Eur Respir J, 1995, 8, 752–754
DOI: 10.1183/09031936.95.08050752
Printed in UK - all rights reserved

Copyright ©ERS Journals Ltd 1995
European Respiratory Journal
ISSN 0903 - 1936

Chronic allergen exposure enhances cholinergic neuro-transmission in sensitized guinea-pigs

N. Kageyama, A. Igarashi, M. Ichinose, M. Miura, H. Yamauchi, M. Tomaki, J. Ishikawa, Y. Sasaki, K. Shirato

Chronic allergen exposure enhances cholinergic neurotransmission in sensitized guinea-pigs. N. Kageyama, A. Igarashi, M. Ichinose, M. Miura, H. Yamauchi, M. Tomaki, J. Ishikawa, Y. Sasaki, K. Shirato. ©ERS Journals Ltd., 1995

ABSTRACT: Airway hyperresponsiveness in asthmatic patients may be related to cholinergic hyperresponsiveness. In this study, we examined whether chronic allergen exposure induces cholinergic hyperresponsiveness in ovalbumin (OA) sensitized guinea-pig airways.

Three weeks after active sensitization, ovalbumin (0.03%, for 3 min, challenged group) or saline inhalation (control group) was repeated every day for 4 weeks. Cholinergic responses were assessed by isometric tracheal contraction after electrical field stimulation (EFS) or exogenously applied acetylcholine (ACh). The contractions were expressed as a percentage of the maximum response to ACh (10^{-3} M) (ACh_{max}). We calculated the effective frequencies producing 25% of ACh_{max} (EF_{25}) from frequency-response curves.

EFS-induced contractile responses were significantly enhanced in the challenged group ($logEF_{25}$=0.66±0.08 (mean±SEM)) compared with the control group ($logEF_{25}$= 1.12±0.16). In contrast, exogenous ACh-mediated contractile tracheal responses were almost the same in both groups.

We conclude that repeated allergen inhalation causes cholinergic airway hyperresponsiveness, presumably due to the facilitation of cholinergic neurotransmission. This mechanism may be involved in the airway hyperresponsiveness in asthmatic airways.

Eur Respir J., 1995, 8, 752–754.

First Department of Internal Medicine, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980, Japan.

Correspondence: K. Shirato
First Department of Internal Medicine
Tohoku University School of Medicine
1-1 Seiryo-machi
Aoba-ku
Sendai 980-77
Japan

Keywords: Acetylcholine
asthma
electrical field stimulation
guinea-pig
neurotransmission

Received: July 13 1994
Accepted after revision February 26 1995

Airway hyperresponsiveness to bronchospastic agents, including cholinergic agonists, is the most typical feature of asthma [1], but the underlying mechanisms are still unknown. Allergen inhalation by sensitized animals causes airway hyperresponsiveness *in vivo* [2, 3]. In *in vivo* models, airway responsiveness to bronchoconstricting agents can be changed by airway smooth muscle contractility itself and/or other factors, such as neural mechanisms. A preliminary report showed that allergen exposure in sensitized guinea-pigs causes subsequent airway hyperresponsiveness to histamine *in vivo*, whereas the response of airway smooth muscle to histamine *in vitro* does not differ from controls [4]. In the guinea-pig, a major portion of the response to histamine is mediated *via* the vagus nerves [5]. It is, therefore, possible that the airway hyperresponsiveness after allergen exposure is of vagal nerve origin rather than due to the airway smooth muscle itself

The aim of this study, therefore, was to examine the effect of chronic allergen exposure both on airway cholinergic nerve function and airway smooth muscle responsiveness to acetylcholine (ACh).

Materials and methods

Animals

Male Dunkin-Hartley guinea-pigs (weight 200–250 g) were actively sensitized by subcutaneous injection of 10 µg ovalbumin dissolved in 0.5 ml of saline containing $Al(OH)_3$ 100 mg, on two consecutive days [6]. Three weeks after the sensitization, the animals were challenged with aerosol saline (control group; n=5) or 0.03% ovalbumin (challenged group; n=6) daily for 4 weeks, in a plexiglass exposure chamber using an ultrasonic nebulizer (output 0.8 $ml \cdot min^{-1}$, for 3 min).

