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Acetylcholine-induced *ex vivo* ATP release from the human nasal mucosa

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ABSTRACT

Objective: The present study aimed at investigating ATP release in response to acetylcholine (Ach) and pharmacologically elucidating the intracellular signal transduction pathway of this reaction in an *ex vivo* experiment.

Methods: The inferior turbinate mucosa was collected from 21 patients with chronic hypertrophic rhinitis who underwent endoscopic turbinectomy. The mucosa was shaped into a filmy round piece, and incubated with chemical(s) in Hank's balanced salt solution for 10 min. After incubation, the ATP concentration was measured by a luciferin-luciferase assay.

Results: The baseline release of ATP without stimulus was 57.2 ± 10.3 fM. The ATP release was significantly increased by stimulation with 100 μM Ach. The Ach-induced ATP release was completely inhibited by removing extracellular Ca^{2+}. Significant inhibition of the Ach-induced ATP release was also observed by the addition of 1 μM atropine, 40 μM 2-APB, 10 μM CBX, and 100 μM PPADS, whereas 30 nM bafilomycin A1 did not affect the ATP release.

Conclusion: These results indicate that the Ach-induced ATP release from the human nasal mucosa is dependent on the pannexin-1 channel and purinergic P2X7 receptor, suggesting that these two molecules constitute a local autocrine/paracrine signaling system in the human nasal epithelium.

1. Introduction

Airway mucociliary transport function is essential for the clearance of inhaled foreign particulate matter along with secreted mucus, and thereby plays an important role in the host defense system. Various inflammatory upper and lower airway diseases are associated with mucociliary dysfunction. It is known that the mucociliary transport function of the airway epithelium is regulated by extracellular adenosine triphosphate (ATP) and intracellular Ca^{2+} through the activation of purinergic receptors on the surface of the epithelial cells [1–3]. It has also been shown that mucociliary transport is promoted by acetylcholine (Ach) in the upper and lower airway epithelium [4,5]. However, the intracellular transduction pathway that connects the Ach receptor and ATP release is scarcely understood in humans, and only partially elucidated even in experimental animals.

ATP release is thought to be mediated by two different mechanisms: vesicle- and channel-mediated pathways. Among the many channel-mediated pathways, the pannexin-1 channel

Abbreviations: Ach, acetylcholine; M_3AchR, M_3 acetylcholine receptor; PLC, phospholipase C; PIP_2, phosphatidylinositol bisphosphate; DAG, diacylglycerol; IP_3, inositol triphosphate; IP_3R, inositol triphosphate receptor; P2X7, purinergic P2X7 receptor.

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is the most convincing candidate for a mediator of ATP release of ATP in a diverse range of cell types, including airway epithelial cells [6]. Pannexins constitute a family of transmembrane channel proteins in vertebrates. They are homologous to the invertebrate gap-junction proteins known as innexins [7], and consist of three subtypes, pannexin-1, pannexin-2, and pannexin-3. Pannexins have no sequence similarity to connexins, the prototypical vertebrate gap-junction proteins [8]. The pannexin genes were originally cloned as gap-junction-related proteins, but their significance *in vivo* has not been fully understood to date.

Several authors have reported ATP release through the pannexin-1 channel [9–11]. Bao et al. [9] observed that oocytes exogenously expressing pannexin-1 release ATP when depolarized by a high concentration of extracellular K^+. Ransford et al. [11] found that cultured airway epithelial cells isolated from the human lung secrete ATP *via* pannexin-1 upon hypotonic stress. Seminario-Vidal et al. [10] also demonstrated hypotonicity-evoked pannexin-1-mediated ATP release from cultured human bronchial epithelial cells and from the excised mouse trachea. With regard to the upper airway, we have recently shown in an *ex vivo* experiment that the human nasal mucosa expresses pannexin-1 and releases ATP through this channel in response to a hypotonic stimulus [12]. Using the same *ex vivo* experimental system, the present study aimed at investigating ATP release in response to Ach and pharmacologically elucidating the intracellular signal transduction pathway of this reaction.

