Antigen-induced hyperreactivity to histamine: role of the vagus nerves and eosinophils

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1Department of Environmental Health Sciences, School of Hygiene and Public Health, and 2Department of Medicine, Johns Hopkins University, Baltimore, Maryland 21205; and 3Departments of Immunology and Medicine, Mayo Clinic, Rochester, Minnesota 55905

Costello, Richard W., Christopher M. Evans, Bethany L. Yost, Kristen E. Belmonte, Gerald J. Gleich, David B. J. Jacoby, and Allison D. Fryer. Antigen-induced hyperreactivity to histamine: role of the vagus nerves and eosinophils. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L709–L714, 1999.—M2 muscarinic receptors limit acetylcholine release from the pulmonary parasympathetic nerves. M2 receptors are dysfunctional in antigen-challenged guinea pigs, causing increased vagally mediated bronchoconstriction. Dysfunction of these M2 receptors is due to eosinophil major basic protein, which is an antagonist for M2 receptors. Histamine-induced bronchoconstriction is composed of a vagal reflex in addition to its direct effect on airway smooth muscle. Because hyperreactivity to histamine is seen in antigen-challenged animals, we hypothesized that hyperreactivity to histamine may be due to increased vagally mediated bronchoconstriction caused by dysfunction of M2 receptors. In anesthetized, antigen-challenged guinea pigs, histamine-induced bronchoconstriction was greater than that in control guinea pigs. After vagotomy or atropine treatment, the response to histamine in antigen-challenged animals was the same as that in control animals. In antigen-challenged animals, blockade of eosinophil influx into the airways or neutralization of eosinophil major basic protein prevented the development of hyperreactivity to histamine. Thus hyperreactivity to histamine in antigen-challenged guinea pigs is vagally mediated and dependent on eosinophil major basic protein.

The function of neuronal M2 muscarinic receptors is decreased in antigen-challenged guinea pigs (16, 30), mice (20), and rats (2), as well as in some patients with asthma (1, 25). Inhibition of the influx of eosinophils into the airways of antigen-challenged guinea pigs with antibodies to either interleukin-5 or the eosinophil adhesion molecule very late activation antigen-4 (VLA-4) preserves the function of the M2 receptor (10, 12). In vitro, eosinophil major basic protein is an antagonist at these receptors (18), whereas in vivo, pretreatment with an antibody to eosinophil major basic protein prevents loss of neuronal M2 muscarinic-receptor function and prevents hyperreactivity to vagal nerve stimulation (11, 21). Histologically, eosinophils are found in association with cholinergic airway nerves after antigen challenge (7). In addition, the number of eosinophils per nerve correlates with the degree of M2-receptor dysfunction (7). In humans, eosinophils are clustered around and along the airways in sections of airway from fatal asthmatics. Extracellular eosinophil major basic protein has also been deposited on the airway nerves (7). Thus loss of function of neuronal M2 muscarinic receptors after antigen challenge of sensitized animals is mediated by eosinophils and eosinophil major basic protein. Eosinophils and M2-receptor function may also contribute to the hyperreactivity associated with asthma.

Exposure of sensitized animals and allergic asthmatics to an antigen characteristically results in hyperreactivity of the airways. Hyperreactivity is commonly measured as increased contractile responses to a variety of agents including histamine (4–6, 29). Histamine constricts airway smooth muscle by a direct effect on the muscle and indirectly by stimulating a reflex that releases acetylcholine from the vagus nerves (8, 19, 24). Because neuronal M2 muscarinic receptors limit vagally induced bronchoconstriction, it would be expected that M2 muscarinic-receptor dysfunction in antigen-challenged animals may increase a vagally mediated reflex. In these studies, we tested whether hyperreactivity to histamine in antigen-challenged animals is the result of an exaggerated vagal reflex. Because antagonism of M2 muscarinic receptors by eosinophil major basic protein may be responsible for the loss of function of these receptors, we also investigated the role of eosinophils in antigen-induced hyperreactivity to histamine.

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METHODS

Specific pathogen-free guinea pigs (Dunkin-Hartley, 200–250 g) were purchased from Hilltop (Scottsdale, PA). Guinea pigs were shipped in filtered crates and housed in laminar flow hoods in clean rooms. All guinea pigs were handled in accordance with the standards established by the US Animal Welfare Acts set forth in the National Institutes 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.

