Chronic allergen exposure enhances cholinergic neurotransmission in sensitized guinea-pigs


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} (EF25) from frequency-response curves.

EFS-induced contractile responses were significantly enhanced in the challenged group (logEF25=0.66±0.08 (mean±SEM)) compared with the control group (logEF25=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.

Keywords: Acetylcholine, asthma, electrical field stimulation, guinea-pig, neurotransmission.
5% CO₂, 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 (Daichi 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) (AChmax). Curves were compared by two-way analysis of variance (ANOVA). The effective frequencies producing 25% of AChmax (EF₂₅) were determined by frequency-response curves. LogEF₂₅ was compared by unpaired Student’s t-test.

**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 logEF₂₅, 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. 1b).

**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 F2α (PGF2α) 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 β2-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