The Difference in Citric Acid-Induced Cough in Congenitally Bronchial-Hypersensitive (BHS) and Bronchial-Hyposensitive (BHR) Guinea Pigs

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Abstract: Cough elicitation and major physiological factors influencing cough occurrence were investigated in congenitally bronchial-hypersensitive (BHS) and -hyposensitive (BHR) guinea pigs exposed to citric acid (0.3 M) aerosol for 10 min. The number of cough in BHS was significantly larger than in BHR, while the latency to cough in BHS was significantly shorter than in BHR. Pretreatment with atropine (0.2%), lidocaine (2%) or salbutamol (0.1%) aerosol and desensitization of C-fibers with capsaicin (100 mg/kg) decreased the cough numbers in both BHS and BHR. The salbutamol, atropine and capsaicin pretreatments prolonged the cough latency in BHS, but only salbutamol prolonged the latency in BHR. After salbutamol pretreatment all BHR guinea pigs exhibited cough, while 66.7% of BHS guinea pigs exhibited it. Vagal blocking by atropine suppressed coughing in both BHS and BHR. Only a small number (33.3%) of BHR guinea pigs and no BHR guinea pigs exhibited a cough response after capsaicin and lidocaine pretreatment whereas many BHS guinea pigs still produced cough after such pretreatment. The present study demonstrated that the cough responsiveness to citric acid aerosol was significantly higher in BHS than in BHR. It was revealed that airway smooth muscle contraction and functional and/or morphological development of airway nervous receptors, especially C-fiber endings, contributed to aggravation of coughing in BHS.

Key words: airway, C-fiber, cough, guinea pig

Introduction

Coughing is normally accompanied by airway constriction which allows an increase in expiratory flow velocity [18]. It is a powerful defensive reflex which is

elicited by the activation of myelinated and nonmyelinated afferent fibers distributed along the larynx and tracheobronchial tree [15]. Moreover, cough is an airway reaction frequently occurring in airway diseases [28] such as bronchitis and asthma.

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Mechanical, chemical and electrical stimuli have been used to elicit cough and discussed with regard to the site and the mechanisms of action in the airway [4, 18]. Among them aerosol of citric acid is a potent tussive agent in humans and guinea pigs [13, 19] and cough induced by citric acid is accompanied by bronchoconstriction [8, 9, 16] and sensory neuropeptide release from the perfused lung [21, 26]. The citric acid-induced cough was enhanced by cigarette smoke exposure [14], but was suppressed by treatment with ruthenium red aerosol which can block the effects of capsaicin [20]. These previous findings suggest that the mechanism by which citric acid stimulates the airway sensory system has a close relationship to capsaicin-sensitive neurons.

Airway hypersensitivity is considered as the most fundamental and common factor in the elicitation of asthma [25, 32], which shows an exaggerated bronchoconstrictive response to various non-specific stimuli as well as specific agents such as histamine and acetylcholine. In patients with asthmatic attack, cough is the most common symptom and is normally accompanied by the occurrence of severe bronchoconstriction [23]. Therefore, it is conceivable that there is a close relationship between cough elicitation and airway hypersensitivity. In fact, this is supported by the finding that the irritation of sensory neurons in the airway is increased in asthma [16]. However, the higher elicitation of cough in asthmatic patients might be produced not only by the higher responsiveness of airway receptors but also by many other factors such as dysfunction of the autonomic nervous system and humoral influence on the airway.

Several kinds of model animals have been used to examine the relationship between airway responsiveness and cough, such as *Ascaris suum*-sensitized guinea pigs [16], cigarette smoke-sensitized guinea pigs [14], metyrapone and ovalbumin-sensitized guinea pigs [32]. BHS (bronchial-hypersensitive) and BHR (bronchial-hyposensitive) strain guinea pigs are spontaneous model animals of airway hyper- and hyposensitivity [22]. They have been developed by genetic selection from Hartley strain guinea pigs, on the basis of measurement of the onset time to falling-down due to hypoxemia resulting from bronchoconstriction induced by inhalation of acetylcholine (0.08%; w/v). It has been known that BHS guinea pigs have significantly greater airway responses to leukotriene D₄ [22], bradykinin [30], histamine

[3] and acetylcholine [31] compared with BHR guinea pigs. These findings indicate that BHS and BHR guinea pigs are good congenital model animals for the investigation of airway hypersensitivity and its related pathophysiology, especially muscarinic functions and the role of C-fibers on the bronchoconstriction.

We considered that it was important for investigating mechanisms of cough to evaluate the relationship between airway hypersensitivity and cough in these animals. The present study was aimed at elucidating whether the cough occurrence was different in BHS and BHR guinea pigs, and also at evaluating the role of airway smooth muscle contraction and the relevant afferent and efferent pathways involved in the elicitation of cough reflex.

