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Short communication

Airway hyperresponsiveness to histamine in mycoplasmal infection: role of histamine *N*-methyltransferase

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Abstract

To elucidate the modulatory role of histamine-degrading enzymes in airway constrictor responses in mycoplasmal infection, we studied hamster tracheal segments under isometric conditions in vitro. Nasal inoculation with *Mycoplasma pneumoniae* potentiated the contractile responses to histamine but not to methacholine. Pretreatment of tissues with the histamine *N*-methyltransferase inhibitor SKF 91488 abolished the infection-induced potentiation, whereas, the diamine oxidase inhibitor aminoguanidine had no effect. The histamine *N*-methyltransferase but not diamine oxidase activity in tracheal tissues was decreased in infected animals. These results suggest that *M. pneumoniae* causes airway hyperresponsiveness to histamine probably through a reduction of endogenous histamine *N*-methyltransferase activity. © 1998 Elsevier Science B.V.

Keywords: Mycoplasmal infection; Histamine metabolism; Airway hyperreactivity; Asthma

1. Introduction

Mycoplasma pneumoniae (*M. pneumoniae*) continues to be a common etiological organism of community-acquired respiratory tract infections. Although there is ample evidence that *M. pneumoniae* infection frequently exacerbates asthma (Seggev et al., 1986; Yano et al., 1994) and airway hyperresponsiveness to histamine (Boldy et al., 1990), the mechanism for this remains uncertain.

Histamine can be metabolized by two major pathways in the body (Zeinger et al., 1976): 50 to 70% of histamine is metabolized by histamine *N*-methyltransferase (EC 2.1.1.8), located in the small intestine, liver, kidney and leukocytes, into *N*-methylhistamine, and the remaining 30 to 45% is metabolized by diamine oxidase (EC 1.4.3.6), also called histaminase, located in intestinal mucosa, placenta, liver, skin, kidney, neutrophils and eosinophils, to imidazole acetic acid. We and others have recently shown that histamine *N*-methyltransferase plays a protective role in the histamine-induced contraction in human bronchi (Tamaoki et al., 1994) and guinea pig trachea (Ohrui et al., 1992). Therefore, to determine whether *M. pneumoniae* infection modifies the contractile responses to histamine and, if so, whether this effect is associated with alterations in histamine *N*-methyltransferase and/or diamine oxidase activity, we studied hamster tracheal segments under isometric conditions in vitro.

2. Methods

2.1. M. pneumoniae infection

The experiments were approved by the Ethical Committee of Tokyo Women's Medical College. Pathogen-free male golden Syrian hamsters, weighing 60–80 g, were anesthetized with a gaseous mixture of nitrous oxide, halothane, and oxygen, and given an intranasal inoculation with *M. pneumoniae* FH strain (1.1×10^7 colony-forming units/ml, 500 μl) or equivalent volume of sterile saline (Cimolai et al., 1992). These hamsters were housed separately for 10 days. In our separate experiment, we confirmed that mycoplasmal infection was established based on the increased antibody titers in serum samples, as determined by an indirect hemagglutinin test, and histologic findings including peribronchial and peribronchiolar infiltration of lymphocytes and polymorphonuclear cells, perivascular edema, intraluminal exudate, and parenchy-

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mal pneumonia. However, apparent infiltration of eosinophils or desquamation of epithelial cells was not observed.

2.2. Contractile responses

The trachea was removed under general anesthesia (sodium pentobarbital, 35 mg/kg, iv) and mounted in 5-ml organ chambers containing Krebs–Henseleit solution consisting of the following composition (in mM): NaCl, 118; KCl, 5.9; $MgSO_4$, 1.2; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; $NaHCO_3$, 25.5; and D-glucose, 5.6, gassed with a mixture of 95% O_2–5% CO_2 at 37°C. Contractile responses were continuously measured isometrically with a force-displacement transducer (Nihon Kohden, JB-652T, Tokyo, Japan) and were recorded on a pen recorder (Nihon Kohden, WT-685G). The tissues were allowed to equilibrate for 60 min while they were washed with Krebs–Henseleit solution every 15 min, and the resting tension was adjusted to 1 g. A contractile response was determined as the difference between peak tension developed and resting tension. All experiments were conducted in the presence of indomethacin (3×10^{-6} M) to avoid prostaglandin release.

