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Involvement of P2X receptor subtypes in ATP-induced enhancement of the cough reflex sensitivity

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Abstract

We examined the effect of inhaled ATP on the chemical irritant-induced coughs to clarify the roles of ionotropic purinergic receptors in these modulations. Although inhalation of 0.1 M citric acid by itself produced only a few coughs in guinea pigs, exposure to ATP, at concentrations of 3–10 μM, for 2 min concentration-dependently increased the number of 0.1 M citric acid-induced coughs. ATP-induced enhancement of the number of citric acid-induced coughs was abolished when animals were pretreated with 2′,3′-*O*-(2,4,6-trinitrophenyl) adenosine 5-triphosphate (TNP-ATP), an antagonist of P2X receptor subtypes $P2X_{1-4}$, at a concentration of 50 μM, for 2 min. However, exposure to pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), an antagonist of P2X receptor subtypes $P2X_{1,2,3,5,7}$, but not of $P2X_4$ receptors, at a concentration of 50 μM, for 2 min, had no effect on the ATP-induced enhancement of the number of citric acid-induced coughs. Furthermore, exposure to reactive blue 2 (RB2, 30 μM, 2 min), an antagonist of P2Y receptors, had no effect on the ATP-induced enhancement of the number of citric acid-induced coughs. Exposure to ATP, at a concentration of 10 μM, for 2 min significantly increased the number of citric acid-induced coughs in capsaicin-pretreated guinea pigs. Furthermore, ATP had no effect on the number of capsaicin-induced coughs in naive animals. These results suggest that although ATP, by itself, does not elicit spontaneous coughs, it likely enhances the cough reflex sensitivity. Furthermore, stimulation of P2X receptors, especially $P2X_4$ receptors, on rapidly adapting receptors may be required for the ATP-induced enhancement of the cough reflex sensitivity.

Keywords: Cough reflex; C-fiber; Rapidly adapting receptor; ATP; Ionotropic purinergic receptor

1. Introduction

Cough is an important respiratory defense mechanism and one of the most important symptoms of pulmonary disorders. Chronic cough is generally defined as cough persisting for 8 weeks or longer. The morbidity associated with chronic cough in humans is likely to be a product of the enhanced frequency and intensity of coughs that occurs as a result of increased excitability in this behavior. Patients with a variety of pulmonary disorders show an enhanced cough sensitivity in response to inhaled irritants, but the frequency and intensity of cough can be elevated. The mechanisms by which the sensitivity, spontaneous frequency and magnitude of cough are increased in airway disease are poorly understood. Therefore, a better understanding of the pathogenic mechanisms of chronic cough is extremely important for the development of new therapeutic strategies to alleviate this stress.

ATP has long been recognized to be an activator of sensory nerves (Brouns et al., 2000). ATP can act on metabotropic and ionotropic purinergic receptors (Burnstock, 2001). The metabotropic receptors are designated P2Y receptors, whereas the ionotropic receptors are referred to as P2X receptors. In the somatosensory system, P2X receptors are most often involved in ATP-induced action potential discharge. In the canine lung, ATP was found to cause a burst of action potentials in vagal C-fibers by a mechanism that was inhibited by nonselective P2X receptor antagonism (Pelleg and Hurt, 1996). In guinea pigs, Undem and his coworkers have found that ATP effectively activates a subset of C-fibers and rapidly adapting receptors. In

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each case, the response is blocked by P2X receptor antagonists (Canning et al., 2004; Undem et al., 2004). Although these results strongly suggest the possibility that ATP may modulate the cough reflex through an excitatory mechanism, the role of ATP in the regulation of cough reflex is not yet clearly defined.

