Substance P-induced airway hyperreactivity is mediated by neuronal M$_2$ receptor dysfunction

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

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Evans, Christopher M., Kristen E. Belmonte, Richard W. Costello, David B. Jacoby, Gerald J. Gleich, and Allison D. Fryer. Substance P-induced airway hyperreactivity is mediated by neuronal M$_2$ receptor dysfunction. Am J Physiol Lung Cell Mol Physiol 279: L477–L486, 2000.—Neuronal muscarinic (M$_2$) receptors inhibit release of acetylcholine from the vagus nerves. Hyperreactivity in antigen-challenged guinea pigs is due to blockade of these M$_2$ autoreceptors by eosinophil major basic protein (MBP) increasing the release of acetylcholine. In vivo, substance P-induced hyperreactivity is vagally mediated. Because substance P induces eosinophil degranulation, we tested whether substance P-induced hyperreactivity is mediated by release of MBP and neuronal M$_2$ receptor dysfunction. Pathogen-free guinea pigs were anesthetized and ventilated. Thirty minutes after intravenous administration of [Sar$^9$,Met(O$_2$)$_{11}$]-substance P, guinea pigs were hyperreactive to vagal stimulation and M$_2$ receptors were dysfunctional. The depletion of inflammatory cells with cyclophosphamide or the administration of an MBP antibody or a neurokinin-1 (NK$_1$) receptor antagonist (SR-140333) all prevented substance P-induced M$_2$ dysfunction and hyperreactivity. Intravenous heparin acutely reversed M$_2$ receptor dysfunction and hyperreactivity. Thus substance P releases MBP from eosinophils resident in the lungs by stimulating NK$_1$ receptors. Substance P-induced hyperreactivity is mediated by blockade of inhibitory neuronal M$_2$ receptors by MBP, resulting in increased release of acetylcholine.

IN THE LUNGS, THE DOMINANT control of airway smooth muscle is provided by the parasympathetic fibers of the vagus nerves (39). These fibers release acetylcholine onto postjunctional muscarinic (M$_3$) receptors to cause airway smooth muscle contraction (43). The release of acetylcholine by airway parasympathetic nerves is inhibited by the activation of neuronal M$_2$ receptors in an autocrine manner (19). In antigen-challenged animals, these neuronal M$_2$ receptors are dysfunctional (20), resulting in increased acetylcholine release (3) and airway hyperreactivity (9, 17). However, M$_3$ receptor function remains unaltered because no change in airway smooth muscle sensitivity to acetylcholine occurs (16).

Inhalation of antigen causes an influx of eosinophils into the airways (11, 13, 44) where they accumulate around airway nerves (11, 15, 16). Blockade of eosinophil accumulation in the airways of antigen-challenged guinea pigs by inhibition of interleukin-5 or very late activation antigen-4 prevents airway hyperreactivity and loss of M$_2$ receptor function (15, 17, 36, 46).

During antigen-induced airway inflammation, eosinophils release major basic protein (MBP) (22, 30). MBP is an allosteric antagonist of M$_2$ receptors in vitro, which can be removed with heparin (29). Treatment of antigen-challenged guinea pigs with heparin restores M$_2$ receptor function (18). Specific removal of MBP with an antibody prevents antigen-induced hyperreactivity via protection of M$_2$ receptor function (16). Thus eosinophil recruitment to the airway nerves and the subsequent release of MBP cause hyperreactivity by blocking inhibitory neuronal M$_2$ receptors in antigen-challenged guinea pigs.

Substance P, which binds neurokinin (NK) receptors, induces vagal reflex-mediated airway hyperreactivity (40). In antigen-challenged guinea pigs, NK$_1$ receptor, but not NK$_2$ receptor, antagonists prevent loss of M$_2$ receptor function and hyperreactivity without affecting eosinophil recruitment (11). Substance P stimulates degranulation of eosinophils in vitro via NK$_1$ receptor stimulation (31). To define the role of substance P in airway hyperreactivity, these studies were designed to test whether intravenous substance P induces airway hyperreactivity via eosinophil degranulation and subsequent M$_2$ receptor dysfunction.

