Function of Pulmonary M_2 Muscarinic Receptors in Antigen-challenged Guinea Pigs Is Restored by Heparin and Poly-L-Glutamate

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Abstract

The effect of heparin and poly-L-glutamate on the function of inhibitory M_2 muscarinic autoreceptors on parasympathetic nerves in the lung was tested in antigen-challenged guinea pigs. After antigen challenge, M_2 receptor function is decreased, thus increasing release of acetylcholine from the vagus and potentiating vagally induced bronchoconstriction. Guinea pigs were anesthetized, tracheostomized, vagotomized, paralyzed, and ventilated. Electrical stimulation of the vagi caused bronchoconstriction and bradycardia. In controls, pilocarpine attenuated vagally induced bronchoconstriction by stimulating neuronal M_2 muscarinic receptors. Conversely, blocking these autoreceptors with gallamine potentiated vagally induced bronchoconstriction. In challenged animals the effects of both drugs were markedly reduced, confirming M_2 receptor dysfunction. 20 min after heparin or poly-L-glutamate, the effects of both pilocarpine and gallamine on vagally induced bronchoconstriction were restored, demonstrating recovery of M_2 receptor function. Neither heparin nor poly-L-glutamate affected vagally induced responses in control animals. Thus antigen-induced dysfunction of M_2 receptors can be reversed by polyanionic polysaccharides (heparin) or polyanionic peptides (poly-L-glutamate). This suggests that a polycationic substance such as eosinophil major basic protein, cationic protein, or peroxidase may be responsible for antigen-induced pulmonary M_2 receptor dysfunction. (*J. Clin. Invest.* 1992.90:2292–2298.)
Key words: airway hyperresponsiveness • eosinophil major basic protein • asthma • allergy

Introduction

Exposure of sensitized animals or humans to antigen causes airway hyperresponsiveness which may persist for several weeks (1–5). Antigen-induced hyperresponsiveness is associated with increased release of acetylcholine from the vagus nerves since vagally induced contraction of airway smooth muscle is potentiated in vivo and in vitro in challenged animals while the response to exogenous acetylcholine is unchanged (6, 7).

In the lungs, release of acetylcholine from the vagus nerves is under the local control of inhibitory muscarinic autoreceptors on the postganglionic nerves (8). These autoreceptors are classified as M_2 muscarinic receptors, while the muscarinic receptors on airway smooth muscle are M_3 receptors (9). Thus, acetylcholine released from the vagus nerve stimulates both M_3 muscarinic receptors on airway smooth muscle, causing contraction, and M_2 muscarinic receptors on the nerves, decreasing further release of acetylcholine.

The negative feedback control of acetylcholine release provided by the M_2 receptor can be demonstrated in vivo by measuring vagally induced bronchoconstriction in the presence of selective muscarinic agonists or antagonists. Blockade of the neuronal M_2 receptors with gallamine potentiates vagally induced bronchoconstriction as much as 10-fold. Conversely, pilocarpine, by stimulating M_2 receptors, decreases vagally induced bronchoconstriction (8).

These neuronal inhibitory M_2 muscarinic receptors have been demonstrated in guinea pigs, rats, cats, dogs, and humans (8, 10–13), and may be hypofunctional in asthmatics (14–15). In the guinea pig, the function of these inhibitory receptors is markedly impaired after acute viral infection (16), acute ozone exposure (17), and antigen challenge of sensitized animals (7). Loss of function of the inhibitory M_2 receptor is characterized by airway hyperresponsiveness to electrical stimulation of the vagus nerve and by failure of pilocarpine to inhibit vagally induced bronchoconstriction. Furthermore, the potentiation of vagally induced bronchoconstriction by gallamine is markedly decreased in treated compared to control animals.

Viral infection, ozone exposure, and antigen challenge all result in an influx of inflammatory cells, especially of eosinophils, into the guinea pig lung (18, 19; Jacoby, D. B., unpublished observation). Eosinophil major basic protein, eosinophil peroxidase, and eosinophil cationic protein are thereby imported into the airways. All of these proteins are strongly positively charged (20, 21). Since other positively charged proteins including poly-L-arginine, poly-L-lysine, basic histone, and protamine have recently been shown to be antagonists for the M_2 receptor (22), it is possible that the positively charged eosinophil proteins might be serving as endogenous M_2 receptor antagonists. The experiments reported here were carried out to determine whether loss of M_2 muscarinic receptor function in the lungs after antigen challenge could be reversed in vivo by using negatively charged substances such as heparin and poly-L-glutamate which bind to and neutralize major basic protein in vitro (23, 24).

