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Short communication

Alteration in Ca^{2+} availability involved in antigen-induced airway hyperresponsiveness in rats

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Abstract

The origin of Ca^{2+} contributing to the enhancement of acetylcholine-induced bronchial smooth muscle constriction in airway hyperresponsiveness induced by antigen challenge was investigated. Under Ca^{2+}-free (concomitant with 10^{-6} M nicardipine) conditions, the contractile responses of bronchial rings to 1 mM acetylcholine were significantly greater in rings from rats with hyperresponsive airways (0.15 ± 0.04 g) than those of rings from normal rats (0.02 ± 0.004 g; $P < 0.05$). The cumulatively administered Ca^{2+} induced a markedly greater bronchoconstriction in rings from rats with hyperresponsive airways in Ca^{2+}-free solution when muscles were pretreated with 1 mM acetylcholine (in the presence of 10^{-6} M nicardipine) than in rings from normal rats, whereas no significant difference in Ca^{2+}-induced bronchoconstriction was observed between the two groups when muscles were pretreated with 60 mM K^+ (in the presence of 10^{-6} M atropine). These findings suggest that enhancement of the availability of Ca^{2+} released from intracellular stores and/or influxed through receptor-operated Ca^{2+} channels in airway smooth muscles might be involved in the airway hyperresponsiveness to acetylcholine in rats.

Keywords: Airway hyperresponsiveness; Bronchus; Ca^{2+} channel, voltage-dependent and receptor-operated; Inositol 1,4,5-trisphosphate; Acetylcholine; (Rat)

1. Introduction

Nonspecific airway hyperresponsiveness is a central feature of allergic bronchial asthma, although the mechanism(s) underlying the development of airway hyperresponsiveness is not clearly understood. The importance of airway hyperresponsiveness in the pathogenesis of asthma has been suggested by its correlation with the severity of this disease (Hargreave et al., 1981). It is thus very important to determine the underlying mechanisms of airway hyperresponsiveness for the therapy of asthma.

Although various results have been reported about the in vitro responsiveness of airway smooth muscles isolated from patients with hyperresponsive airways (Roberts et al., 1984; Cerrina et al., 1986; Goldie et al., 1986; De Jongste et al., 1987; Bai, 1990), it has also been reported that airways of asthmatic patients are hyperresponsive both in vivo and in vitro (Roberts et al., 1984; De Jongste et al., 1987; Bai, 1990). For instance, airway smooth muscles obtained from in vivo hyperresponsive patients have in vitro hyperresponsiveness to methacholine (Roberts et al., 1984). Likewise, we have recently demonstrated, both in vivo and in vitro airway hyperresponsiveness to acetylcholine in rats that were actively sensitized and repeatedly challenged with aerosolized antigen (Chiba and Misawa, 1993).

To understand the mechanisms of the increased responsiveness to acetylcholine, we examined the effect of extracellular Ca^{2+} removal on acetylcholine-induced constriction of bronchial rings from rats with hyperresponsive airways. We also investigated the Ca^{2+}-induced constriction of bronchial rings from rats with hyperresponsive airways after the rings were depolarized with acetylcholine or high K^+ in Ca^{2+}-free medium. The isolated bronchi from rats with hyperresponsive airways were compared with bronchial rings from non-sensitized normal animals.

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2. Materials and methods

Male Wistar rats (6 weeks of age, specific pathogen-free, 170–190 g) were purchased from Charles River Japan and housed under standard laboratory conditions with free access to food and water. The animals were sensitized with 2,4-dinitrophenylated *Ascaris suum* extract (DNP-Asc, 2 mg protein s.c.) together with *Bordetella pertussis* (2×10^{10}) as an adjuvant and were given a booster injection of DNP-Asc (0.5 mg protein i.m.) 5 days later. Eight days after the first immunization, the rats were challenged by inhaling DNP-Asc (6 mg protein/ml, 5–6 ml), via an ultrasonic nebulizer (TUR-3000, Nihon Kohden, Tokyo, Japan), for 20 min in a conscious state in a Plexiglas box (300 × 200 mm, height: 150 mm). Then the animals were subjected to 3 times repeated antigenic challenge every 48 h with the same inhalational challenge method described above (Chiba and Misawa, 1993).