METHODS

Animals. Specific pathogen-free female Dunkin-Hartley guinea pigs (250–350 g; supplied by Hilltop Animal Farms, Scottsdale, PA) were used. All animals were shipped in filtered crates and kept in high-efficiency particulate-filtered...
SUBSTANCE P-INDUCED M₂ DYSFUNCTION IN THE AIRWAYS

L478

Spectramed), and baseline Ppi was 101 measured at the trachea with a DTX pressure transducer (Viggo-Spectramed, Oxnard, CA), and the heart rate was derived from the blood pressure tracing with a tachograph.

Animals were ventilated through a tracheal cannula with a positive-pressure, constant-volume rodent respirator (Harvard Apparatus, South Natick, MA) at a tidal volume of 10 ml/kg and a respiratory rate of 100 breaths/min. Sucinylcholine (10 mg/kg iv), a non-depolarizing muscle relaxant, was constantly infused as a paralyzing agent. Pulmonary inflation pressure (Ppi) was measured at the trachea with a DTX pressure transducer (Viggo-Spectramed), and baseline Ppi was 101 ± 2 mmHgO (mean ± SE). All signals were recorded on a polygraph (Grass Instrument, Quincy, MA). Bronchoconstriction was measured as the increase in Ppi above the basal inflation pressure produced by the ventilator (12). The sensitivity of the method was increased by taking the output Ppi signal from the driver of one channel to the input of the preamplifier of a different channel on the polygraph. Thus increases in Ppi as small as 2–3 mmHgO were recorded accurately. All animals were chemically sympathectomized by guanethidine pretreatment (10 mg/kg iv) to deplete norepinephrine (5).

[Sar⁹,Met(O²)¹¹]substance P, a stable substance P analog, was used in these experiments. For the remainder of this paper, [Sar⁹,Met(O²)¹¹]substance P will be referred to as SM-substance P. SM-substance P has a similar affinity for NK₁ and for NK₂ receptors as substance P (41). SM-substance P (100 ng/kg) was administered intravenously to pathogen-free guinea pigs. This dose of SM-substance P induced a transient decrease in blood pressure of ~20 mmHg, which lasted no more than 5 min, but it did not cause significant bronchoconstriction. Thirty minutes later, airway hyperreactivity and M₂ receptor function were measured (see below).

Some pathogen-free guinea pigs receiving SM-substance P were pretreated with cyclophosphamide (30 mg/kg ip every other day for 1 wk before the experiment; Ref. 21). We have demonstrated previously that this dose of cyclophosphamide does not affect hyperreactivity or M₂ receptor function in control animals (21). Other pathogen-free guinea pigs (control and those receiving SM-substance P) were pretreated with rabbit anti-guinea pig MBP antiserum (3.0 ml ip 1 h before SM-substance P; Ref. 35). SM-substance P has a greater affinity for NK₂ than for NK₁ receptors (41). To confirm that the effects of SM-substance P were mediated by NK₂ receptors, the NK₁ receptor antagonist SR-140333 (1 mg/kg ip 1 h before SM-substance P; Ref. 1) or the NK₂ receptor antagonist SR-48968 (1 mg/kg iv 5 min before SM-substance P; Ref. 7) was used.

Studies of vagal hyperresponsiveness. Anesthetized, ventilated, and paralyzed pathogen-free guinea pigs were used. Both vagi were cut, and the distal ends were placed on platinum-stimulating electrodes. Electrical stimulation of both vagus nerves produced frequency-dependent bronchoconstriction (measured as an increase in Ppi above baseline) and bradycardia. The vagus nerves were stimulated at frequencies ranging from 2 to 15 Hz for 5 s at 120-s intervals, keeping both pulse duration (0.1 ms) and voltage (10 V) constant among groups. Increases in Ppi were recorded on a Grass polygraph as described above. At the end of experiments, vagally induced bronchoconstriction was abolished by administration of atropine (1 mg/kg iv), demonstrating that these responses were mediated via release of acetylcholine.