Methods

Sensitization and challenge with antigen. Specific pathogen-free guinea pigs (Dunkin Hartley; 200–250 g) were injected intraperitoneally with

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either saline (control) or 10 mg/kg ovalbumin every other day for three injections. 3 wk after the first injection, they were exposed to an aerosol of 5% ovalbumin for 5 min on each of four consecutive days. This regimen has previously been shown to increase the response to vagal stimulation in sensitized animals (6) by impairing M_2 receptor function (7).

Anesthesia. 24 h after the last aerosol challenge, the animals were anesthetized with urethane (1.5 g/kg, intraperitoneally). None of the experiments lasted for longer than 3 h while this dose of urethane produced a deep anesthesia lasting 8–10 h (25). However, because paralyzing agents were used, the depth of anesthesia was monitored by observing for fluctuations in heart rate and blood pressure. Guinea pigs were handled in accordance with the standards established by the USA Animal Welfare Acts set forth in 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.

Measurement of pulmonary inflation pressure (Ppi).[1] Once the guinea pigs were anesthetized, both external jugular veins were cannulated for the administration of drugs. Both vagi were cut and placed on shielded electrodes immersed in a pool of liquid paraffin. The electrodes were connected to a stimulator (SD9; Grass Instrument Co., Quincy, MA). A heating blanket was used to maintain body temperature at 37°C.

The trachea was cannulated and the animals were paralyzed with suxamethonium (infused at 10 μg/kg per min) and ventilated using a positive pressure, constant volume animal ventilator (Harvard Apparatus, South Natick, MA). Ppi was measured at the trachea using a pressure transducer (Viggo-Spectramed, Oxnard, CA). Flow was measured using a pneumotach (Fleish/00; OEM Medical, Richmond, VA) with a differential pressure transducer (Grass Instrument Co.) and this signal was integrated to measure tidal volume. All signals were recorded on a polygraph (Grass Instrument Co.). A carotid artery was cannulated for measurement of blood pressure using a pressure transducer (Viggo-Spectramed), and the heart rate was derived from the blood pressure using a tachograph. All signals were recorded on a polygraph (Grass Instrument Co.). pO_2 and pCO_2 were measured using arterial blood samples at the beginning and end of each experiment (170 pH/blood gas analyzer; Corning Glass Inc., Corning, NY).

A positive pressure of 85–100 mm H_2O was needed for adequate ventilation of the animals. Given constant flow and volume, bronchoconstriction was measured as the increase in Ppi over the baseline inflation pressure (26). Since the increase in Ppi after vagal stimulation reflects primarily an increase in lung resistance with only a small change in dynamic compliance (10), an increase in Ppi will be referred to as bronchoconstriction. The Ppi signal from the driver was fed into the input of the preamplifier of a second channel on the polygraph, and the baseline Ppi was subtracted electrically. Thus Ppi was recorded on one channel and increases in Ppi were recorded on a separate channel at a higher sensitivity. Using this method it was possible to accurately measure increases in Ppi as small as 2 mm H_2O above baseline.

Simultaneous stimulation of both vagus nerves (2–15 Hz, 0.2-ms pulse duration, 5–20 V, 45 pulses per train) at 1-min intervals, caused bronchoconstriction (measured as an increase in Ppi) and bradycardia. After establishing a stable baseline response to vagal stimulation, either heparin (2,000 U/kg), poly-L-glutamate (10 mg/kg), or saline was injected intravenously. Electrical stimulation of the vagi was continued every minute for the next half-hour.

30 min after either heparin or poly-L-glutamate and before administration of either pilocarpine or gallamine control responses to electrical stimulation of the vagus nerves were obtained. Bronchoconstriction in response to stimulation of the vagus nerves was matched in control and sensitized guinea pigs by adjusting the voltage (within a range of 5–20 V). The frequencies used (2 Hz for pilocarpine experiments or 15 Hz for gallamine experiments), pulse duration (0.2 ms) and length of time that the stimulator was on (22 s for pilocarpine experiments or 3 s for gallamine experiments) were not different between groups. Thus the effects of pilocarpine and gallamine on vagally induced bronchoconstriction could be compared between groups without concern about different initial bronchoconstrictor responses.

Once the parameters for vagally induced bronchoconstriction were set and several consistent responses were obtained, either pilocarpine (1–100 μg/kg i.v.) or gallamine (0.1–10 mg/kg i.v.) was given in cumulative doses, and the effects on vagally induced bronchoconstriction and bradycardia were measured. 30 and 100 μg/kg of pilocarpine produced a transient bronchoconstriction. Therefore, the effect of these doses of pilocarpine on vagally induced bronchoconstriction was measured after the Ppi had returned to baseline.