Materials and Methods

Animals

Forty-seven BHS and 47 BHR guinea pigs were used in this study. They weighed 800–1,000 g and were provided by The Institute of Bio-Active Science, Nippon Zoki Pharmaceutical Co., Ltd. (Hyogo, Japan).

Experimental procedures

Unanesthetized, unrestrained guinea pigs were placed individually in a transparent chamber (diameter 24.5 cm) which allowed the guinea pigs to move freely during exposure to aerosols of drugs. The room temperature was maintained at 18–24°C. Room air with drug aerosols was introduced into the chamber at 1.0 l/min through the inlet and exhausted through the outlet of the chamber. Pressure changes in the chamber due to the spontaneous normal breathing and coughing of the guinea pig were measured by a pressure transducer (TOYODA, PD104, 0.1F-3006) which was connected to the chamber. The pressure signal was amplified by a DC amplifier (TOYODA, AA6010) and recorded on a pen recorder (NEC San-ei, RECTI-HORIZ-8K). Cough was evaluated as a large swing in the pressure change, accompanied by a set of rapid expiration and inspiration. Typical traces of spontaneous breathing and cough events are shown in Fig. 1.

Drugs were nebulized to particles ranging in diameter from 1 to 8 μ m via an ultrasonic nebulizer (Omron, NE-U12; 0.13 ml/min) placed between the chamber and an air pump (NISSO, θ 6000). During the exposure

animals were continuously watched by a trained investigator, and the number of coughs and the time to onset of cough (latency) were determined. A cough can be distinguished from a sneeze on the basis of sound and behavior [7, 14].

The guinea pigs were exposed to citric acid (0.3 M; Junsei Chemical Co., Ltd., Tokyo) for 10 min. Animals were exposed to salbutamol (0.1%, w/v; Wako pure Chemical Industries Ltd., Tokyo, Japan), atropine (0.2%, w/v; Tokyo Chemical Industries Co., Ltd., Tokyo) or lidocaine (2%, w/v; Fujisawa Pharmaceutical Co., Ltd., Tokyo) aerosol for 5 min in the 5 min preceding the inhalation of citric acid. Salbutamol was used to relax the airway smooth muscle by its β_2 agonistic action. Atropine was used to block the vagal efferent pathway. Lidocaine was used to block the airway sensory nerve fibers by its local anesthetic action.

Capsaicin pretreatment

Capsaicin pretreatment was performed to evaluate the possible role of vagal C-fiber endings and their axonal reflexes in the elicitation of cough. A total dose of 100 mg/kg of capsaicin (Sigma Chemical Co., St. Louis, MO, USA) was divided into four portions (20 + 20 + 30 + 30; mg/kg) and was 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.

Statistical analyses

The number of coughs during the 10 min exposure to citric acid and the latency to cough were compared between the two strains by one-way analyses of variance (ANOVA). P values less than 0.05 were considered statistically significant.

Results

Cough response to citric acid in BHS and BHR

BHS and BHR guinea pigs were individually exposed to nebulized citric acid (0.3 M) for 10 min. A concentration of 0.3 M citric acid was selected for inducing coughs, since in our preliminary study this concentration was satisfactory for eliciting coughs in guinea pigs. The number of coughs exhibited by BHS and BHR guinea pigs are compared in Fig. 2: 16.9 ± 2.0 (counts/10 min, mean $\pm$ SEM) in BHS, and 9.9 ± 1.9 (counts/10 min, mean $\pm$ SEM) in BHR, respectively. The number of coughs in BHS guinea pigs was significantly larger than in BHR ($p < 0.05$). The latency to cough in BHS guinea pigs was significantly shorter than in BHR ($p < 0.01$): 182.0 ± 28.1 (sec, mean $\pm$ SEM) in BHS, and 375.0 ± 36.9 (sec, mean $\pm$ SEM) in BHR; Fig. 3.

The effects of various drugs on citric acid induced-cough in BHS and BHR

The number of citric acid induced coughs pretreated with salbutamol (0.1%), lidocaine (2%), atropine (0.2%)

Fig. 1. The traces show spontaneous respiration and an episode of cough, electrically recorded from the internal pressure changes in the chamber before and during exposure to aerosol of citric acid (0.3 M) of a conscious guinea pig. The upward direction of the trace represents the inspiration.

Fig. 2. The number of coughs induced by citric acid aerosols (0.3 M) in 10 min. The number of coughs in BHS was significantly larger than in BHR, * $p < 0.05$. Each bar represents the mean $\pm$ SEM of 14–15 separate experiments.

