVA caused a 10- to 20-fold increase in the production of PGD₂ and thromboxanes and an approximate threefold increase in PGE₂ and PGI₂ (measured as the metabolite, 6-keto-PGF₂α). As expected, indomethacin significantly inhibited the antigen-induced increase in prostaglandin release from the bronchus (Table 1).

Table 2. Summary of the effects of prostaglandins on the active and synaptic membrane properties of bronchial parasympathetic ganglia neurons

<table>
<thead>
<tr>
<th>Membrane properties</th>
<th>PGD₂ (n = 8)</th>
<th>PGE₂ (n = 6)</th>
<th>PGI₂ (n = 5)</th>
<th>PGF₂₀ (n = 6)</th>
<th>TxA₂ (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input impedance, mΩ</td>
<td>60 ± 12.9</td>
<td>40.0 ± 5.8</td>
<td>36.7 ± 4.7</td>
<td>45 ± 5</td>
<td>41 ± 4</td>
</tr>
<tr>
<td>mΩ</td>
<td>65.0 ± 10.5</td>
<td>37.5 ± 4.8</td>
<td>35.6 ± 7.7</td>
<td>46 ± 4</td>
<td>36.2 ± 6</td>
</tr>
<tr>
<td>Accommodation, spikes/1.0 nA</td>
<td>2.8 ± 0.5</td>
<td>3.0 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>2.8 ± 0.5</td>
<td>3.6 ± 0.3</td>
</tr>
<tr>
<td>Cumulative spike</td>
<td>173 ± 16</td>
<td>197 ± 32</td>
<td>147 ± 27</td>
<td>230 ± 35</td>
<td>147 ± 27</td>
</tr>
<tr>
<td>AHP duration, ms</td>
<td>125 ± 21*</td>
<td>64 ± 6*</td>
<td>151 ± 28</td>
<td>239 ± 37</td>
<td>151 ± 27</td>
</tr>
<tr>
<td>fEPSP amplitude, mv</td>
<td>13 ± 3(n = 6)</td>
<td>10 ± 4(n = 4)</td>
<td>12 ± 3(n = 4)</td>
<td>11 ± 2</td>
<td>14 ± 2</td>
</tr>
<tr>
<td></td>
<td>12 ± 4</td>
<td>11 ± 3</td>
<td>12 ± 2</td>
<td>17 ± 3*</td>
<td>13 ± 3</td>
</tr>
</tbody>
</table>

*P < 0.05 when compared with control value. AHP, after hypolarizing potential; fEPSP, fast excitatory postsynaptic potentials; TxA₂, thromboxane A₂.

Data represent absolute values (± SE) of each membrane property measured before and during (italics) bath application of 0.1 μM of the prostanoïd shown at the top of the column. See text for details regarding measurement of properties.
effect was blocked by indomethacin. In phasic neurons, a prolonged, suprathreshold depolarizing pulse (500 ms, 1.0 nA) elicited 3 ± 0.2 (n = 12) action potentials, followed by accommodation to the stimulus (Fig. 1A). After antigen challenge (OVA, 10 μg/ml, 5 min), the same neurons elicited a greater than sixfold increase in the number of action potentials to the same stimulus (Fig. 1B; n = 12, P = 0.01), an effect that does not reverse during washout of OVA (lasting 40.3 ± 10.4 min; n = 12).

In the presence of indomethacin (3 μM), antigen challenge (OVA, 10 μg/ml) had no effect on accommodation eliciting 5 ± 2 action potentials [P = 0.4 compared with control (pre-OVA) response, n = 8; Fig. 1C]. In phasic neurons from control animals (injected with regular serum), OVA (10 μg/ml) had no effect on accommodation properties (P = 0.8; n = 6). For the afterhyperpolarization duration following single or multiple action potentials, we recorded either no change in some neurons (n = 4) or a decrease of 21 or 37% (after 4 action potentials at 25 Hz) in the remaining cells after antigen challenge (P = 0.1, n = 6).