Sensitization and challenge. Guinea pigs were injected intraperitoneally with 10 mg/kg of ovalbumin on days 1, 3, and 5. Three weeks later, the sensitized guinea pigs were exposed to an aerosol of 5% ovalbumin for 5 min either on a single occasion or daily for 4 days. Sensitization was confirmed by demonstrating that ovalbumin (250 mg/kg iv) administered at the end of the experiment in some randomly chosen animals caused a rapid sustained rise in pulmonary inflation pressure ($P_{pi}$). In contrast, ovalbumin had no effect on $P_{pi}$ in nonsensitized animals.

Eosinophil-blocking antibodies. Sensitized animals were pretreated with either rabbit polyclonal antibody to eosinophil major basic protein (1 ml ip) or control rabbit serum (1 ml ip) 1 h before antigen challenge (11). In other experiments, sensitized guinea pigs were pretreated with HP1/2 (4 mg/kg ip), a mouse anti-human antibody to VLA-4, 1 h before each of the four antigen challenges (12, 33).

Measurement of $P_{pi}$. The experiments were carried out 18–24 h after the exposure of sensitized guinea pigs to ovalbumin or for the nonchallenged control group on day 26. The guinea pigs were anesthetized with urethane (1.5 mg/kg ip). None of the experiments lasted longer than 3 h, although this dose of urethane produces a deep anesthesia lasting 8–10 h (17). However, because paralyzing agents were used, the depth of anesthesia was monitored by observing for fluctuations in heart rate and blood pressure.

Once the guinea pigs were anesthetized, cannulas were placed into both jugular veins for the administration of drugs. Each animal's body temperature was maintained at 37°C with a homeothermic heating blanket (Harvard, Cambridge, MA).

The animals were ventilated with a positive-pressure constant-volume animal ventilator (Harvard) and were paralyzed with succinylcholine chloride (infused at 10 µg·kg$^{-1}$·min$^{-1}$). $P_{pi}$ was measured with a pressure transducer (Spectromed DTX, Oxnard, CA). All signals were displayed on a Grass polygraph (Quincy, MA).

The baseline $P_{pi}$ of the anesthetized guinea pigs was 70–150 mmH$_2$O. Bronchoconstriction was measured as the increase in $P_{pi}$ over the baseline $P_{pi}$ produced by the ventilator (9, 13). A change in $P_{pi}$ probably reflects changes in both resistance and compliance (3, 9). The sensitivity of the method was increased by taking the output $P_{pi}$ signal from the driver to the input of the preamplifier of a second channel on the polygraph. Thus $P_{pi}$ was recorded on one channel, and increases in $P_{pi}$ were recorded on a separate channel at a greater amplification. With this method, increases in pressure as small as 2 mmH$_2$O could be recorded accurately.

Histamine-induced bronchoconstriction. All animals were pretreated with guanethidine (10 mg/kg iv), and 30 min later, increasing doses of histamine sulfate (1–20 nmol/kg iv) were administered. There was an interval of at least 5 min before each dose of histamine. The rise in $P_{pi}$ above baseline in response to histamine was recorded and compared between groups of animals.

Animals served as their own controls, and bronchoconstrictive response to histamine was compared before and after vagotomy. Experiments were also performed in animals in which the vagi were intact and the bronchoconstriction to histamine was compared with histamine-induced bronchoconstriction in animals studied only after vagotomy.

Reagents. Atropine, guanethidine, histamine, pilocarpine, normal rabbit serum, succinylcholine chloride, and urethan were all purchased from Sigma (St. Louis, MO). Ovalbumin (grade II) was also purchased from Sigma. Methoctramine was purchased from ICN Chemicals (Aurora, OH). The antibody to VLA-4 was a generous gift from Dr. R. Lobb (Biogen, Cambridge, MA). The antibody to eosinophil major basic protein was isolated as previously described (22, 31).

Statistics. Differences in baseline pulmonary inflation and responses to methoctramine were compared between groups with an analysis of variance. Differences in the increase in $P_{pi}$ between groups of animals were compared with an analysis of variance for repeated measures.

RESULTS

With the vagus nerves intact, the baseline $P_{pi}$ was 99.2 ± 6.7 mmH$_2$O in control animals, 97.8 ± 4.9 mmH$_2$O in single antigen-challenged animals, and 107.5 ± 7.5 mmH$_2$O in repeatedly challenged animals. In animals studied only after the vagus nerves were cut, the $P_{pi}$ was 100.8 ± 7.3 mmH$_2$O in control animals, 107.5 ± 6.7 mmH$_2$O single antigen-challenged animals, and 105 ± 9.2 mmH$_2$O in repeatedly challenged animals. None of the baseline $P_{pi}$ values were significantly different from each other.