In some experiments bronchoconstriction and bradycardia were elicited using intravenous acetylcholine (ACh). At the end of each experiment atropine (1 mg/kg i.v.) blocked all responses to intravenous ACh and to vagal nerve stimulation, demonstrating that ACh and vagally induced bronchoconstriction and bradycardia were mediated via muscarinic receptors. At the end of each experiment, ovalbumin (1 mg/kg i.v.) was injected to demonstrate that the sensitized animals were indeed sensitized.

Drugs. Atropine, gallamine, ovalbumin, pilocarpine, suxamethonium, heparin, poly-L-glutamate, and urethane were purchased from Sigma Chemical Co., St. Louis, Mo. All drugs were dissolved and diluted in 0.9% NaCl.

Statistics. The effects of heparin, poly-L-glutamate, and saline on dose-response curves to pilocarpine, acetylcholine, and gallamine in antigen-challenged and control guinea pigs were compared using a two-way analysis of variance for repeated measures. The initial bronchoconstriction and bradycardia responses to stimulation of the vagus nerves were compared between control and challenged guinea pigs using unpaired Student's t tests. The effects of heparin and poly-L-glutamate on vagally induced bronchoconstriction and bradycardia were analyzed using paired Student's t tests. A P value of < 0.05 was considered significant.

Results

In all animals that were injected with ovalbumin intraperitoneally, intravenous ovalbumin at the end of the experiment caused a sustained bronchoconstriction (in excess of 500 mm H_2O), thus demonstrating that the animals were sensitized. None of the animals that had been injected intraperitoneally with saline responded to intravenous ovalbumin.

Baseline Ppi (85–100 mm H_2O) and baseline heart rate (250–310 beats/min) were the same in control animals and in animals that were sensitized and challenged with ovalbumin. Electrical stimulation of both vagus nerves (2 or 15 Hz, 0.2 ms pulse duration, 5–20 V, 45 pulses per train) produced bronchoconstriction (measured as an increase in Ppi) and bradycardia (see far left section of Fig. 1). Both of these responses to vagal nerve stimulation were transient and were rapidly reversed after electrical stimulation was stopped. Vagally induced bronchoconstriction and bradycardia were completely blocked by atropine (1 mg/kg), indicating that they were mediated via the release of acetylcholine onto muscarinic receptors.

In guinea pigs that were not sensitized or challenged administration of heparin had no effect on either vagally induced bronchoconstriction (increase of 18.8±4.5 mm H_2O before heparin vs. 18.0±4.2 mm H_2O 20 min after heparin) or bradycardia (fall of 172±27 beats/min before heparin vs. 163±31 beats/min 20 min after heparin). In contrast, heparin decreased the response to vagally induced bronchoconstriction in sensitized, challenged animals. This decrease began 6–7 min