Following the equilibration period, histamine (Sigma, St. Louis, MO, USA) or methacholine (Wako Pure, Tokyo) was added to the chamber in a cumulative manner at concentrations ranging from 10^{-8} to 10^{-3} M in half-molar increments at 5-min intervals or 2 min after stable plateau was achieved, whichever was the longer period. Because the contractile responses to methacholine were not altered by *M. pneumoniae* infection, we studied only histamine-induced contractions in the following experiments. To study the role of histamine-degrading enzymes, the tissues were incubated for 20 min with SKF 91488 (*S*-[4-(*N*,*N*-dimethylamino)-butyl] isothiourea, 10^{-4} M, a gift from Smith Kline, Philadelphia, PA, USA), an inhibitor of histamine *N*-methyltransferase (Beaven and Shaff, 1979), or aminoguanidine (10^{-4} M), an inhibitor of diamine oxidase (Schuler, 1952), and the histamine concentration-response curves were generated in a similar manner.

At the end of these experiments, each tracheal segments was blotted on a gauze pad and weighed. Active tensions were normalized for tissue weight and expressed as grams tension per gram of tissue weight. To characterize the concentration-response curves, we determined the maximal contractile response (E_{max}) and the negative logarithm of molar concentration of agonist required to produce 50% of E_{max} (pD_2) by linear regression analysis.

2.3. Measurement of histamine N-methyltransferase and diamine oxidase activities

The activity of histamine *N*-methyltransferase was measured in tracheal tissues according to the method by Fukuda et al. (1991). Briefly, tissues were homogenized with four volumes of ice-cold phosphate-buffered saline (PBS, 0.05 M, pH 7.4) containing 1-mM dithiothreitol and 1% polyethyleneglycol with a glass homogenizer. The homogenate was centrifuged at 4°C ($120\,000 \times g$, 1 h), and the supernatant was dialyzed three times for 8 h against 100 volumes of the buffer. The reaction of histamine *N*-methyltransferase was carried out at 37°C for 20 min in 0.5 ml of a mixture of 0.1 ml of the supernatant, 0.3 ml of 0.1 M PBS containing 0.1-mM pargyline and 0.1-mM aminoguanidine (pH 7.4), 0.05 ml of 1 mM histamine and 0.05 ml of *S*-adenosyl-L-methionine. After incubation, N^{τ}-methylhistamine was separated from histamine by a high-performance liquid chromatography on a weak cation exchanger (Toyo-Soda, TSKgel CM2SW, Tokyo), with 37.5-mM citric acid, 1.25% imidazole and 20% acetonitrile, as the mobile phase at a flow rate of 1.0 ml/min. The fluorescence intensity of the reaction mixture was then