Tissue preparation

On the final day of inhalation, the animals were anaesthetized with intraperitoneal urethane (2 $g \cdot kg^{-1}$). Tracheal strips were placed in a tissue bath (10 ml) containing Krebs-Henseleit solution, containing (mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.5 and glucose 5.05, at 37°C, and bubbled with 95% O_2,

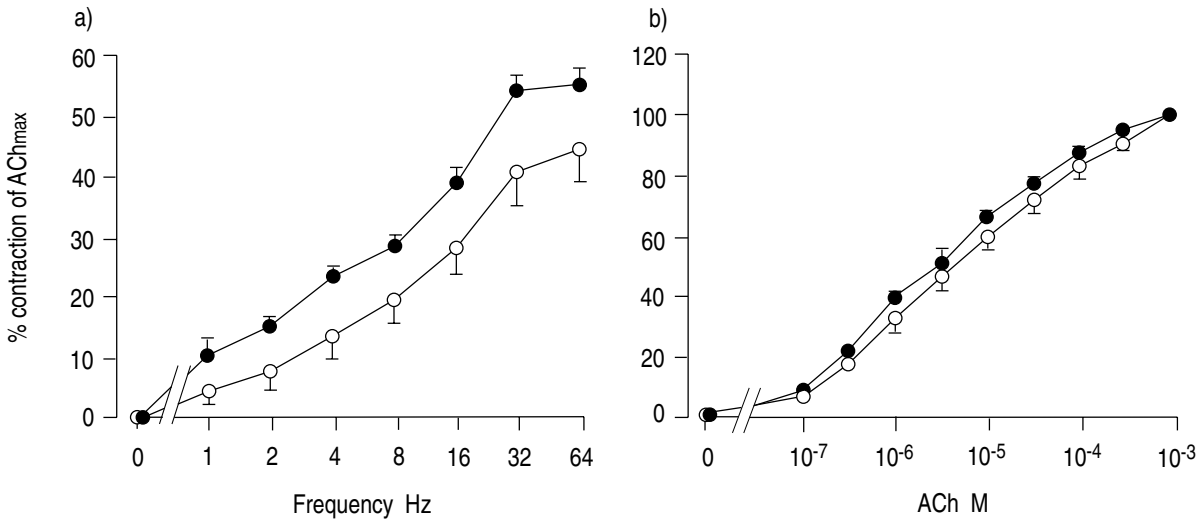

Fig. 1. – a) Electrical field stimulation frequency response curves; and b) exogenous acetylcholine (ACh) dose-response relationships in control (○; n=5) and challenged (●; n=6) groups. All points are means±SEM. There was significant difference ($p<0.05$) between the two groups by two-way analysis of variance (ANOVA).

5% CO_2, and with pH 7.4. Indomethacin (10^{-5} M) was present throughout the experiment in order to prevent the production of cyclooxygenase products that might influence airway tone. Isometric tension was measured by a pressure transducer (UL-10GR, Minebea Co. Ltd, Tokyo, Japan) and was recorded on a polygraph (Rectigraph-8K, NEC San-ei Instruments, Ltd, Tokyo, Japan). An initial tension of 1 g, which was found to be optimal for measuring changes in tension, was applied to the tracheal strips. Sensitization was assessed by adding albumin to the organ bath to give a final concentration of 0.01% albumin, and by recording the change in tension at the end of each experiment.

Methods

Electrical field stimulation was delivered by two parallel platinum wires connected to an electrical stimulator. Biphasic square wave impulses of supramaximal voltage (50 v) and 0.5 ms pulse duration were applied for 10 s every 4 min at frequencies ranging 1–64 Hz.

Cumulative dose-response curves to ACh (10^{-7} to 10^{-3} M) were constructed.

The following drugs were used: acetylcholine chloride (Daiichi pharmaceutical Co. Ltd, Osaka, Japan); indomethacin (Sumitomo chemical Co., Osaka, Japan); atropine sulphate (Tanabe Pharmaceutical, Osaka, Japan); urethane and ovalbumin (Sigma Chemical Co., St. Louis, MO, USA); saline (0.9% sodium chloride) (Otsuka Chemical Co., Tokyo, Japan).