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

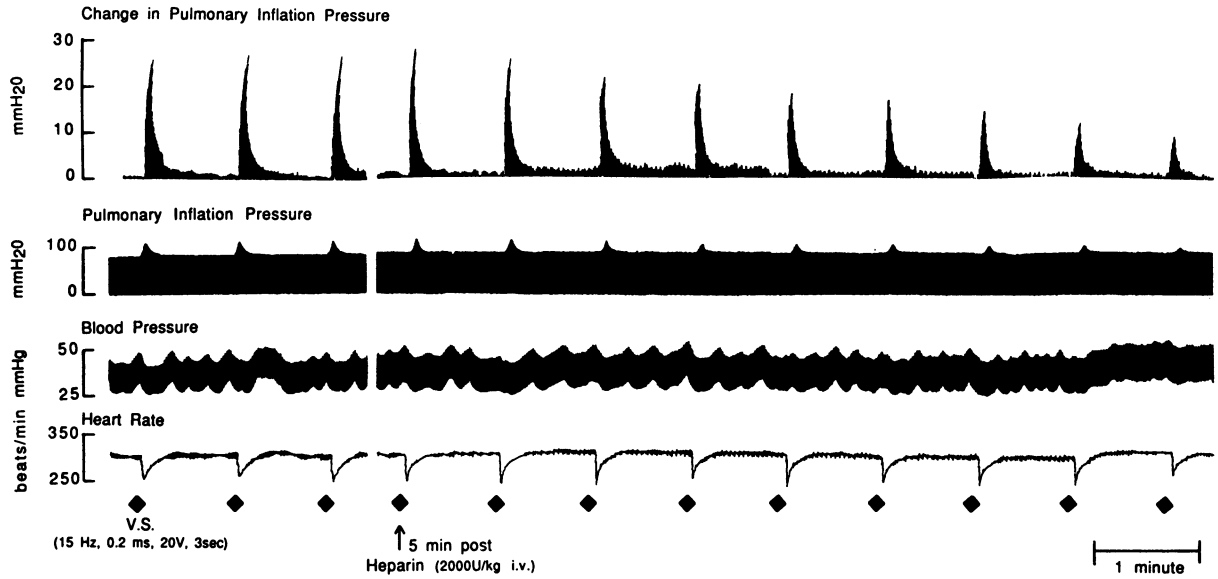

Figure 1. Heparin inhibits vagally induced bronchoconstriction in sensitized guinea pigs challenged with ovalbumin. Electrical stimulation of both vagus nerves (V.S. and at the black diamonds) causes bradycardia (measured as a fall in heart rate) and bronchoconstriction (measured as a rise in pulmonary inflation pressure). The first three responses to vagal nerve stimulation on the left of the figure are in the absence of heparin. The remaining responses are 6–14 min after 2,000 U heparin/kg i.v. Starting at 6 min after heparin vagally induced bronchoconstriction is decreased. In contrast, vagally induced bradycardia is unaltered by heparin. Heparin did not alter either baseline pulmonary inflation pressure or heart rate.

after heparin was injected, and reached a plateau within 10–15 min (see Fig. 1). Poly-L-glutamate caused a similar decrease in vagally induced bronchoconstriction in sensitized, challenged animals. At 20 min after heparin or poly-L-glutamate, vagally induced bronchoconstriction was significantly decreased to 60 and 53%, respectively, of the original response (Fig. 2). In the heart, vagally induced bradycardia was not altered by either heparin or poly-L-glutamate (Figs. 1 and 2).

In control animals, pilocarpine (1–100 μg/kg i.v.) inhibited vagally induced bronchoconstriction in a dose-dependent fashion by stimulating M_2 muscarinic receptors on the pulmonary parasympathetic nerves (*open squares,* Figs. 3 and 4). In contrast, pilocarpine had no significant effect on the response to vagal stimulation in sensitized, challenged animals (*filled squares,* Figs. 3 and 4). After treatment with either heparin or poly-L-glutamate the ability of pilocarpine to inhibit vagally induced bronchoconstriction in challenged guinea pigs was completely restored. There was no significant difference between control and challenged groups: $P = 0.0001$). 30 min after heparin (2,000 U/kg i.v.) the effect of pilocarpine on vagally induced bronchoconstriction was completely restored (*closed triangles,* there was no significant difference between control and challenged + heparin groups). In the absence of pilocarpine, vagally induced bronchoconstriction (2 Hz, 0.2 ms, 10–20 V, 45 pulses/train) was not significantly different between the three groups of animals (controls, 18±3.3; challenged, 18±5.8; and challenged + heparin, 23±5.5 mm H_2O). Results are expressed as percentage of vagally induced bronchoconstriction before pilocarpine. Each point is the mean of five animals with SEM shown by vertical bars. ■, Challenged; ▲, challenged + heparin (2,000 U/kg); □, control.

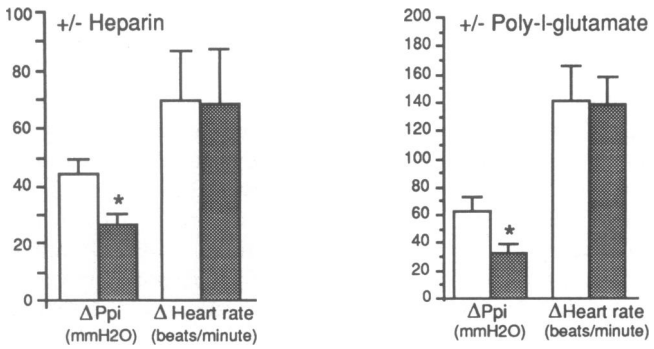

Figure 2. Heparin and poly-L-glutamate inhibit vagally-induced bronchoconstriction but not vagally-induced bradycardia in sensitized guinea-pigs challenged with ovalbumin. Electrical stimulation of the vagus nerves causes bronchoconstriction, measured as an increase in Ppi in mm H_2O, and bradycardia, measured as a change in heart rate in beats/minute (*open columns*). Twenty minutes post heparin (2,000 U/kg i.v.) or poly-L-glutamate (10 mg/kg) (hatched columns), vagally-induced bronchoconstriction was inhibited, while vagally-induced bradycardia was unaltered. Data shown are mean with s.e. mean shown by vertical bars, n = 7 for heparin and 5 for poly-L-glutamate. *P value less than 0.05, using paired Student's t-test. □, Control; ■, heparin or poly-L-glutamate.