To test whether a cationic substance increased responsiveness to vagal stimulation, some animals were treated with heparin. Animals were treated with SM-substance P, and 30 min later, a frequency-response curve was generated. Heparin (3,000 U/kg iv) was then administered (3,000 U/kg iv), and 20 min later, the frequency-response curve was repeated. To ensure that there was no tachyphylaxis to repeated vagal nerve stimulation, some animals were treated with heparin without first generating a frequency response as described above. The frequency-response curves in these animals were identical to the data obtained in animals stimulated before and after heparin, and these data have been combined.

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Studies of M₂ receptor function. After the pilocarpine dose-response and frequency-response experiments, the sensitivity of airway smooth muscle to acetylcholine (1–10 µg/kg iv) was tested. In vagotomized guinea pigs, acetylcholine-induced bronchoconstriction is due to M₂ receptor stimulation (4, 43).

Bronchoalveolar lavage. At the end of the experiment, bronchoalveolar lavage was performed via the tracheal cannula. The lungs were lavaged with five aliquots of 10 ml of phosphate-buffered saline (PBS). The recovered lavage fluid (35–45 ml) was centrifuged. Total cells were counted under a Neubauer Hemocytometer (Hauser Scientific, Horsham, PA). Aliquots of the cell suspension were cytospun onto glass...
controls. Heparin acutely restored M2 receptor function in inhibition of vagally induced bronchoconstriction in bronchoconstriction by 15% in the SM-substance P-carpine (100 μg/kg) only inhibited vagally induced bronchoconstriction by 15% in the SM-substance P-carpine (100 μg/kg) only inhibited vagally induced bronchoconstriction in controls. Heparin acutely restored M2 receptor function in SM-substance P-treated animals, such that the effect of 100 μg/kg pilocarpine was similar to that in control animals (Fig. 1).

Depletion of inflammatory cells by pretreatment of pathogen-free guinea pigs with cyclophosphamide (30 mg/kg ip) prevented SM-substance P-induced loss of neuronal M2 receptor function (Fig. 1). Similarly, pretreatment with either the antibody to MBP (Fig. 2) or the NK2 antagonist (Fig. 3) restored responsiveness to pilocarpine, and pretreatment with cyclophosphamide ( alone, n = 4) prevented loss of responsiveness to pilocarpine. *Statistical significance from control and cyclophosphamide pretreated.

In control guinea pigs, increasing doses of pilocarpine (0.1–100 μg/kg) inhibited vagally induced bronchoconstriction in a dose-dependent manner in control animals (Fig. 1). However, in guinea pigs treated with SM-substance P, pilocarpine did not significantly inhibit vagally induced bronchoconstriction, indicating neuronal M2 receptor dysfunction (Fig. 1). The highest dose of pilocarpine (100 μg/kg) only inhibited vagally induced bronchoconstriction by 15% in the SM-substance P-treated animals compared with a greater than 60% inhibition of vagally induced bronchoconstriction in controls. Heparin acutely restored M2 receptor function in SM-substance P-treated animals, such that the

Drugs and reagents. Acetylcholine, cyclophosphamide, guanethidine, PBS, pilocarpine, SM-substance P, sodium chloride, succinylcholine, and urethan were purchased from Sigma (St. Louis, MO). Rabbit anti-guinea pig MBP antibody was produced as described previously (35, 48). The NK receptor antagonists SR-140333 and SR-48968 were the generous gifts of Dr. Xavier Emonds-Alt (Sanofi Reserche, Montpellier, France). All drugs were dissolved and diluted in 0.9% NaCl or PBS with the exception of SR-140333, which was dissolved in DMSO (10 mg/ml stock solution) and then diluted in PBS. Appropriate controls with this vehicle were carried out, and they demonstrated that DMSO had no effect on any of the parameters measured here.