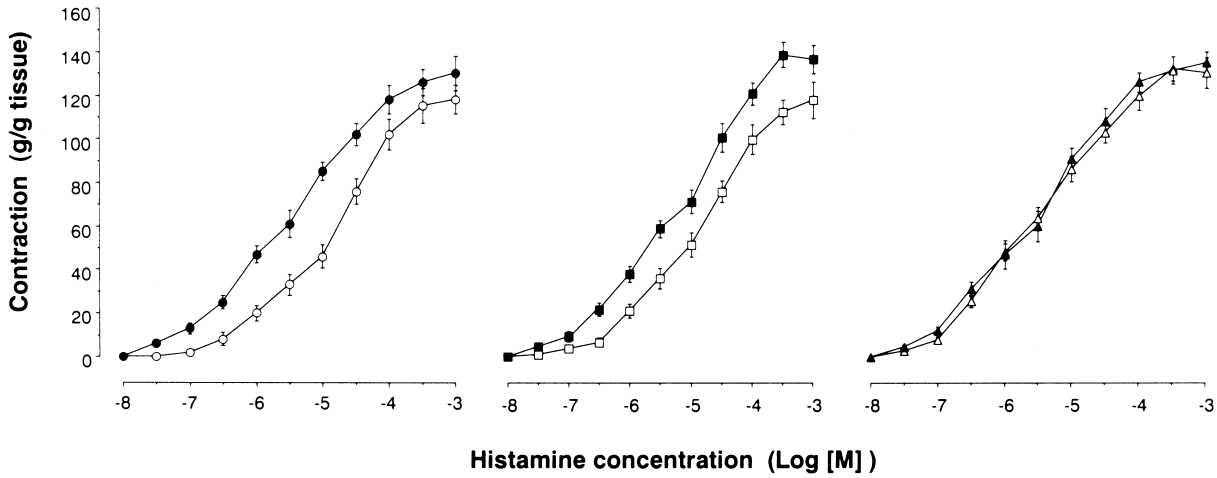

Fig. 1. Contractile responses of tracheal segments to histamine in *M. pneumoniae*-infected hamsters (closed symbols) and saline-treated control hamsters (open symbols) in the absence (left panel) and presence of aminoguanidine (10^{-4} M, middle panel) or SKF 91488 (10^{-4} M, right panel). Responses are expressed as grams tension per gram of tissue weight. Values are means ± S.E.; $n = 11$ for each point.

measured using a post-column derivatization with *o*-phthalaldehyde and 2-mercaptoethanol, and the histamine *N*-methyltransferase activity was expressed as pmol of N^{τ}-methylhistamine formed per hour per milligram of protein.

To assay diamine oxidase activity, [1,4-^{14}C]putrescine (Amersham, Tokyo) was used as a substrate (Kusche and Lorenz, 1983). [^{14}C]Putrescine was mixed with unlabeled putrescine to yield a specific activity of 0.22 μCi/μmol, and this was then mixed with PBS to form a 4.5-mM putrescine solution. The reaction of diamine oxidase was carried out at 37°C for 30 min in a mixture of 0.1 ml of the supernatant of the tracheal homogenate and 0.05 ml of the putrescine solution. After incubation, 1 ml of 1 M NaOH was added and the mixture was extracted with 6-ml toluene including 0.35% 2,5-diphenyloxazole. The toluene phase was then counted in a liquid scintillation spectrometer (Packard Instruments, 460 CD, Downers Grove, IL, USA).

2.4. Statistics

All values were expressed as means ± S.E. Comparative statistical analysis was performed using analysis of variance followed by either Turkey's test for multiple comparisons or by Student's *t*-test; *n* refers to the number of hamsters from which the tissues were taken, and $P < 0.05$ was considered statistically significant.

3. Results

As demonstrated in Fig. 1, contractile responses of tracheal segments to histamine were greater in the hamsters infected with *M. pneumoniae* than in those received saline alone. *M. pneumoniae* infection caused a leftward displacement of the histamine concentration-response curves, so that the pD_2 values increased from 4.9 ± 0.2 to 5.7 ± 0.2 ($P < 0.01$, $n = 11$), but the difference in the maximal contraction did not reach a significant level. The contractile responses to methacholine were similar in the saline-treated and *M. pneumoniae*-infected animals, with the pD_2 values of 5.5 ± 0.3 and 5.6 ± 0.4, respectively ($n = 9$ for each, data not shown). Incubation of tissues with aminoguanidine did not alter the contractile responses to histamine in saline-treated hamsters, and in the presence of aminoguanidine *M. pneumoniae* infection potentiated the histamine-induced contraction (pD_2: 4.8 ± 0.2 vs. 5.5 ± 0.2; $P < 0.01$, $n = 11$). In contrast, SKF 91488 per se increased the contractile responses to histamine in saline-treated animals (pD_2: 4.8 ± 0.3 vs. 5.5 ± 0.3; $P < 0.01$, $n = 11$), and this effect was not further potentiated by *M. pneumoniae*.

The activity of histamine *N*-methyltransferase in tracheal tissues was 850 ± 284 pmol/mg protein per h ($n = 9$) in the saline-treated hamsters and 136 ± 73 pmol/mg protein per h ($n = 9$) in the *M. pneumoniae*-infected animals (Fig. 2). There was a significant difference between these values ($P < 0.01$). The diamine oxidase activity, on the other hand, was not altered by *M. pneumoniae* infection.