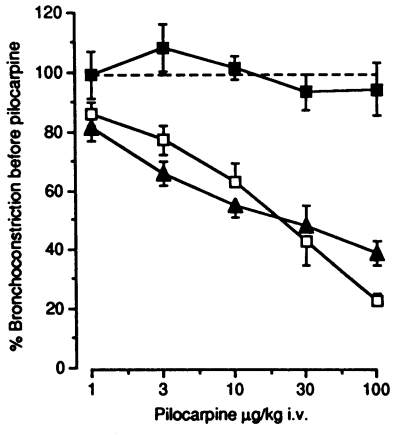

Figure 3. Heparin restores the response to pilocarpine in ovalbumin-challenged guinea pigs. Pilocarpine (1–100 μg/kg i.v.) significantly inhibited vagally induced bronchoconstriction in control guinea pigs (*open squares,* $P = 0.001$). After antigen challenge the effect of pilocarpine on vagally induced bronchoconstriction was abolished (*closed squares,* there was a significant difference between control and challenged groups: $P = 0.0001$). 30 min after heparin (2,000 U/kg i.v.) the effect of pilocarpine on vagally induced bronchoconstriction was completely restored (*closed triangles,* there was no significant difference between control and challenged + heparin groups). In the absence of pilocarpine, vagally induced bronchoconstriction (2 Hz, 0.2 ms, 10–20 V, 45 pulses/train) was not significantly different between the three groups of animals (controls, 18±3.3; challenged, 18±5.8; and challenged + heparin, 23±5.5 mm H_2O). Results are expressed as percentage of vagally induced bronchoconstriction before pilocarpine. Each point is the mean of five animals with SEM shown by vertical bars. ■, Challenged; ▲, challenged + heparin (2,000 U/kg); □, control.

tween the effect of pilocarpine on vagally induced bronchoconstriction in control animals and in challenged animals who had received either heparin (*closed triangles,* Fig. 3) or poly-L-glutamate (*closed circles,* Fig. 4).

Gallamine (0.1–10 mg/kg i.v.) potentiated vagally induced bronchoconstriction in control animals due to blockade of neuronal M_2 muscarinic receptors (*open squares,* Fig. 5). The effect of gallamine was quite marked: 10 mg/kg increased vagally induced bronchoconstriction fivefold. In contrast, potentiation of vagally induced bronchoconstriction by gallamine was significantly attenuated in sensitized, challenged animals (*closed squares,* Fig. 5). In the challenged animals treated with heparin, the ability of gallamine to potentiate vagally induced bronchoconstriction was completely restored to control levels (*closed triangles,* Fig. 5). There was no significant difference between the effect of gallamine on vagally induced bronchoconstriction in control animals and in challenged animals who had received heparin. Furthermore, in control animals, heparin did not significantly affect gallamine-induced potentiation of vagally induced bronchoconstriction (the effect of 0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg gallamine on vagally induced bronchoconstriction in control guinea pigs after heparin was: 170±10, 190±20, 330±30, 400±40, and 530±40, respectively. Data are expressed as percentage of bronchoconstriction before gallamine, means±SEM are given).

In the heart, gallamine caused a dose-related inhibition of vagally induced bradycardia. The effect of gallamine on vagally induced bradycardia was not different between control, control animals treated with heparin, challenged, or challenged animals treated with heparin (data not shown).

Neither heparin nor poly-L-glutamate altered the response of postjunctional M_3 muscarinic receptors in the lung or postjunctional M_2 muscarinic receptors in the heart to cholinergic agonists. In nonchallenged guinea pigs, intravenous ACh (1–7 μg/kg) caused dose-related bronchoconstriction and bradycardia that were not altered by heparin (bronchoconstriction data shown in Fig. 6). Furthermore, in challenged guinea pigs, there

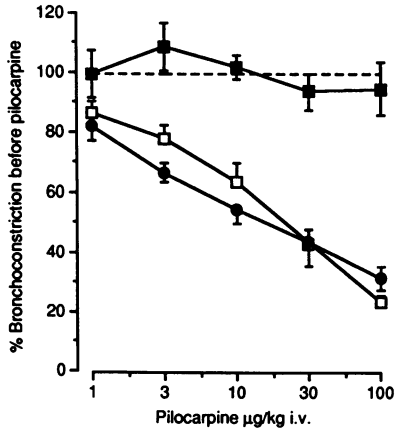

Figure 4. Poly-L-glutamate restores the response to pilocarpine in ovalbumin-challenged guinea pigs. Data from control (*open squares*) and antigen-challenged (*closed squares*) groups is identical to that expressed in Fig. 3. 30 min after poly-L-glutamate (10 mg/kg i.v.) the effect of pilocarpine on vagally induced bronchoconstriction was completely restored (*closed circles,* there was no significant difference between control and challenged + poly-L-glutamate groups). In the absence of pilocarpine vagally induced bronchoconstriction (2 Hz, 0.2 ms, 10–20 V, 45 pulses/train) was not significantly different among the three groups of animals (controls, 18±3.3; challenged, 18±5.8; and challenged + poly-L-glutamate, 14.5±6.3 mm H_2O). Results are expressed as in Fig. 3. Each point is the mean of four to five animals with SEM shown by vertical bars. ■, Challenged; ●, challenged + poly-L-glutamate 10 mg/kg; □, control.