Therefore, the present study was designed to investigate the effect of inhaled ATP on the chemical irritant-induced coughs, and to clarify the roles of ionotropic purinergic receptors in these modulations.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Tokyo Animal Laboratory Inc., Tokyo, Japan) weighing about 300–350 g were used. The animals were housed in groups of four per cage under a 12-h light–dark cycle with food and water available continuously. These studies were carried out in accordance with the Guidelines for the Use of Laboratory Animals as adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Antitussive assay

The cough reflex was induced as previously described (Kamei et al., 1989; Kamei and Kasuya, 1992). Briefly, animals were exposed to a nebulized solution of capsaicin (30 μM) or citric acid (0.1 M) under conscious and identical conditions using a body plethysmograph. Capsaicin was dissolved to a concentration of 30 mg/ml in a 10% ethanol and 10% Tween 80 saline solution. The solution was diluted with saline. Citric acid was dissolved to a concentration of 0.1 M in a saline solution. The guinea pigs were exposed to capsaicin and citric acid for 6 and 10 min, respectively.

2.3. Capsaicin pretreatment

A total dose of 100 mg/kg capsaicin was divided into four portions (20, 20, 30, 30 mg/kg) and subcutaneously injected over 4 days under anaesthesia with ketamine (50 mg/kg, i.m.). Terbutaline (0.1 mg/kg, s.c.) and aminophylline (25 mg/kg, i.p.) were given to counteract the respiratory impairment associated with capsaicin injection.

2.4. Drugs

Adenosine 5-triphosphate (ATP), pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), an antagonist of P2X receptor subtypes $P2X_{1,2,3,5,7}$, but not of $P2X_4$ receptors and 2′,3′-*O*-(2,4,6-trinitrophenyl) adenosine 5-triphosphate (TNP-ATP), an antagonist of P2X receptor subtypes $P2X_{1-4}$ were purchased from Sigma Chemical Co. Procion blue HB (reactive blue 2, RB2), an antagonist of P2Y receptors was purchased from ACROS Organics (Geel, Belgium). All drugs were dissolved in saline (0.9% NaCl). The guinea pigs were exposed to ATP aerosol for 2 min during the 5 min preceding the inhalation of citric acid or capsaicin. Animals were exposed to PPADS, TNP-ATP or RB2 aerosol for 2 min during the 5 min preceding the inhalation of ATP aerosol. The aerosols were produced by means of an ultrasonic nebulizer (Nihon Kohden, TUR-3200). About 0.6 ml of solution was nebulized per minute and all animals were identical conditions, using a body plethysmograph. The doses of PPADS, TNP-ATP and RB2 were determined as referred to the results previously described (Sokolova et al., 2003; Sperlagh et al., 2000).

2.5. Statistics

Data are expressed as the means±S.E. The statistical significance of differences was assessed by the Mann–Whitney *U*-test. A level of probability of 0.05 or less was considered significant.

3. Results

3.1. Effect of ATP on the number of citric acid-induced coughs

A concentration of 0.1 M citric acid was selected for inducing coughs, since our preliminary study showed that this concentration by itself produced only few coughs in guinea pigs (1.90±.6 coughs/10 min, $n=6$). As shown in Fig. 1A, exposure to ATP, at concentrations of 3–10 μM, for 2 min concentration-

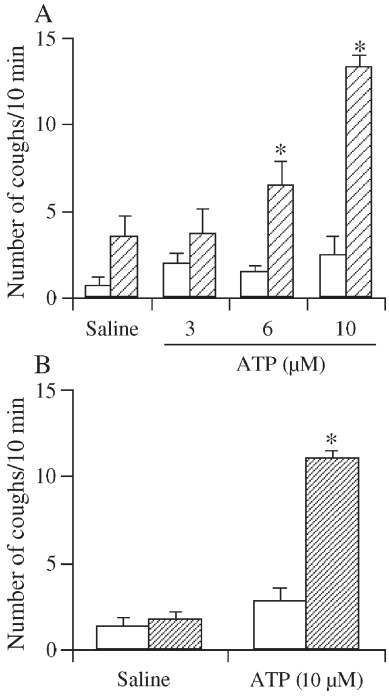

Fig. 1. Concentration-dependent effect of ATP on the number of citric acid-induced coughs in naive guinea pigs (A) and effect of ATP (10 μM) on the number of citric acid-induced coughs in capsaicinized guinea pigs (B). The guinea pigs were exposed to ATP aerosol for 2 min during the 5 min preceding the inhalation of citric acid. The number of coughs during 10 min of exposure to citric acid was counted before (open column) and after exposure to saline or ATP (hatched column). Each column represents the mean with S.E. of 6 animals. *$P<0.05$ vs. the value before exposure to ATP.