The animals were killed by a blow to the head and exsanguinated, and the trachea and bronchus were immediately removed and carefully cleaned of adhering connective tissues. A length of about 5 mm of the left main bronchus was isolated (8–9 cartilages) and the resultant tissue ring preparation was then suspended in a 10 ml organ bath by two stainless-steel wires (0.3 mm diameter) passed through the lumen. For all tissues, one end was fixed to the bottom of the organ bath while the other was connected to a force-displacement transducer (TB-612T, Nihon Kohden) for the measurement of isometric force. A resting tension of 1.0 g was applied. The buffer solution contained modified Krebs-Henseleit solution with the following composition (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and glucose 10.0. The buffer solution was maintained at 37° C and oxygenated with 95% O_2–5% CO_2. After an equilibration period, the organ bath solution was replaced with Ca^{2+}-free 10^{-6} M nicardipine containing solution with the following composition (mM): NaCl 122.4, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, glucose 10.0 and EGTA 0.01. Fifteen minutes later, 1 mM acetylcholine was added and, after attainment of a plateau (almost baseline level) response to acetylcholine, a cumulative concentration-response curve for Ca^{2+} (0.1–5.0 mM) was made. A higher concentration of Ca^{2+} was added after the response to the previous concentration reached a plateau. In another series of experiments, bronchial smooth muscles were depolarized with 60 mM K^+, instead of acetylcholine, in the presence of 10^{-6} M atropine and in the absence of nicardipine in the Ca^{2+}-free solution. All these functional studies were performed in the presence of 10^{-6} M indomethacin. This concentration of indomethacin had no effect on baseline tension and acetylcholine- and high K^+-induced constriction of bronchial rings from both groups (data not shown).

The following drugs were used: 2,4-dinitrobenzene sulfonic acid sodium salt (Tokyo Kasei Co., Tokyo, Japan); acetylcholine chloride (Daiichi Pharmaceutical Co., Tokyo, Japan) and nicardipine, atropine sulfate, ethylene glycol-bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA), dimethyl sulfoxide (DMSO), indomethacin (Sigma, St. Louis, MO, USA). Indomethacin and nicardipine were dissolved in 10 mM Na_2CO_3 and DMSO, respectively, to a concentration of 10^{-4} M and administered in the organ bath in a final concentration of 10^{-6} M.

All the data are expressed as the means with S.E. The statistical significance of differences was determined by Dunnett's *t*-test. Values of $P < 0.05$ were taken to indicate a significant difference between groups of data.

3. Results

Our previous study revealed that the sensitization procedure used in the present study had no significant effect by itself on the acetylcholine responsiveness of rat main bronchial preparations (Chiba and Misawa, 1993). So in the present study, the bronchial responsiveness of repeatedly antigen-challenged rats and non-sensitized normal control rats was compared. The concentration of nicardipine used completely blocked the high K^+ (10–90 mM)-induced bronchoconstriction in Ca^{2+}-containing normal Krebs solution, indicating that voltage-dependent Ca^{2+} channels (VDCs) were completely blocked in this condition.

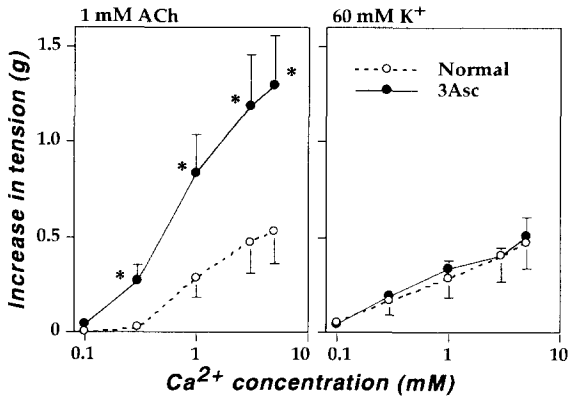

Fig. 1. Cumulative concentration-response curves for Ca^{2+} of bronchial rings from nonsensitized normal control (Normal; ○) and repeatedly antigen challenged (3Asc; ●) rats. Bronchial rings were preincubated with 10^{-6} M nicardipine and 1 mM acetylcholine (left panel) or depolarized with isotonic 60 mM K^+ (right panel) in Ca^{2+}-free, 0.01 mM EGTA solution. Values are means ± S.E. from 8 (Normal) and 5 (3Asc) experiments. * $P < 0.05$.