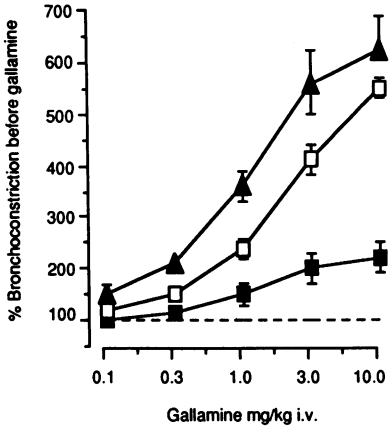

Figure 5. Heparin restores the response to gallamine in ovalbumin-challenged guinea pigs. Gallamine (0.1–10 mg/kg i.v.) significantly potentiated vagally induced bronchoconstriction in control guinea pigs (*open squares,* P = 0.01). After antigen challenge the effect of gallamine on vagally induced bronchoconstriction was attenuated (*closed squares,* there was a significant difference between control and challenged groups: P = 0.05). 30 min after heparin (2,000 U/kg i.v.) the effect of gallamine on vagally induced bronchoconstriction was completely restored (*closed triangles,* there was no significant difference between control and challenged + heparin groups). In the absence of gallamine vagally induced bronchoconstriction (15 Hz, 0.2 ms, 10–20 V, 45 pulses/train) was not significantly different among the three groups of animals (controls, 25±5.4; challenged, 19±0.4; and challenged + heparin, 17.5±1.0 mm H_2O). Results are expressed as percentage of vagally induced bronchoconstriction before gallamine. Each point is the mean of five animals with SEM shown by vertical bars. ▲, challenged + heparin (2,000 U/kg); □, control; ■, challenged.

was no significant difference between pilocarpine induced bronchoconstriction or bradycardia after treatment with either saline (control) heparin or poly-L-glutamate (Fig. 7).

Discussion

In control animals pilocarpine inhibited and gallamine potentiated vagally induced bronchoconstriction due to stimulation (pilocarpine) and blockade (gallamine) of inhibitory M_2 muscarinic receptors on the parasympathetic nerves in the lung. These data confirm previous findings (8, 27). It has been dem-

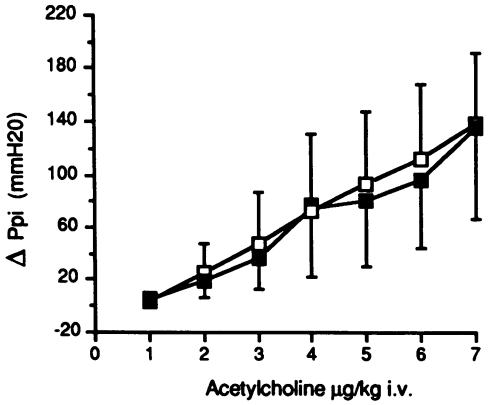

Figure 6. Heparin does not affect the function of M_3 muscarinic receptors on airway smooth muscle. Acetylcholine (1–7 μg/kg i.v.) causes a dose-related bronchoconstriction, measured as an increase in Ppi (*open squares*). 30 min after heparin (2,000 U/kg i.v.; *closed squares*) acetylcholine-induced bronchoconstriction was identical to the response obtained in the absence of heparin. Each point is the mean of three animals with SEM shown by vertical bars. □, Control; ■, + heparin (2,000 U/kg).