dependently increased the number of citric acid-induced coughs. A significant increase in the number of coughs was observed when animals were exposed to 6 and 10 μM of ATP (6 μM: pre, 1.5±0.4 coughs/10 min, post, 6.5±1.4 coughs/10 min, $n=6$; 10 μM: 2.5±1.1 coughs/10 min, post, 13.3±0.7 coughs/10 min, $n=6$). However, the vehicle for ATP (saline) by itself had no significant effect on the number of citric acid-induced coughs (pre, 0.7±0.5 coughs/10 min, post, 3.5±1.2 coughs/10 min, $n=6$).

3.2. Effect of ATP on the number of citric acid-induced coughs in capsaicin-pretreated guinea pigs

Exposure to capsaicin (30 μM) for 6 min produced 15.2±0.3 coughs ($n=6$) in naive guinea pigs. On the other hand, only a small number of coughs (2.1±0.3 coughs, $n=6$) were produced when capsaicin-pretreated guinea pigs were exposed to capsaicin (30 μM) for 6 min.

As shown in Fig. 1B, exposure to ATP, at 10 μM, for 2 min significantly increased the number of citric acid-induced coughs in capsaicin-pretreated guinea pigs, as observed in naive guinea pigs.

3.3. Effect of ATP on the number of capsaicin-induced cough

Exposure to ATP, at 10 μM, for 2 min had no significant effect on the number of capsaicin (30 μM)-induced coughs (before ATP, 16.4±0.5 coughs/6 min; after ATP, 16.2±0.9 coughs/6 min, $n=6$).

3.4. Effects of ionotropic purinergic receptor antagonists on ATP-induced enhancement of the number of citric acid-induced coughs

As shown in Fig. 2, the ATP-induced enhancement of the number of citric acid-induced coughs was abolished when animals were pretreated with TNP-ATP, at a concentration of 50 μM, for 2 min. However, either PPADS or RB2 had no effect on the ATP-induced enhancement of the number of citric acid-induced coughs.

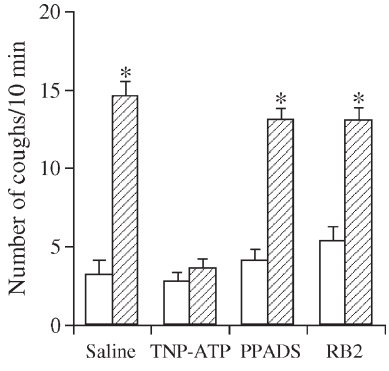

Fig. 2. Effects of P2X and P2Y receptor antagonists on the ATP-induced enhancement of the number of citric acid-induced coughs. The guinea pigs were exposed to ATP (10 μM) aerosol for 2 min during the 5 min preceding the inhalation of citric acid. The guinea pigs were exposed to PPADS (50 μM), TNP-ATP (50 μM) or RB2 (30 μM) aerosol for 2 min during the 5 min preceding the inhalation of ATP aerosol. The number of coughs during the 10 min of exposure of citric acid was counted before (open column) and after exposure to ATP (hatched column). Each column represents the mean with S.E. of 6 animals. *$P<0.05$ vs. the value before exposure to ATP.

4. Discussion

In the present study, we demonstrated that ATP concentration-dependently and significantly enhanced the number of citric acid-induced coughs in guinea pigs and this effect was completely blocked by pretreatment with inhaled TNP-ATP, but not PPADS aerosol. Furthermore, exposure to RB2, an antagonist of P2Y receptors, had no effect on the ATP-induced enhancement of the number of citric acid-induced coughs. P2X receptors are a family of cation channels gated by extracellular ATP (Jahr and Jessell, 1983; Krishtal et al., 1983). To date, seven P2X subunits ($P2X_1$ to $P2X_7$) have been cloned (North and Surprenant, 2000; Khakh et al., 2001), and six ($P2_1$ to $P2X_6$) are expressed on primary sensory neurons (Collo et al., 1996; Vulchanova et al., 1996, 1997, 1998; Xiang et al., 1998; Li et al., 1999; Novakovic et al., 1999). PPADS is considered to be an antagonist of P2X receptor subtypes $P2X_{1,2,3,5,7}$, but not of $P2X_4$ (Khakh et al., 2001), whereas TNP-ATP is considered to be an antagonist for P2X receptor subtypes $P2X_{1-4}$ (Virginio et al., 1998; Khakh et al., 2001). On the other hand, Canning et al. (2004) reported that inhalation of the selective $P2X_1$ and $P2X_3$ receptor agonist α, β-methylene ATP does not readily evoke coughing. Taken together, these results suggest that stimulation of P2X receptors, especially $P2X_4$ receptors in the airways is required for ATP-induced enhancement of the cough reflex.