Effect of histamine on $P_{pi}$. Preliminary studies indicated that when the vagus nerves were intact, doses of histamine > 20 nmol/kg frequently caused a fatal bronchoconstriction, in particular in antigen-challenged animals. Thus, in the experiments reported here, the maximum dose of histamine was 20 nmol/kg iv.

With the vagus nerves intact, histamine (1–20 nmol/kg iv) induced a dose-dependent increase in $P_{pi}$ in control animals (Fig. 1, □). Vagotomy did not alter the response to histamine in these control animals (Fig. 1, ■). In antigen-sensitized guinea pigs, histamine-induced bronchoconstriction was significantly increased after either a single antigen challenge ($P = 0.0001$; Fig. 1, ○) or repeated antigen challenges ($P = 0.0001$; Fig. 2, ○) compared with their respective control animals. There was no difference in the response to histamine between control and antigen-challenged guinea pigs after vagotomy (Fig. 1).

In the above experiments, each animal served as its own control, with dose-response curves being performed before and after vagotomy. Preliminary studies showed that with no intervention there was no difference in the magnitude of the response to three repeated dose-response curves to histamine. To further control for tachyphylaxis as an explanation for the differences in the response to histamine before and after vagotomy, experiments were performed in separate groups of animals where histamine dose-response curves were carried out only once. In these experiments, the response to histamine was identical to that seen in Fig. 1.

Effect of muscarinic-receptor antagonists and agonists on histamine-induced bronchoconstriction. Pretreatment of nonvagotomized antigen-challenged ani-
mals with the nonselective muscarinic-receptor antagonist atropine (1 mg/kg iv) completely reversed histamine hyperreactivity compared with antigen-challenged-only animals (P = 0.001; Fig. 3, ▼). When the vagus nerves were cut, there were no differences in the response to histamine among any of the groups of animals (data not shown).

Pretreatment with an antibody to eosinophil major basic protein. The effect of histamine on P\textsubscript{pa} was tested in antigen-sensitized guinea pigs pretreated with the antibody to eosinophil major basic protein before a single antigen challenge (n = 5; Fig. 5, ▼). When the vagus nerves were intact, the antibody to major basic protein completely attenuated histamine hyperreactivity in antigen-challenged animals (Fig. 5, ○) compared with that in antibody-pretreated antigen-challenged animals (P = 0.01; Fig. 5, ▼).

In contrast, pretreatment with control (normal) rabbit serum had no inhibitory effect on hyperreactivity to histamine in antigen-challenged guinea pigs (1–20 nmol histamine/kg; data not shown). In these experiments, the maximum increase in P\textsubscript{pa} in response to 20 nmol/kg of histamine was 295 ± 29 mmH\textsubscript{2}O in rabbit serum-treated guinea pigs (n = 3) compared with 287 ± 23 mmH\textsubscript{2}O in non-serum-treated antigen-challenged guinea pigs (Fig. 5, ▼).

**DISCUSSION**

In antigen-challenged animals, the response to histamine was significantly greater than that in control animals when the vagus nerves were left intact. When...
Vagal reflexes were eliminated either by vagotomy (Fig. 1) or by pretreatment with atropine (Fig. 3), there was no difference in the response to histamine between control and antigen-challenged animals even at the highest dose tolerated by the challenged animals (20 nmol/kg iv). With the vagus nerves cut, there were no differences between control and antigen-challenged animals, indicating that the increased reactivity to histamine in antigen-challenged guinea pigs is not due to an effect on airway smooth muscle. Thus, in guinea pigs, antigen-induced hyperreactivity to histamine is vagally mediated.

Experiments in control and antigen-challenged animals were performed by assessing the response to histamine before and after vagotomy, with each animal serving as its own control. This control overcomes bias introduced by the variability in the response to antigen among guinea pigs. The response to histamine was also compared between animals that were only administered vagus nerves intact, either before or after vagotomy.

Under normal circumstances, neuronal M2 muscarinic receptors limit acetylcholine release from the vagus nerves. These M2 muscarinic receptors are dysfunctional after antigen challenge in guinea pigs (16, 30), mice (20), and rats (2) as well as in some humans with asthma (1, 25). The presence of functional M2 muscarinic receptors in control animals limits the magnitude of the vagal reflex response. When these receptors are stimulated with pilocarpine, histamine-induced bronchoconstriction is inhibited. Conversely, when these receptors are blocked with an M2-selective antagonist such as methoctramine, the response to
histamine is potentiated. It is likely that methoctramine is potentiating the reflex portion of the histamine response because the potentiation is blocked with atropine. The presence of functional, inhibitory M₂ receptor antagonism may explain why there is no significant vagally mediated response to histamine in control animals.