Statistics. All data are expressed as means ± SE. Acetylcholine, frequency, and pilocarpine responses were analyzed using two-way analyses of variance for repeated measures. Baseline heart rates, blood pressures, Ppi, and changes in Ppi were analyzed using paired Student’s t-test (Statview 4.5; Abacus Concepts, Berkeley, CA; P ≤ 0.05 was considered significant).

RESULTS

In the absence of pilocarpine, electrical stimulation of both vagus nerves caused bronchoconstrictions, which were not significantly different among groups (control, 22.0 ± 2.1; SM-substance P, 21.6 ± 1.8; SM-substance P with cyclophosphamide pretreatment, 28.9 ± 4.6; SM-substance P with antibody to MBP pretreatment, 26.0 ± 2.0; SM-substance P with NK1 antagonist pretreatment, 24.8 ± 2.6; and NK2 antagonist treatment, 19.3 ± 3.2 mmHg). In the absence of pilocarpine, the voltages used to obtain the above bronchoconstrictions were variable but not significantly different among groups (control, 18.3 ± 7.7; SM-substance P, 16.6 ± 4.6; SM-substance P with cyclophosphamide pretreatment, 7.7 ± 4.3; SM-substance P with antibody to MBP pretreatment, 9.9 ± 2.2; SM-substance P with NK1 antagonist pretreatment, 10.5 ± 0.9; and NK2 antagonist treatment, 19.3 ± 7.1 V). Vagal stimulation also caused transient bradycardia, which was not significantly altered by SM-substance P (data not shown). Responses to electrical stimulation of the vagus nerves were cholinergic because they were abolished by administration of atropine (1 mg/kg).

In control guinea pigs, increasing doses of pilocarpine (0.1–100 μg/kg) inhibited vagally induced bronchoconstriction in a dose-dependent manner, demonstrating the presence of functional M2 receptors (Fig. 1). However, in guinea pigs treated with SM-substance P, pilocarpine did not significantly inhibit vagally induced bronchoconstriction, indicating neuronal M2 receptor dysfunction (Fig. 1). The highest dose of pilocarpine (100 μg/kg) only inhibited vagally induced bronchoconstriction by 15% in the SM-substance P-treated animals compared with a greater than 60% inhibition of vagally induced bronchoconstriction in controls. Heparin acutely restored M2 receptor function in SM-substance P-treated animals, such that the

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onist (Fig. 3B), also prevented SM-substance P-induced M2 receptor dysfunction. In these experiments, neither the antibody to MBP (data not shown; n = 2) nor the NK1 antagonist SR-140333 (Fig. 3A) affected M2 receptor function in control guinea pigs.

In control guinea pigs, electrical stimulation of the distal ends of the cut vagi at increasing frequencies (2–15 Hz, 0.1 ms, 10.0 V for 5 s at 120-s intervals) produced frequency-dependent bronchoconstriction, measured as an increase in $P_{pl}$. SM-substance P significantly potentiated vagally induced bronchoconstriction compared with that in control animals (Fig. 4). Administration of heparin after SM-substance P reversed the SM-substance P-induced potentiation of vagally in-
duced bronchoconstriction (Fig. 4A). Heparin acutely reduced vagally induced bronchoconstriction below control levels; this difference was not statistically significant ($P = 0.6240$).

Pretreatment with antibody to MBP prevented potentiation of vagally induced bronchoconstriction in SM-substance P-treated guinea pigs (Fig. 4B). Likewise, pretreatment with the NK$_1$ receptor antagonist (Fig. 5) also prevented potentiation of vagally induced bronchoconstriction in SM-substance P-treated guinea pigs. In control guinea pigs, neither antibody to MBP (Fig. 4B) nor SR-140333 (Fig. 5) affected airway reactivity to vagal stimulation. We attempted to test the effects of the NK$_2$ antagonist SR-48968 on SM-substance P-induced potentiation of vagally induced bronchoconstriction. In animals given the NK$_2$ antagonist (1 mg/kg ip), the responses to vagal stimulation were markedly inhibited so that we were unable to measure the reactivity of the vagus nerves after SM-substance P (data not shown).