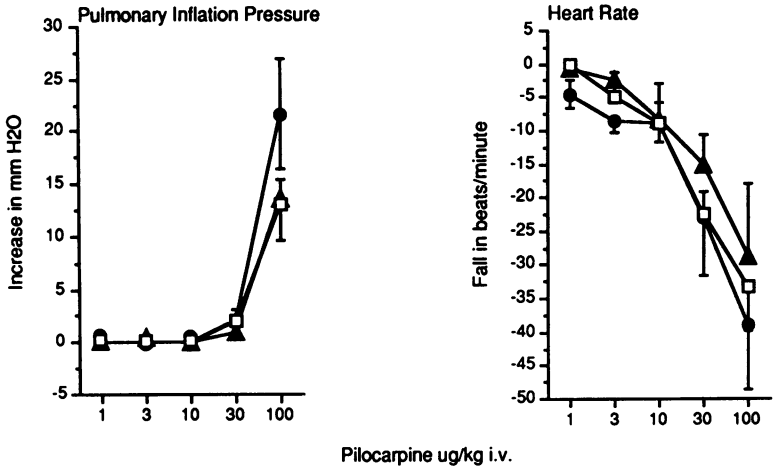

Figure 7. Neither heparin nor poly-L-glutamate affects the function of M_3 muscarinic receptors in the lung or M_2 muscarinic receptors in the heart. The muscarinic agonist pilocarpine (1–100 μg/kg i.v.; *open squares*) caused dose-related bronchoconstriction, measured as an increase in Ppi (*left*), and bradycardia, measured as a fall in heart rate (*right*) in guinea pigs challenged with antigen. These responses to pilocarpine were not significantly different in challenged guinea pigs treated with either heparin (2,000 U/kg i.v.; *closed triangles*) or poly-L-glutamate (10 mg/kg i.v.; *closed circles*). Each point is the mean of five animals with SEM shown by vertical bars. ▲, + Heparin; □, challenged; ●, + poly-L-glutamate.

onstrated that the function of neuronal muscarinic receptors is dependent upon the frequency at which the vagus nerves are firing. Autoreceptors function best and the effect of antagonists is most apparent at higher frequencies of stimulation (7, 8). Conversely, it is easier to demonstrate the effects of agonists at lower frequencies of stimulation. For these reasons the effects of gallamine and pilocarpine on vagally induced bronchoconstriction were tested using 15 and 2 Hz, respectively.

Both pilocarpine-induced inhibition and gallamine-induced potentiation of vagally induced bronchoconstriction were markedly attenuated after antigen challenge. Thus, in challenged guinea pigs the neuronal M_2 receptors could no longer be stimulated by exogenous agonists since pilocarpine did not inhibit vagally induced bronchoconstriction. Likewise, the ability of endogenous acetylcholine to stimulate these receptors is markedly impaired since the potentiation of vagally induced bronchoconstriction by gallamine was attenuated. The loss of M_2 receptor mediated negative feedback causes hyperresponsiveness to electrical stimulation of the vagus (7). These results confirm that the function of neuronal M_2 receptors in the lung is impaired after antigen challenge (7).

Administration of either heparin or poly-L-glutamate completely reversed the loss of M_2 muscarinic receptor function in the lungs of challenged guinea-pigs. 20 min after either heparin or poly-L-glutamate the neuronal receptor in antigen-challenged guinea pigs could once more be stimulated by exogenous agonists since pilocarpine inhibited vagally induced bronchoconstriction (Figs. 3 and 4). Similarly, the ability of endogenous acetylcholine to stimulate neuronal M_2 muscarinic receptors was also completely restored since gallamine once more potentiated vagally induced bronchoconstriction (Fig. 5). Furthermore, the return of the ability of endogenous ACh to stimulate neuronal M_2 receptors was reflected in a > 50% decrease in the bronchoconstrictor response to vagal stimulation (Figs. 1 and 2). The effects of heparin and poly-L-glutamate on vagally induced bronchoconstriction could not be accounted for by changes in baseline resistance since baseline Ppi was the same in antigen challenged and control guinea-pigs in the absence and presence of heparin or poly-L-glutamate.

It is possible that other mechanisms contribute to increased vagally induced bronchoconstriction in antigen-challenged animals. Airway inflammation might decrease airway neutral endopeptidase activity, as seen after viral infection (28), thereby increasing the effects of tachykinins on parasympathetic nerves (29). Furthermore, both inflammatory cells and airway tissues may release prostaglandins and other mediators that may affect the response to nerve stimulation (30). However the magnitude of the fall in vagally induced bronchoconstriction after heparin or poly-L-glutamate treatment and the coincident return to normal of M_2 receptor function lead us to believe that loss of M_2 receptor function is the most important mechanism causing increased response to vagal stimulation.

In contrast to results obtained in challenged guinea pigs neither heparin nor poly-L-glutamate altered the function of neuronal M_2 receptors in the lungs of control guinea-pigs. Neither compound inhibited vagally induced bronchoconstriction or changed the effect of gallamine on vagally induced bronchoconstriction in nonchallenged guinea pigs.

The response of postjunctional M_3 muscarinic receptors in the lung was not affected by heparin since dose-response curves to acetylcholine were superimposable in the absence or presence of heparin in control guinea pigs (Fig. 6). It has been previously demonstrated that the function of the postjunctional M_3 muscarinic receptors in the lungs is not altered from control by antigen challenge in guinea pigs (7). Thus, it was not surprising that polyanionic compounds such as heparin and poly-L-glutamate had no effect on M_3 receptor function in challenged pigs (see left side of Fig. 7). Since neither heparin nor poly-L-glutamate had any effect on M_3 muscarinic receptors in the lungs, restoration of the effects of pilocarpine and gallamine on vagally induced bronchoconstriction in challenged guinea pigs is not due to any postjunctional effect of heparin or poly-L-glutamate in the lungs.