In antigen-sensitized and -challenged guinea pigs, eosinophils are selectively recruited to cholinergic nerves (7). The influx of eosinophils into the lungs of antigen-challenged guinea pigs can be inhibited by pretreatment with an antibody to VLA-4 (12, 26, 33). In antigen-challenged animals, inhibiting the influx of eosinophils into the airways prevents loss of neuronal M₂ muscarinic-receptor function and prevents the development of hyperreactivity (10, 12, 26). In vitro, eosinophil major basic protein is an antagonist for M₂ muscarinic receptors (18). In vivo, neutralizing major basic protein with heparin (12) or with an antibody to major basic protein (11) also prevents loss of M₂ receptor function in antigen-challenged guinea pigs. The antibody to major basic protein does not inhibit recruitment of eosinophils to the nerves (11); it acts by neutralizing the eosinophil product, major basic protein (22, 31). These studies demonstrate that loss of neuronal M₂ muscarinic-receptor function in antigen-challenged guinea pigs is due to blockade of M₂ receptors by eosinophil major basic protein.

Because antigen-induced hyperreactivity to histamine is vagally mediated, the role of eosinophil major basic protein in antigen-induced hyperreactivity was tested. Pretreatment of single antigen-challenged guinea pigs with the antibody to eosinophil major basic protein, but not with control rabbit serum, completely prevented hyperreactivity 24 h later. These data demonstrate that hyperreactivity to histamine 24 h after antigen challenge is mediated by eosinophil major basic protein. However, inhibition of eosinophil influx into the airways with the antibody to VLA-4 did not prevent hyperreactivity to histamine 24 h after antigen challenge. Thus, although eosinophil major basic protein is critical to developing hyperreactivity, recruitment of eosinophils into the lungs is not required, suggesting that the major basic protein must have come from resident eosinophils.

In contrast, when sensitized animals were pretreated with the antibody to VLA-4 and challenged repeatedly with antigen over 4 days, hyperreactivity to histamine after antigen challenge was prevented. One explanation for these findings may be that degranulation of resident eosinophils in the guinea pig mediates the hyperreactivity seen 24 h after a single antigen challenge, whereas maintenance of hyperreactivity to histamine requires recruitment of additional eosinophils from the peripheral circulation to the airway nerves.

In control nonsensitized animals, including guinea pigs (24), rabbits (19), and dogs (23, 36), histamine has been shown to cause bronchoconstriction by a direct effect on airway smooth muscle in addition to a vagal reflex response because sectioning the vagus nerves inhibited the histamine-induced bronchoconstriction by up to 50%. In contrast, sectioning the vagus in our control animals did not alter the histamine-induced bronchoconstriction. However, in previous studies (13, 15), it is noteworthy that the animals had been paralyzed with gallamine, which is a selective antagonist for M₂ muscarinic receptors. When we blocked the neuronal M₂ receptors with methoctramine (Fig. 4), there was a considerable vagal response to histamine in control animals.

In summary, histamine-induced bronchoconstriction is mediated by a direct effect on airway smooth muscle in control animals, although there is a vagal component when the neuronal M₂ receptors are inhibited. In antigen-challenged guinea pigs, hyperreactivity to histamine is vagally mediated. Furthermore, this vagally mediated hyperreactivity is dependent on release of major basic protein from resident eosinophils. Because in antigen-challenged guinea pigs there is loss of function of the neuronal M₂ muscarinic receptors, which is also eosinophil major basic protein mediated, the results of this study suggest that histamine hyperreactivity seen after antigen challenge is due to antagonism of neuronal M₂ muscarinic receptors by eosinophil major basic protein.

In humans, the response to histamine in vivo does not correlate with in vitro responses, suggesting that the hyperresponsiveness does not reflect an intrinsic abnormality of the airway smooth muscle (27, 34). In some humans with asthma, the function of the neuronal M₂ muscarinic receptors is impaired while histologically eosinophils are localized to airway nerves (7). Thus antagonism of M₂ muscarinic receptors by eosinophil major basic protein may also be a mechanism for the hyperreactivity to agents such as histamine in patients with asthma.

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