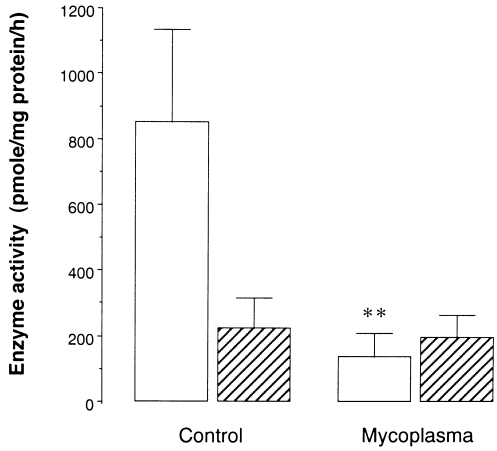

Fig. 2. Activities of histamine *N*-methyltransferase (open columns) and diamine oxidase (shaded columns) in tracheal tissues from *M. pneumoniae*-infected hamsters and saline-treated control hamsters. Values are expressed as means ± S.E.; $n = 9$ for histamine *N*-methyltransferase and $n = 8$ for diamine oxidase. $**P < 0.01$, significantly different from corresponding control values.

4. Discussion

Our in vitro studies demonstrate that the alteration of endogenous histamine *N*-methyltransferase activity may be important in airway hyperresponsiveness to histamine caused by *M. pneumoniae* infection. This conclusion is derived from the following findings. First, intranasal inoculation with *M. pneumoniae* potentiated the contractile responses of hamster tracheal segments to histamine without affecting those to methacholine. Second, this potentiation was abolished under the condition that the histamine *N*-methyltransferase activity had been blocked by the specific inhibitor SKF 91488 (Beaven and Shaff, 1979). Third, we measured the activity of histamine *N*-methyltransferase by high-performance liquid chromatography on post-column derivatization with *o*-phthalaldehyde and found that the enzyme activity of hamster tracheal tissues was greatly decreased by *M. pneumoniae* infection. Therefore, the observed potentiation of histamine-induced contraction may be attributable to the reduction of histamine *N*-methyltransferase activity and the consequent inhibition of histamine degradation and the increased availability of histamine at the airway smooth muscle receptors.

It has been known that histamine is metabolized by two major enzymes, histamine *N*-methyltransferase and diamine oxidase, located on a variety of mammalian tissues and inflammatory cells (White et al., 1987). The former catalyzes methyl transfer from *S*-adenosyl-L-methionine to histamine to form *N*-methylhistamine, which is further metabolized by monoamine oxidase to *N*-methylimidazole

acetic acid, and the latter also metabolizes histamine to *N*-methylimidazole acetic acid. In this study, we found that aminoguanidine at a concentration sufficient to inhibit diamine oxidase activity (Schuler, 1952) did not alter the contractile responses to histamine in saline-treated control animals, indicating that diamine oxidase may play a less important role in the histamine metabolism in the hamster trachea. In contrast, SKF 91488 augmented the histamine-induced contraction in the control animals but had no effect in *M. pneumoniae*-infected animals. These results suggest that histamine is metabolized principally through the histamine *N*-methyltransferase pathway, as is also true in the guinea pig and human airways (Ohrui et al., 1992; Tamaoki et al., 1994), and that *M. pneumoniae* may have specifically inhibited the histamine *N*-methyltransferase activity. This notion is further supported by the finding that the diamine oxidase activity was not significantly affected by *M. pneumoniae* infection.

The airway epithelium has been shown to inhibit bronchoconstrictor responses to a variety of stimuli by releasing epithelium-derived relaxing factor and by metabolizing tachykinins with neutral endopeptidase. Concerning the histamine metabolism, Ohrui et al. (1992) recently showed the presence of histamine *N*-methyltransferase activity and its mRNA in the guinea pig tracheal epithelium by in situ hybridization. Although apparent desquamation of airway epithelium was not observed in our *M. pneumoniae*-infected hamsters, the epithelial histamine *N*-methyltransferase could have been functionally damaged by *M. pneumoniae*. It is also possible that histamine *N*-methyltransferase localized to other cells such as endothelial cells may be involved.

Previous studies have shown that there is a close relation between respiratory tract infection with *M. pneumoniae* and asthma exacerbation (Seggev et al., 1986; Yano et al., 1994) or airway hyperresponsiveness to histamine (Boldy et al., 1990). The potential ability of mycoplasma cell components to alter normal pulmonary physiology has been known and is exemplified by the ciliostatic, hemagglutinating and proteolytic activities of cell extracts (Chandler and Barile, 1980). Moreover, Seggev et al. (1996) have recently shown that *M. pneumoniae*-specific IgE plays a role in the exacerbation of asthma. However, further studies may be required to determine which toxic products from replicating organism are responsible for the inhibition of histamine *N*-methyltransferase activity and the development of airway hyperresponsiveness.

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