Analysis

Data are expressed as mean±SEM. The contractions were expressed as a percentage of the maximum tissue response to ACh (10^{-3} M) (ACh_{max}). Curves were compared by two-way analysis of variance (ANOVA). The effective frequencies producing 25% of ACh_{max} (EF_{25}) were determined by frequency-response curves. $LogEF_{25}$ was compared by unpaired Student's t-test.

Table 1. – Effect of repeated antigen exposure on EFS-induced bronchoconstriction

	Control	OA
F_{max} %	45.2±5.8	55.7±2.5
$LogEF_{25}$	1.12±0.16	0.66±0.08*

F_{max}: maximal contractile response to the largest frequency of EFS given expressed as a percentage of the maximal contraction to ACh (10^{-3} M) (ACh_{max}); EFS: electrical field stimulation; ACh: acetylcholine; OA: ovalbumin; EF_{25}: effective frequency producing 25% of ACh_{max}. Differences between two groups were compared using student's t-test for the unpaired data. *: $p<0.05$.

Results

Electrical field stimulation of guinea-pig trachea evoked a rapid contraction, which was cholinergic in origin because atropine (10^{-5} M) completely inhibited these responses. In the challenged group, the electrical field stimulation-induced tracheal contractile response curve was significantly shifted to the left compared with that of the control group (fig. 1a). The $logEF_{25}$, i.e. the threshold of electrical field stimulation, was significantly lower in the challenged group (0.66±0.08) than in the control group (1.12±0.16) ($p<0.05$) (table 1). The dose-response curves to exogenous ACh were not significantly different between the two groups (fig. lb).

Discussion

We have demonstrated that chronic allergen exposure enhances vagal cholinergic bronchoconstrictor response after electrical field stimulation in sensitized guinea-pigs. Because the exogenously applied ACh-induced response was not changed by allergen inhalation, it is suggested that neurotransmission through cholinergic efferent nerves is enhanced by chronic allergen exposure and subsequent airway inflammation.

Another possible mechanism by which cholinergic responsiveness in the airways might be increased after

allergen exposure is damage or loss of epithelial cells, since these cells release epithelial derived relaxant factor [7]. However, because the direct ACh-induced contraction was not influenced by allergen inhalation, this possibility is unlikely. For the same reason, an increase in smooth muscle responsiveness by allergen exposure is also unlikely.

Airway hyperresponsiveness to pharmacological stimuli is one of the characteristic features of asthma [1]. A previous report, which compared the responses to histamine *in vivo* and *in vitro* in human airways, failed to show a significant relationship between the two, suggesting that airway hyperresponsiveness does not result from an abnormality in the airway smooth muscle [8]. A preliminary animal study also showed that allergen exposure causes airway hyperresponsiveness to histamine *in vivo* without affecting the airway smooth muscle contraction to histamine *in vitro* [4]. Bronchoconstricting agents, including histamine, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and bradykinin, have been shown to cause airway smooth muscle contraction not only by a direct action on the muscle but also by cholinergic mechanisms. McCaig [9] reported that allergen exposure enhances cholinergic tracheal contraction in sensitized guinea-pigs, but did not examine the contractile response after exogenous ACh. Therefore, the site of cholinergic hyperresponsiveness remained unknown.

The present study shows that allergen exposure enhances cholinergic nerve function without affecting the smooth muscle response, suggesting that cholinergic hyperresponsiveness after allergen challenge is due to presynaptic mechanisms. It is, therefore, possible that the airway hyperresponsiveness observed in asthmatic patients is also due, at least in part, to enhanced cholinergic neurotransmission.