We subsequently tested the effect of the NK$_2$ antagonist on airway responsiveness to vagal stimulation in control guinea pigs. In these guinea pigs, repeated vagal stimulation at 10 Hz, 10 V, 0.1 ms, for 5 s at 120-s intervals caused a baseline change in $P_{pl}$ of 98.3 ± 3.9 mmH$_2$O ($n = 4$). Once a baseline response to vagal stimulation was achieved, the NK$_2$ antagonist SR-48968 was given (1 mg/kg iv). This dose caused a 79% inhibition of vagally induced bronchoconstriction over a 12-min period of repeated stimulation at the same pulse parameters and intervals (Fig. 6A). There was not, however, a change in vagally induced bradycardia at this time (Fig. 6B).

Intravenous acetylcholine (1–10 $\mu$g/kg) induced dose-dependent bronchoconstriction in all groups re-
Regardless of treatment. In all of the guinea pigs, both vagi were cut to eliminate any possible vagal reflex. There were no significant differences in the response to intravenous acetylcholine in vagotomized guinea pigs treated with the NK2 antagonist vs. animals not receiving the NK2 antagonist (Fig. 7). Additionally, in vagotomized animals treated with intravenous SM-substance P, there were no significant differences in acetylcholine-induced bronchoconstriction among groups (Fig. 8).

Administration of SM-substance P did not significantly alter the number of inflammatory cells recovered from bronchoalveolar lavage fluid of pathogen-free guinea pigs (Table 1). Cyclophosphamide treatment caused a marked decrease in the total numbers of leukocytes returned in bronchoalveolar lavage. Eosinophils, neutrophils, and lymphocytes comprised less than 2% of the returned cells in the cyclophosphamide-treated animals compared with 25% in control animals (n = 2; data not shown).

DISCUSSION

Intravenous SM-substance P did not significantly alter heart rate, systolic and diastolic blood pressure, or baseline P_{pi}. Likewise, SM-substance P did not cause any significant differences among the populations of inflammatory cells returned in bronchoalveolar lavage (Table 1). In addition, substance P did not change the responsiveness of the airway smooth muscle to acetylcholine (Fig. 6). Although intravenous muscarinic agonists can elicit a vagal reflex (4), there was no involvement of the vagus nerves here because both vagus nerves were cut.

In the heart, vagally induced bradycardia is mediated by M2 receptors (32). Unlike vagally induced bronchoconstriction in the lungs, SM-substance P did not significantly affect acetylcholine in control guinea pigs because the muscarinic agonist pilocarpine inhibited vagally induced bronchoconstriction in a dose-dependent manner. In contrast, in animals treated with intravenous SM-substance P, pilocarpine no longer inhibited vagally induced bronchoconstriction (Fig. 1). Thus SM-substance P causes dysfunction of neuronal M2 receptors in the lungs.

Neuronal M2 receptors were functioning to inhibit release of acetylcholine in control guinea pigs because the muscarinic agonist pilocarpine inhibited vagally induced bronchoconstriction in a dose-dependent manner. In contrast, in animals treated with intravenous SM-substance P, pilocarpine no longer inhibited vagally induced bronchoconstriction (Fig. 1). Thus SM-substance P causes dysfunction of neuronal M2 receptors in the lungs.

Cyclophosphamide pretreatment prevented SM-substance P-induced loss of M2 receptor, suggesting inflammatory cell involvement (Fig. 1). The lungs of pathogen-free guinea pigs contain resident eosinophils (10, 11, 15, 17). We have previously shown that eosinophils cause dysfunction of neuronal M2 receptors by releasing MBP (9, 16, 29). Heparin reversed SM-substance P loss of neuronal M2 receptor function, demonstrating a reversible role of cationic proteins in tachykinin-mediated loss of M2 receptor function (Fig. 1; Ref. 10). In addition, specific inhibition of eosinophil MBP also protected M2 receptor function in the SM-substance P-treated guinea pigs, indicating that the positively charged MBP released by eosinophils is the cause of neuronal M2 receptor dysfunction (Fig. 2). Because none of these treatments affect M2 receptor function in control animals (Fig. 2; Refs. 18, 21), SM-substance P-induced loss of neuronal M2 receptor function must be mediated via release of eosinophil MBP.
Intravenous SM-substance P also causes hyperreactivity to electrical stimulation of the vagus nerves. Vagally induced bronchoconstriction is significantly potentiated in the animals treated with SM-substance P compared with control guinea pigs (Fig. 4). Because there is no change in the sensitivity of the airway smooth muscle to acetylcholine, SM-substance P-induced hyperreactivity is mediated entirely through the vagus nerves (Fig. 6). These data agree with a study by Omini et al. (40) showing that substance P-induced hyperreactivity is vagally mediated.