Fig. 3. The latency to cough induced by citric acid aerosols (0.3 M). There was significant difference between BHS and BHR, * $p < 0.01$. Each bar represents the mean $\pm$ SEM of 14–15 separate experiments.

or capsaicin (100 mg/kg) and percent decrease compared with vehicle challenge are shown in Fig. 4. The latency to these coughs and percent increase compared with vehicle challenge are shown in Fig. 5. The individual number of guinea pigs which exhibited coughs is described as a percentage in Table 1. Only a small number (33.3%) of BHR guinea pigs and no BHR guinea pigs exhibited a cough response after capsaicin and lidocaine pretreatments, respectively. However, many BHS guinea pigs (75%, 60%) still evoked cough response after these pretreatments. The number of coughs in BHS was significantly decreased by prior inhalation of salbutamol, atropine and lidocaine, decreasing to 2.2 counts/10 min ($p < 0.01$), 8 counts/10 min ($p < 0.01$) and 1.2 counts/10 min ($p < 0.01$) compared with the vehicle challenge, respectively. In BHR guinea pigs, the number of coughs was also decreased to 2.0 counts/10 min ($p < 0.01$) and 8.0 counts/10 min ($p < 0.01$) by salbutamol and atropine, respectively. Citric acid induced coughs were completely inhibited by lidocaine in BHR. The capsaicin pretreatment decreased the number of coughs to 3.5 counts/10 min ($p < 0.01$) in BHS and 1.2 counts/10 min ($p < 0.01$) in BHR.

The latency to cough was calculated in the individuals which coughed, but could not be calculated in the individuals which did not cough during the 10 min. The latency to cough in BHS was increased to 386.5 sec ($p < 0.05$), 347.3 sec ($p < 0.01$), 300.3 sec by prior inhala-

Table 1. Effects of relaxing agent of airway smooth muscle, vagal blocking, local anesthesia, and capsaicin pretreatment on citric acid induced-cough in BHS and BHR guinea pigs

	BHS	BHR
Vehicle	100% (8/8)	100% (9/9)
Salbutamol (0.1%)	66.7% (4/6)	100% (4/4)
Atropine (2%)	66.7% (4/6)	87.5% (7/8)
Lidocaine (2%)	60.0% (3/5)	0% (0/5)
Capsaicin (100 mg/kg)	75.0% (6/8)	33.3% (2/6)

The values show the percentage of the number of animals that coughed.

tion of salbutamol, atropine and lidocaine, compared with the vehicle challenge. In BHR the latency was shown as 541 sec ($p < 0.01$) and 481.6 sec in salbutamol and atropine inhalation, but could not be calculated in lidocaine inhalation because of the absence of coughing. The capsaicin pretreatment prolonged the latency to 290.3 sec ($p < 0.05$) in BHS guinea pigs, but did not significantly change the latency in BHR.

Discussion

Airway hypersensitivity is induced by complex mechanisms involving changes in the functions of airway sensory neurons and the autonomic nervous system, and the actions of humoral factors on both the nervous system and airway smooth muscle, as well as changes

Fig. 4. The effect of salbutamol (0.1%), atropine (0.2%), lidocaine (2%) or capsaicin (100 mg/kg) on the number of coughs induced by citric acid aerosol (0.3 M) during 10 min. **A:** Pretreatment with salbutamol (0.1%), atropine (0.2%), lidocaine (2%) or capsaicin significantly decreased the number of coughs in both BHS and BHR. **B:** Each bar shows the percentage of decrease compared to vehicle challenge. Each bar represents the mean $\pm$ SEM from 5–9 separate experiments: * $p < 0.01$ compared with vehicle challenge, # $p < 0.05$ compared with BHR.

in metabolism within the smooth muscle cells.

BHS and BHR guinea pigs used in the present study have as characteristics airway hypersensitivity and hyposensitivity associated with their muscarinic functions represented by M_2 and M_3 receptors and also AChE. Such muscarinic functions are closely linked with the vagal reflex derived from the airway [10, 11]. Furthermore, a larger contribution of airway C-fibers on the

airway contraction was observed in BHS compared with BHR in a preliminary experiment. It is known that citric acid-induced cough is produced by the effect of citric acid on capsaicin-sensitive sensory neurons [6, 7], while the bronchoconstriction induced by citric acid in guinea pigs involves a muscarinic mechanism [2, 6]. It is, therefore, interesting to investigate the relationship between cough occurrence and airway sensitivity

Fig. 5. The effect of salbutamol (0.1%), atropine (0.2%), lidocaine (2%) or capsaicin (100 mg/kg) on the latency to cough induced by citric acid aerosol (0.3 M). A: Pretreatment with salbutamol (0.1%), atropine (0.2%) or capsaicin increased the latency to cough in both BHS and BHR. B: Each bar shows the percentage of increase compared to vehicle challenge. The latency to cough with pretreatment of lidocaine was not determined, since cough was not detected during 10 min. Each bar represents the mean $\pm$ SEM of 5–9 separate experiments: * $p < 0.05$ compared with vehicle challenge, # $p < 0.01$ compared with BHR.

in these model animals with different muscarinic functions and the role of C-fibers in the airway.