From the present study, the mechanisms which caused the facilitation of cholinergic neurotransmission cannot be determined. A previous study has shown that antigen inhalation causes a dysfunction of muscarinic M2 receptors in pulmonary parasympathetic nerves and enhances cholinergic neurotransmission in guinea-pigs [10]. Furthermore, another report has shown that antigen challenge can lead to enhanced excitability of guinea-pig bronchial ganglion neurons by increasing membrane resistance, depolarizing the membrane potential, and decreasing the action potential accommodative properties of the neuron [11]. These mechanisms may be involved in the mechanisms of cholinergic nerve facilitation in the present study. Because β-agonists have been reported to modulate the cholinergic neurotransmission prejunctionally in canine [12], ferret [13], and human airways [14], sympathetic mechanism may be an alternative explanation of the cholinergic hyperresponsiveness observed in the present study. Sympathetic dysfunction by chronic allergen challenge may be involved in the mechanism of the facilitation of ACh release observed in our study.

In conclusion, we have shown that chronic allergen exposure increases cholinergic nerve function without affecting the airway smooth muscle contractile response to ACh, possibly due to the facilitation of cholinergic neurotransmission. Because the vagal cholinergic nervous system is the dominant neural bronchoconstrictor mechanism in all animals, including humans, and plays an important role in the regulation of airway tone [15], the hyperfunction of this system after allergen inhalation and subsequent airway inflammation may be involved in the pathogenesis and airway hyperresponsiveness of asthma.

Acknowledgement: The authors thank B. Bell for reading the manuscript.

References

1. Boushey HA, Holtzman MJ, Sheller JR, Nadel JA. Bronchial hyperreactivity. *Am Rev Respir Dis* 1980; 121: 389–413.
2. Marsh WR, Irvin CG, Murphy KR, Behrens BL, Larsen GL. Increases in airway reactivity to histamine and inflammatory cells in bronchoalveolar lavage after the late asthmatic response in an animal model. *Am Rev Respir Dis* 1985; 131: 875–879.
3. Chung KF, Becker AB, Lazarus SC, Frick OL, Nadel JA, Gold WM. Antigen-induced airway hyperresponsiveness and pulmonary inflammation in allergic dogs. *J Appl Physiol* 1985; 58: 1347–1353.
4. Stewart AG, Fennessy MR. A model of bronchial hyperreactivity using ovalbumin-sensitized guinea-pigs. *Clin Exp Pharmacol Physiol* 1983; 10: 205.
5. Hulbert WC, McLean T, Wiggs B, Paré PD, Hogg JC. Histamine dose-response curves in guinea-pigs. *J Appl Physiol* 1985; 58: 625–634.
6. Andersson P. Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs. *Allergy* 1980; 35: 65–71.
7. Flavahan NA, Aarhus LL, Rimele TJ, Vanhoutte PM. The respiratory epithelium inhibits bronchial smooth muscle tone. *J Appl Physiol* 1985; 58: 834–838.
8. Vincenc KS, Black JL, Yan K, Armour CL, Donnelly PD, Woolcock AJ. Comparison of *in vivo* and *in vitro* responses to histamine in human airways. *Am Rev Respir Dis* 1983; 128: 875–879.
9. McCaig DJ. Comparison of autonomic responses in the trachea isolated from normal and albumin-sensitive guinea-pigs. *Br J Pharmacol* 1987; 92: 809–816.
10. Fryer AD, Wills-Karp M. Dysfunction of M2-muscarinic receptors in pulmonary parasympathetic nerves after antigen challenge. *J Appl Physiol* 1991; 71: 2255–2261.
11. Myers AC, Undem BJ, Weinreich D. Influence of antigen on membrane properties of guinea-pig bronchial ganglion neurons. *J Appl Physiol* 1991; 71: 970–976.
12. Ito Y, Tajima K. Dual effects of catecholamines on pre- and post-junctional membranes in the dog trachea. *Br J Pharmacol* 1982; 75: 433–440.
13. Skoogh BE, Svedmyr N. β_2-adrenoceptor stimulation inhibits ganglionic transmission in ferret trachea. *Pulm Pharmacol* 1989; 1: 167–172.
14. Rhoden KJ, Meldrum LA, Barnes PJ. Inhibiton of cholinergic neurotransmission in human airways by β_2-adrenoceptors. *J Appl Physiol* 1988; 65: 700–705.
15. Barnes PJ. Neural control of human airways in health and disease. *Am Rev Respir Dis* 1986; 134: 1289–1314.