2. Methods

2.1. Patients and sample collection

The inferior turbinate mucosa was collected from 21 patients with chronic hypertrophic rhinitis who underwent endoscopic turbinectomy. The study included 17 male and 4 female patients, aged 17–79 years, with an average age of 49.0 years. Total and/or specific serum IgE levels were positive in 15 patients. Bilateral inferior turbinate bones were resected together with the lateral mucosa of the turbinate under general anesthesia. Informed consent was obtained from all patients, and the study was approved by the institutional review board of the University of Occupational and Environmental Health.

2.2. Chemicals

Ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) was purchased from Dojindo (Kumamoto, Japan). Ach was from MP Biomedicals (Santa Ana, CA). Carbenoxolone (CBX; a pannexin-1 blocker) was bought from Sigma (St Louis, MO). Atropine sulfate and pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS; a purinergic P2X receptor antagonist) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Bafilomycin A1 (a vesicular transport inhibitor) was purchased from Bivotica (Goettingen, Germany). 2-Aminoethoxydiphenyl borate (2-APB; an inositol trisphosphate (IP_3) receptor antagonist) was from Tocris Bioscience (Bristol, UK).

2.3. Measurement of ex vivo ATP release from the nasal mucosa

Ex vivo ATP release from the nasal mucosa was measured according to Ohbuchi et al. [12] with modification. The lateral surface of the collected turbinate was shaved to prepare a film of the mucosal surface layer immediately after sample collection. A circular punch (inner diameter = 4 mm) was used to cut round mucosal pieces out of the filmy tissue. The cutout mucosal pieces were preincubated with Hank's balanced salt solution (HBSS; 8000 (in mg/L) NaCl, 400 KCl, 350 $NaHCO_3$, 140 $CaCl_2$, 100 $MgCl_2{\cdot}6H_2O$, 100 $MgSO_4{\cdot}7H_2O$, $7H_2O$, 60 KH_2PO_4, 47.8 Na_2HPO_4, 1000 glucose) for 30 min, washed and then incubated in a 12-well culture plate containing 4 mL medium in each well with chemical(s) for 10 min. In the 12-well culture plate, 3 wells were used for each mucosal piece; the first for preincubation, the next for washing, and the last for stimulation. The incubation medium was HBSS and the experiment was performed at room temperature, unless otherwise indicated. After incubation, 100 μL of medium was collected by an ATP water-testing device, AQUASNAP (Hygiena, Camarillo, CA), and the ATP concentration was measured by a luciferin-luciferase assay using a SystemSURE luminometer (Hygiena, Camarillo, CA). Patients' backgrounds were similar in each chemical group.

2.4. Statistical analysis

Data were expressed as the mean ± SEM. The statistical significance of differences was analyzed by a two-tailed Wilcoxon signed-rank test. *P*-values less than 0.05 were considered significant.

3. Results

The baseline release of ATP without stimulus was 57.2 ± 10.3 fM ($n = 16$), ranging from 12 to 146 fM. The ATP release was significantly increased to 168.9 ± 20.9 fM ($n = 16$) by stimulation with 100 μM Ach ($P = 0.0004$; Fig. 1).

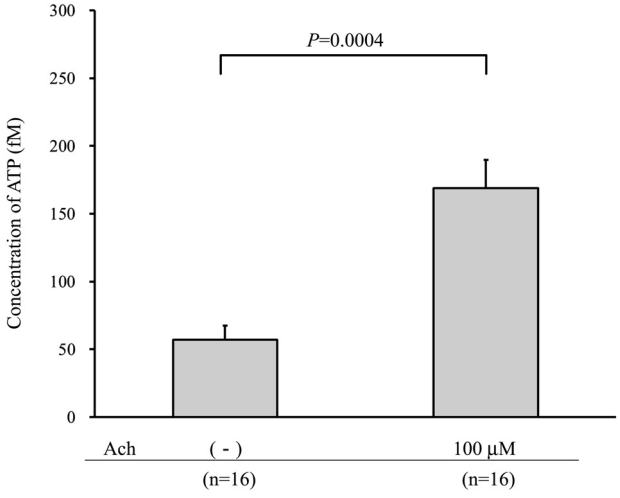

Fig. 1. ATP release from the nasal mucosa in response to ATP. The baseline release of ATP without stimulus was 57.2 ± 10.3 fM ($n = 16$). The ATP release was significantly increased by stimulation with 100 μM Ach.

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