Cough frequency and latency to cough have been used as useful parameters in various studies on tussive or antitussive effects of drugs for assessing the strength of cough elicitation and sensitivity to stimulants [5]. In this study, therefore, these two parameters were used for the analysis of citric acid-induced coughs. The

present study demonstrated that there were significant differences in both the number of coughs and the latency to cough between BHS and BHR strain guinea pigs, showing that BHS was more susceptible induced to cough by citric acid inhalation than BHR.

Intravenous administration of salbutamol, a β_2 agonist known as a potent bronchodilator, inhibited the increase of pulmonary airway resistance induced by histamine or

bradykinin in guinea pigs [1], and also the inhalation of salbutamol inhibited the bronchoconstriction induced by histamine aerosols [24]. In the present study, pretreatment with salbutamol aerosol significantly decreased the number of coughs and increased the latency to cough induced by citric acid in both strains of guinea pigs; the number of coughs showed an 89.4% decrease in BHS and an 85.8% decrease in BHR compared to vehicle challenge, and the latency to cough was showed a 140.1% increase in BHS and a 69.4% increase in BHR compared to vehicle challenge. There were no significant differences in the number of coughs and latency between BHS and BHR after pretreatment with salbutamol, despite BHS showing significantly greater coughing than BHR without pretreatment of salbutamol. Furthermore, the percentage of the number of animals that elicited cough was smaller in BHS than in BHR. These findings suggest that the airway smooth muscle contraction is fundamentally important for inducing cough in guinea pigs, when all the difference in the airway sensitivity is substantially abolished by the relaxation of the airway smooth muscle. Therefore, airway contractility affects the frequency of cough induction.

It is known that citric acid induced cough is produced by effects on bronchoconstriction involving with the muscarinic mechanism [2, 7]. Vagal blocking by atropine aerosols significantly decreased the number of coughs in both BHS and BHR. The number of coughs showed a 60.7% decrease in BHS and a 76.1% decrease in BHR compared to vehicle challenge, but there was no significant difference between the percentage of decrease in BHS and that in BHR. Atropine aerosols increased the latency to cough in BHS, but not significantly in BHR. The percentage of the number of animals that elicited cough was somewhat smaller in BHS than in BHR. These results suggest that vagal blocking by atropine suppress citric acid-induced coughing in BHS and BHR, revealing that the vagal reflex has an important role in the induction of cough; however the relationship between airway sensitivity and vagal reflex was not clarified in this study.

Many studies on the effects of local anesthesia in the airway suggest that the airway nervous receptors are primarily associated with the occurrence of cough in human [8], rabbits [12] and asthmatic subjects [27, 29]. In this study, aerosolized lidocaine inhibited the citric acid-induced cough in the two strains of guinea pigs; the number

of coughs showed a 94.1% decrease in BHS, and a 100% decrease in BHR. Lidocaine completely inhibited cough in BHR, but not completely in BHS, as shown by the fact that 60% of BHS guinea pigs still coughed. These results suggest that the cough reflex is more easily induced in BHS than in BHR. The cough reflex could be elicited in BHS by some of the airway sensory nerves which were not blocked by lidocaine. Airway sensory nerves may be functionally or morphologically developed in airway hypersensitiveness and such sensory nerves may be an enhancing factor of coughing.

Capsaicin stimulates primarily neuropeptide-containing non-myelinated C-fiber afferents. Although capsaicin in low concentrations produces cough, high concentrations of capsaicin are neurotoxic and cause nerve cell death known as "desensitization" of C-fibers [17]. In this study, citric acid induced-coughs were inhibited by "desensitization" in the two strains of guinea pigs; the number of coughs showed an 82.8% decrease in BHS, and a 91.7% decrease in BHR. This finding suggests that citric acid induced-cough is mediated through the activation of capsaicin-sensitive C-fiber endings. Since 75.0% of BHS guinea pigs and 33.3% of BHR guinea pigs coughed, cough in BHS is more difficult to suppress by pretreatment with a high concentration of capsaicin than in BHR. This indicates that the cough reflex induced by citric acid in desensitized BHS can be induced by some non-desensitized nerve fibers during citric acid inhalation. C-fiber endings may be functionally and/or morphologically developed in airway hypersensitiveness and C-fibers may be influential for the severity of cough.

All the results in the present study demonstrated that BHS guinea pigs have the higher sensitivity to irritation of the airway nervous receptors, especially C-fiber endings. Also the present study confirmed that the airway contraction is primarily important for inducing cough.

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