SM-substance P-induced hyperreactivity is mediated by the release of MBP. Pretreatment with antibody to eosinophil MBP prevented SM-substance P-induced hyperreactivity, and administration of heparin to SM-substance P-treated guinea pigs acutely reversed vagal hyperreactivity (Fig. 4). Neither the antibody to MBP (Fig. 4B) nor heparin (18) affects reactivity in control animals. Treatments that protect or restore neuronal M2 receptor function after SM-substance P also prevent or reverse vagally mediated hyperreactivity in the absence of any change in airway smooth muscle sensitivity to acetylcholine. Thus loss of M2 receptor function is the cause of hyperreactivity in SM-substance P-treated guinea pigs. Both of these effects are dependent on release of MBP from eosinophils.

After treatment with SM-substance P, the response to high-frequency vagal stimulation was markedly increased (Fig. 4), whereas the response to low-frequency stimulation was not. This further suggests that M2 receptor dysfunction is responsible for the hyperresponsiveness. Inhibition of acetylcholine release by neuronal M2 receptors is much greater at high-frequency nerve stimulation since there is more acetylcholine released to stimulate the receptors (19, 20).

Therefore, increased bronchoconstriction due to loss of neuronal M2 receptor function is more apparent at higher frequencies than at lower frequencies. Conversely, the effects of exogenous agonists on M2 receptors (as in our pilocarpine experiments) are more readily apparent at lower frequencies when they are not competing for receptors with endogenous acetylcholine.

SM-substance P-induced loss of neuronal M2 receptor function was prevented by pretreatment with a NK2 receptor antagonist (Fig. 3A) but not with a NK1 antagonist (Fig. 3B). In addition, the NK2 receptor antagonist prevented SM-substance P-induced hyperreactivity (Fig. 5). We were not able to test the effect of the NK2 antagonist on hyperreactivity since the NK2 antagonist blocked vagally induced bronchoconstriction in the absence of SM-substance P (Fig. 6A). Neither NK antagonist treatment affected the sensitivity of airway smooth muscle to acetylcholine (Figs. 7 and 8).

Because the NK2 receptor antagonist inhibited vagally induced bronchoconstriction in the absence of exogenous SM-substance P, it appears that the endogenous tachykinins enhance vagal neurotransmission in pathogen-free guinea pigs via NK2 receptors (26, 49). This may explain the conflicting results over the roles of NK1 and NK2 receptors found in models of hyperreactivity (6, 45). It has been suggested that NK2 receptors mediate inflammation, whereas our data suggest that tachykinins directly enhance neurotransmission via NK2 receptors independently of inflammation and presumably also independently of antigen challenge.

Substance P can initiate degranulation of eosinophils (31) and does so by stimulating NK1 receptors (14). Recovery of eosinophils in the bronchoalveolar fluid...
lavage of control guinea pigs indicates that they have eosinophils resident within their airways (Table 1). Eosinophil MBP is an endogenous allosteric antagonist for M2 receptors in vitro (29). Although, substance P can induce eosinophil degranulation directly via the NK1 receptors (14), an indirect pathway that may include the release of mediators from other inflammatory cells, such as mast cells, macrophages, or neutrophils (33, 42), cannot be excluded.