In the canine lung, ATP has been found to cause a burst of action potentials in vagal C-fibers (Pelleg and Hurt, 1996). The P2X receptor antagonist PPADS markedly attenuated the action of ATP (Pelleg and Hurt, 1996). Furthermore, the P2Y receptor antagonist Reactive Blue 2 did not affect the action of ATP on vagal C-fibers (Pelleg and Hurt, 1996). In guinea pigs, it has been reported that ATP effectively activates not only a C-fiber from no dose neurons, but also rapidly adapting receptors (Canning et al., 2004; Undem et al., 2004). In each case, the response is blocked by P2X receptor antagonist. These results indicate that ATP can act on P2X receptor subtypes to activate either C-fibers or rapidly adapting receptors. The sensory afferent nerves within the respiratory tract are through to consist of two types: rapidly adapting receptors which respond to mechanical stimuli and mucus within the airways and C-fiber receptors which are responsive to chemical stimulation and react to agents such as capsaicin (e.g., Harrison, 2004). Activation in C-fibers is thought to result in the antidromic release of neuropeptides such as substance P and neurokinin A, which in turn activate the rapidly adapting receptors to feed into the central cough mechanisms. Evidence has been presented to support the hypothesis that C-fibers and rapidly adapting receptors regulate coughing. C-fiber-selective stimulants such as bradykinin and capsaicin are effective at evoking cough (Karlsson and Fuller, 1999). Rapidly adapting receptors are also activated by many stimuli that evoke coughing, and the results

of vagal cooling experiments are consistent with the notion that rapidly adapting receptors, but not C-fibers, are responsible for the cough reflex (Tatar et al., 1988; Widdicombe, 2003). Thus, it is necessary to clarify whether ATP activates C-fibers or rapidly adapting receptors to enhance the citric acid-induced coughs. While the inhalation of citric acid stimulates both C-fibers and rapidly adapting receptors, capsaicin appears to stimulate only C-fibers and both of these agents have been shown to induce cough in several species (Undem et al., 2002; El-Hashim et al., 2004). Although capsaicin produced coughs at low concentrations, high concentrations of capsaicin are neurotoxic and cause nerve cell death—this is characterized by the desensitization of C-fibers (Korpas and Tomori, 1979). In this study, we demonstrated that capsaicin-induced coughs were inhibited by the desensitization of C-fibers. However, ATP-induced enhancement of the number of citric acid-induced coughs was not abolished in C-fibers-desensitized guinea pigs. Furthermore, we also observed that inhaled ATP had no effect on the number of capsaicin-induced coughs in naive animals. These findings suggest that ATP activates C-fiber-independent pathways, namely rapidly adapting receptors, to enhance citric acid-induced coughs. On the other hand, in the present study, we observed that sensitivity to capsaicin-induced coughs was reduced, whereas sensitivity to citric acid-induced coughs was not changed by the desensitization of C-fibers. Thus, it is possible that the nature of citric acid (0.1 M)-induced coughs might not totally be dependent to the transient receptor potential vanilloid 1 (TRPV1), but might be dependent to other channels such as acid-sensing ion channels.

In conclusion, our present results suggest that although ATP, by itself, did not elicit spontaneous coughs, it is likely to enhance the cough reflex sensitivity. Furthermore, stimulation of P2X receptors, especially $P2X_4$ receptors, on rapidly adapting receptors may be required for the ATP-induced enhancement of the cough reflex sensitivity.

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