We determined whether another COX inhibitor, piroxicam, structurally unrelated to indomethacin, would also inhibit antigen-induced inhibition of accommodation. Piroxicam (0.05 μM) effectively inhibited OVA-induced production of prostaglandins in bronchial tissue (Table 1). Piroxicam mimicked the effects of indomethacin on antigen-induced changes in action potential accommodation. In the presence of piroxicam (0.05 μM), antigen challenge (OVA, 10 μg/ml) had no effect on action potential accommodation in phasic neurons (Fig. 1D; n = 8), eliciting 4 ± 1 action potentials. These results are summarized in Fig. 1E.

To determine whether prostaglandins released during antigen challenge affected synaptic transmission, the effects of antigen (10 μg/ml of OVA) on the amplitude of the fEPSP was determined with and without COX inhibitors. In control neurons, vagus nerve stimulation elicited fEPSPs that were subthreshold for action potential formation with an amplitude of 13 ± 3 mV (n = 6, Fig. 2A). After antigen challenge (10 μg/ml of OVA), the fEPSP were significantly increased to 19 ± 2 mV (n = 6; P = 0.02). In two experiments when QX-314 was not used to block regenerative spikes (see METHODS), this increase in fEPSP amplitude was sufficient to drive the cell to threshold for action potential generation (n = 2; e.g., Fig. 2B). After antigen challenge (10 μg/ml OVA) in the presence of indomethacin (3 μM), fEPSPs were not increased, averaging 14 ± 3 mV (n = 6). Likewise, OVA challenge in the presence of piroxicam (0.05 μM) did not increase fEPSP amplitude (12 ± 4 mV, P = 0.4, n = 5, Fig. 2C). The effects of antigen on excitatory potentials, with and without COX inhibitors, are summarized in Fig. 2E. In ganglia neurons from serum control animals (injected with regular serum), OVA (10 μg/ml) had no effect on fEPSP amplitudes (P > 0.05; n = 4).

The effects of exogenous prostaglandins on active and synaptic membrane properties were also determined. Among the prostaglandins found to be increased by OVA exposure (Table 1), only PGD2 (0.1 μM) mimicked the effect of antigen challenge on action potential accommodation (Fig. 3A; Table 2). Exposing the tissue to PGE2, PG12, PGF2α, or thromboxane A2 (0.1 μM) as well as the thromboxane mimetic U-46619 (0.1 μM, n = 4; data not shown) had no effect on action potential accommodation. In addition, PGD2 (0.1 μM) decreased the duration of the cumulative action AHP by 28 ± 1% (Table 2). A more profound decrease in AHP duration was observed after bath application of PGE2 (0.1 μM; n = 6, Fig. 3B; Table 2).

None of the prostanoids found to be significantly elevated on OVA exposure (PGD2, PGE2, thromboxane A2, or prostacyclin) mimicked the effect of OVA in causing an increase in fEPSP amplitude. Paradoxically, PGF2α mimicked the effect of OVA in this regard, causing a 28 ± 17% increase in fEPSP amplitude (P <
DISCUSSION

The data provide direct evidence that the immediate hypersensitivity response in guinea pig airway tissue is associated with an increase in synaptic efficacy, as directly assessed by quantifying the amplitude of fEPSPs, in bronchial parasympathetic ganglia. In addition, the results support our previous finding that antigen challenge causes a substantial decrease in the accommodative properties of bronchial ganglion neurons. It has previously been shown that the frequency of action potentials arising from preganglionic neurons is far greater than the frequency of action potentials leaving the ganglia along postganglionic fibers (8, 11).

In other words, the bronchial parasympathetic ganglia appear to be sites at which parasympathetic activity is filtered. This occurs because many of the preganglionic action potentials entering the bronchial parasympathetic ganglia evoke fEPSPs that are below threshold for action potential discharge in the ganglion neurons. The increase in synaptic efficacy (increase in fEPSP amplitude) and decrease in accommodation would likely lead to a decrease in the filtering capacity of the ganglia and consequently an overall increase in parasympathetic activity at effector tissue in the airway (smooth muscle, glands, etc.).

We previously reported that histamine was responsible for antigen-induced membrane depolarization and increases in $R_i$ in guinea pig bronchial ganglia but had no effect on action potential properties (12). Results from the present study support the hypothesis that prostaglandins are the primary autacoids responsible for antigen-induced decreases in accommodation and increases in fEPSP amplitude in bronchial ganglia. Pretreatment of the bronchi with the COX inhibitor indomethacin prevented both the antigen-induced increase in synaptic efficacy and decrease in accommodation.