In total, these studies provide a potential in vivo mechanism for substance P-induced hyperreactivity. It is the result of M2 receptor blockade by MBP, and the release of MBP by eosinophils is NK1 receptor mediated. Loss of M2 receptor function increases the release of acetylcholine in response to electrical stimulation of the vagus nerves (3, 28), which increases bronchoconstriction (4, 16, 17). Thus SM-substance P-induced hyperreactivity is the direct result of increased release of acetylcholine due to loss of neuronal M2 receptor function (Fig. 9).

In antigen-challenged guinea pigs, hyperreactivity to vagal stimulation (10, 16, 17) and to histamine (9, 10) is mediated entirely via increased release of acetylcholine from the vagus nerves. This increased release of acetylcholine from the vagi is due to loss of neuronal M2 receptor function via blockade of the M2 receptors by eosinophil MBP (9, 15–17). A role for tachykinins in this pathway has been suggested by the finding that NK1 antagonists prevent hyperactivity and loss of neuronal M2 receptor function in antigen-challenged guinea pigs without inhibiting eosinophil influx into the airways (10).

It has generally been assumed that the absence or presence of eosinophils in the lungs is enough to implicate a role for eosinophils in a particular response such as hyperreactivity. However, this is clearly not the case. Although eosinophils were present in tachykinin antagonist-treated antigen-challenged guinea pigs, neuronal M2 receptors were functional and the animals were not hyperreactive (10). Eosinophil MBP had been demonstrated previously to be the mechanism for loss of M2 receptor function in antigen-challenged guinea pigs (16). Therefore, the conclusion from these studies could not be that eosinophils were not important to antigen-induced airway hyperreactivity induced by M2 receptor dysfunction. Rather, it is activation and not the mere presence of eosinophils that is important. It was demonstrated previously in primates that although antigen-induced hyperreactivity was associated with a decrease in eosinophils, extracellular eosinophil peroxidase was increased, demonstrating that the eosinophils had been activated (24). In the experiments reported here, SM-substance P-induced hyperreactivity and M2 receptor dysfunction were also not associated with a change in eosinophil number (Table 1). However, the eosinophils were activated as indicated by the ability of the antibody to MBP to inhibit hyperreactivity (Fig. 4). Thus the number of eosinophils present in the lungs is less important than the activation state of the eosinophils (Fig. 9).

There are several points to consider based on the observation that activation is more important than the presence of eosinophils. First, the experiments described here were carried out in pathogen-free guinea pigs. Although guinea pigs have been described as hypereosinophilic, the presence of eosinophils in the lungs does not contribute to hyperreactivity unless the eosinophils are activated, in this case by SM-substance P. Second, although antigen challenge of sensitized, pathogen-free guinea pigs causes recruitment of additional eosinophils into the lungs, there are already enough eosinophils resident in pathogen-free guinea pigs to cause the same degree of M2 receptor dysfunction and hyperreactivity as in the antigen-challenged animals (Figs. 1 and 5; Ref. 16).

In humans, administration of substance P induces hyperreactivity to inhaled methacholine (8). Methacholine-induced bronchoconstriction has been assumed to be due to a direct effect on the airway smooth muscle. However, it has been demonstrated that there is a vagal reflex component to methacholine (4, 47). Neuronal M2 receptors inhibit release of acetylcholine from the vagal parasympathetic nerves in humans (37) and are dysfunctional in some patients with asthma (2, 38). There are significant numbers of eosinophils in the airways of asthmatics (25, 27), and we have demonstrated an association of eosinophils with airway nerves in patients who have died of asthma (11). Therefore, the potentiation of agonist-induced bronchoconstriction by substance P in humans with asthma may also be mediated by eosinophil MBP and M2 receptors.

In conclusion, SM-substance P-induced hyperreactivity in pathogen-free guinea pigs is mediated by release of eosinophil MBP and subsequent loss of neuronal M2 receptor function. In addition, we have demonstrated that it is not the presence of eosinophils, but the release of eosinophil MBP, that is critical to the development of hyperreactivity.

REFERENCES