Antigen challenge–induced changes in M_2 muscarinic receptor function appear to be limited to the lungs since M_2 receptor function in the heart is not altered (7). Since antigen challenge was carried out by inhalation it is not surprising that systemic alterations in muscarinic receptor function did not occur. Likewise, neither heparin nor poly-L-glutamate altered M_2 muscarinic receptor function in the heart in either control or in challenged guinea pigs.

In contrast, both heparin and poly-L-glutamate inhibit vagally induced bronchoconstriction in challenged but not in control guinea pigs (Figs. 1 and 2). Vagally induced bronchoconstriction is inhibited within 6–7 min of administration of either substance with the effect plateauing 10–15 min later. Since the time course of inhibition of vagally induced bronchoconstriction is similar for both drugs it is tempting to speculate

that inhibition of vagally induced bronchoconstriction by heparin and poly-L-glutamate is due to dissociation of an endogenous antagonist from the M_2 muscarinic receptor.

Heparin and poly-L-glutamate are structurally very different from each other. Heparin is a polysaccharide, while poly-L-glutamate is a polypeptide. What these compounds do have in common is that they are both strongly negatively charged. Because of their charge these compounds will precipitate positively charged proteins in vitro (23) and in vivo (24), and this effect may account for their actions in the experiments reported here.

It has recently been demonstrated that strongly positively charged proteins such as poly-L-arginine and poly-L-lysine are antagonists at M_2 muscarinic receptors (22). Furthermore, eosinophil major basic protein, which is also strongly positively charged, will displace the muscarinic ligand ^{3}H-*N*-methylscopolamine from M_2 muscarinic receptors in the heart, an effect that can be reversed by heparin (31).

Major basic protein is found in eosinophil granules (32) and eosinophils are increased in guinea pig lungs after antigen challenge (19). There is a strong correlation between the presence of inflammatory cells in the lung and development of hyperresponsiveness after antigen challenge (4, 18, 33). Furthermore antigen-induced hyperresponsiveness is inhibited by depletion of inflammatory cells (34). Thus, there is a clear correlation between the presence of inflammatory cells in the lungs and the development of hyperresponsiveness after antigen challenge. It is possible that products of inflammatory cells, such as major basic protein or some other positively charged substance, are acting as endogenous antagonists at the neuronal M_2 muscarinic receptor in the lungs of challenged guinea pigs. Blockade of the neuronal M_2 muscarinic receptor would lead to increased release of acetylcholine from the vagus nerves, and to increased vagally induced bronchoconstriction. Heparin or poly-L-glutamate may inactivate this endogenous antagonist, restore the function of the neuronal M_2 receptor, and subsequently reverse the vagally induced hyperresponsiveness.

In support of this theory, Gundel et al. (35) have demonstrated that major basic protein given intratracheally in primates causes both an increase in resistance and airway hyperresponsiveness. Furthermore, both of these effects were prevented by intratracheal administration of poly-L-glutamate (24). Since the vagus nerves were intact in these experiments it is possible that major basic protein was increasing resistance by acting as an antagonist at the neuronal M_2 muscarinic receptor.

Inhibitory M_2 muscarinic receptors have been shown to control release of acetylcholine from human pulmonary parasympathetic nerves and there is evidence that these receptors are not functioning in some asthmatic patients (14, 15). Eosinophils are present in the lungs of asthmatics (36), and major basic protein is present in their sputum (37). There is also a significant, positive correlation between the number of eosinophils present in the lung, the amount of major basic protein recovered in lavage, and airway hyperresponsiveness in asthmatics (38). Thus, in asthmatic patients hyperresponsiveness may, in part, be caused by loss of M_2 muscarinic receptor function due to the presence of major basic protein or some other positively charged substance acting as an endogenous inhibitor of the neuronal M_2 muscarinic receptor.

These studies confirm that the normal control of acetylcholine release by inhibitory M_2 muscarinic receptors on the pulmonary parasympathetic nerves is impaired after antigen challenge. Furthermore, acute administration of the polyanionic substances heparin and poly-L-glutamate can reverse the effects of antigen challenge on these neuronal M_2 autoreceptors. Thus, both heparin and poly-L-glutamate decrease vagally induced bronchoconstriction in antigen-challenged guinea pigs since acetylcholine released from the vagus nerves is now able to stimulate inhibitory M_2 muscarinic autoreceptors to decrease further release of acetylcholine. A possible mechanism for this effect is that polycationic substances in the lung, such as eosinophil major basic protein, eosinophil peroxidase, and eosinophil cationic protein, are acting as endogenous inhibitors of the M_2 muscarinic receptor in antigen-challenged guinea pigs. The polyanionic substances heparin and poly-L-glutamate restore M_2 receptor function by binding to and neutralizing these polycationic substances.

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