We previously reported that accommodation in phasic neurons could be reduced either by activation of the potassium current with characteristics similar to A-current or by inhibiting calcium-activated potassium current(s) (9). That PGD$_2$ decreased the AHP duration after repetitive action potentials (an indicator of calcium-activated potassium current) suggests this current may be associated with the decrease in accommodation. However, that PGE$_2$ inhibited the afterhyperpolarization after repetitive action potentials, but did not affect accommodation, indicates that inhibition of the calcium-activated potassium current responsible for AHP is not associated with the antigen-induced decrease in accommodation. Similarly, in guinea pig gallbladder ganglia, PGE$_2$ attenuates the AHP in parasympathetic neurons and also has no effect on accommodation (5). On the basis of our previous study (9), it is possible that effect of PGD$_2$ (and antigen) on accommodation involves activation of the A-current; the results presented in the present study may provide

Fig. 4. PGF$_{2\alpha}$ potentiates synaptic transmission. A: vagus nerve-stimulated fEPSPs are evoked in a control neuron. Trace shows 10 superimposed consecutive traces. B: vagus nerve-stimulated fEPSPs are potentiated, some to threshold, after application of PGF$_{2\alpha}$(0.1 μM). C: summary of the effects of exogenous application of PGF$_{2\alpha}$ on fEPSP amplitude recorded in bronchial parasympathetic ganglia neurons. *P < 0.05 compared with control.

0.05, n = 6; Fig. 4), occasionally to action potential threshold (2 of 6 neurons).
evidence for a unique role for PGD₂ in regulation of the A-current and action potential accommodation.

The results shed some light on the nature of the particular COX product responsible for the antigen-induced decrease in accommodation. Antigen exposure leads to significant increases in the production and release of PGD₂, PGE₂, thromboxane, and prostacyclin. Among these eicosanoids, however, only PGD₂ was capable of mimicking the effect of antigen on decreasing action potential accommodation. These data, along with the data obtained with the COX inhibitors, provide strong support for the hypothesis that antigen-induced changes in accommodation are secondary to the formation of PGD₂. The cellular source of the PGD₂ cannot be discerned from the data, but mast cells are likely candidates. These data may also provide a possible mechanism for prostaglandin-induced increases in acetylcholine release from prejunctional nerve fibers in the lower airways (7). However, other studies have suggested inhibitory effects of prostaglandins on airway cholinergic parasympathetic activity (2, 4, 5).

The nature of the prostaglandin responsible for the antigen-induced increase in fEPSP amplitude is less obvious. When studied at a concentration of 0.1 μM (so that some receptor selectivity could be appreciated), only PGF₂α, affected fEPSP amplitude. However, unlike our previously reported study on antigen-induced prostaglandin release from guinea pig tracheal tissue (20), PGF₂α, was not significantly elevated in bronchial superfusate on antigen exposure. This paradox may be explained by the possibility that antigen did indeed increase PGF₂α, production within or near the ganglia, and this level was greater than the PGF₂α, levels in the superfusate. It is possible that the method of extraction and assay may have differed from those previously used (20). Alternatively, from our exogenously applied prostaglandin experiments, perhaps concentrations >0.1 μM are needed for other prostaglandins to mimic the effect of antigen challenge on fEPSPs. It should be noted, however, that a concentration of 0.1 μM PGD₂ or PGE₂ was sufficient to affect other electrophysiological membrane properties of the ganglion neurons (Table 1).

In conclusion, the COX enzyme(s), activated during specific antigen challenge, initiate(s) the release of large quantities of prostaglandins in guinea pig bronchial tissue. Some of these prostaglandins differentially affect membrane properties of intrinsic ganglia neurons. Antigen-induced PGD₂ production appears to effectively inhibit action potential accommodation in ganglion neurons, such that many more action potentials can be evoked during prolonged stimulation. PGF₂α, is effective at directly increasing synaptic efficacy in the bronchial ganglia, as noted by a substantial increase in fEPSP amplitude. Such changes in excitability, considered along with the effects of endogenously released histamine (12), may contribute to increases in parasympathetic tone in the lower airways associated with allergen exposure.

This work was supported by the National Heart, Lung, and Blood Institute (A. C. Myers, B. J. Undem).

REFERENCES