Under Ca^{2+}-free (concomitant with 10^{-6} M nicardipine and 0.01 mM EGTA) conditions, 1 mM acetylcholine generated only a transient phasic constriction in all preparations used. The generated tension of bronchial rings from repeatedly antigen-challenged rats (0.15 ± 0.04 g, $n = 5$) was significantly ($P < 0.05$) greater than that from nonsensitized normal control rats (0.02 ± 0.004 g, $n = 8$).

The left panel of Fig. 1 shows the concentration-response curves for Ca^{2+} of rat bronchial rings that were preincubated with 10^{-6} M nicardipine and 1 mM acetylcholine under Ca^{2+}-free, 0.01 mM EGTA conditions. The addition of Ca^{2+} induced a concentration-dependent constriction in both groups. The contractile responses to Ca^{2+} of the acetylcholine-stimulated muscles were markedly increased after repeated antigenic challenge. However, the responses to Ca^{2+} of the bronchial muscles depolarized with 60 mM K^+ (in the absence of nicardipine and presence of 10^{-6} M atropine) were the same in both groups (the right panel of Fig. 1).

4. Discussion

Airway smooth muscles are predominantly innervated by vagal efferent nerves, which release acetylcholine when stimulated and subsequently activate muscarinic acetylcholine receptors. The activation of muscarinic receptors existing on airway smooth muscle, which are mainly thought to be of the M_3 subtype (Yang et al., 1993), results in smooth muscle contraction by increasing the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), i.e. through Ca^{2+} release from the sarcoplasmic reticulum and Ca^{2+} influx from voltage-dependent (VDCs) and receptor-operated Ca^{2+} channels (ROCs) (Barnes, 1990; Montano et al., 1993; Rodger and Pyne, 1992). Therefore, the increased response to acetylcholine of isolated bronchus from rats with airway hyperresponsiveness (Chiba and Misawa, 1993) may be attributable to an enhanced Ca^{2+} mobilization in these airways.

In the present study, no significant difference between groups in the contractile responses to cumulatively administered Ca^{2+} was observed in bronchial muscles that were predepolarized with 60 mM K^+ (Fig. 1, right panel), suggesting that no change in VDC function occurs in bronchial smooth muscles of rats with hyperresponsive airways. This finding also suggests that the contractility of the two bronchial tissues was similar. Taking this into consideration, it is unlikely that an enhanced VDC function is involved in the increased acetylcholine responsiveness of the bronchus isolated from repeatedly antigen-challenged rats. This might have relevance to the early finding that VDC-blocking drugs such as nifedipine are ineffective as therapy for asthma (Ferrari et al., 1989). The Ca^{2+}-induced constriction of muscles that were preincubated with 1 mM acetylcholine in the presence of 10^{-6} M nicardipine in Ca^{2+}-free 0.01 mM EGTA solution was much greater in the hyperresponsive airways than that in the normal group (Fig. 1, left panel). Furthermore, under these conditions, the contractile response to acetylcholine of bronchial rings from repeatedly antigen-challenged rats was always significantly greater than that from control rats (see Results). These findings strongly suggest that enhanced Ca^{2+} release from intracellular stores and/or influx through ROCs rather than through VDCs might be involved in the increased responsiveness to acetylcholine of isolated bronchus from rats with hyperresponsive airways.

It has been thought that activation of muscarinic M_3 receptors promotes inositol 1,4,5-trisphosphate (IP_3) generation via activation of the effector enzyme, phospholipase C, in airway smooth muscles. It has also been suggested that IP_3 stimulates the release of Ca^{2+} from the sarcoplasmic reticulum, and inositol 1,3,4,5-tetrakisphosphate (IP_4; the metabolite of IP_3 produced by IP_3 kinase) may be responsible for opening putative ROCs (Luckhoff and Clapham, 1992; Rodger and Pyne, 1992), although the later process has not yet been demonstrated in muscle cells. Alternatively, it has been reported that activation of protein kinase C by diacylglycerol, another metabolite of phosphatidylinositol by phospholipase C, enhances the Ca^{2+} sensitivity of smooth muscle contraction (Ozaki et al., 1990; Sato et al., 1992). Thus, although we do not yet understand whether an increment in $[Ca^{2+}]_i$ level or an enhancement of Ca^{2+} sensitivity occurs, we speculate that the resultant enhanced phosphatidylinositol turnover in airway smooth muscles, via the increased Ca^{2+} efficacy, might be involved in airway hyperresponsiveness in rats.

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