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Role of cyclin-dependent kinase 5 in capsaicin-induced cough

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Abstract

The role of cyclin-dependent kinase 5 (Cdk5) in the capsaicin-induced cough reflex was examined in mice. Pretreatment with inhaled roscovitine, a selective Cdk5 inhibitor, at concentrations of 0.3 to 3 mM inhibited the number of capsaicin-induced coughs in a concentration-dependent manner. Pretreatment with inhaled roscovitine, at a concentration of 3 mM also slightly but significantly inhibited the number of citric acid-induced coughs. The number of capsaicin-induced coughs was significantly reduced when C-fibers were desensitized by the pretreatment with capsaicin. The number of citric acid-induced coughs was slightly but significantly reduced in capsaicin-pretreated mice as compared with that in naive mice. Although the inhalation of roscovitine at a concentration of 3 mM significantly reduced the number of citric acid-induced coughs in naive mice to the level observed in capsaicin-pretreated mice, roscovitine had no effect on the number of citric acid-induced coughs in capsaicin-pretreated mice. These results suggest that Cdk5-dependent factors are involved in C-fiber-mediated cough signaling.

Keywords: Cyclin-dependent kinase 5 (Cdk5); Cough reflex; Capsaicin; TRPV1; Substance P

1. Introduction

It is well known that cough occurs in healthy people as physiological defense mechanism that clears foreign particles and excessive bronchial secretions from the airways, and is also a common symptom of a variety of respiratory diseases (Irwin et al., 1981). The cough reflex is triggered by the stimulation of myelinated rapidly adapting receptors and/or unmyelinated C-fibers within the larynx, trachea and proximal bronchi (Karlsson, 1996; Widdicombe, 1998). Although cough is well known to have a beneficial role in the defense of the host persistent chronic cough can lead to physical exhaustion and is associated with significant morbidity (e.g., sleep loss, irritability) (O'Connell, 1998). However, the detailed mechanism of persistent chronic cough is not well defined.

Stimulation of capsaicin-sensitive bronchopulmonary C-fibers is involved in eliciting the cough reflex (Karlsson, 1996). It has been reported that capsaicin binds to the cytosolic domain of transient receptor potential vanilloid 1 (TRPV1) receptors because of its lipophilic nature (Jung et al., 1999). Substance P, which is contained in afferent C-fiber endings within the airway epithelium and smooth muscle layer, is released by the activation of afferent C-fibers. Bonham et al. (1996) reported that substance P stimulates rapidly adapting receptors in guinea pigs. This stimulation of rapidly adapting receptors by substance P is a potential link between the two airway defense systems, both of which elicit bronchoconstriction, mucus secretion and cough. Such a link, whereby C-fiber-receptor stimulation leads to the release of substance P and the subsequent stimulation of rapidly adapting receptors, has been previously proposed to explain the overlap of stimuli and reflex effects of both afferent systems (Widdicombe, 1995); i.e., the activation of TRPV1 receptors by capsaicin causes the release of substance P and the subsequent stimulation of rapidly adapting receptors, which may enhance cough reflexes. However, little is known about the signal transduction mechanisms in the afferent C-fibers that are responsible for the modulation of cough transmission.

Cyclin-dependent kinase 5 (Cdk5) is a proline-directed serine/threonine kinase that has been implicated in the development and diseases of the mammalian nervous system. Recently, Pareek et al. (2006) demonstrated that Cdk5 co-localized with TRPV1 receptors, which suggests that Cdk5

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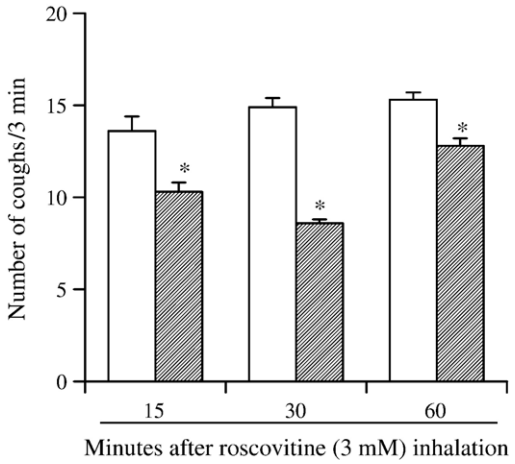

Fig. 1. Time course of the effect of roscovitine on capsaicin-induced coughs in mice. Mice were exposed to roscovitine (3 mM) aerosol for 2 min during the 15, 30 or 60 min preceding the inhalation of capsaicin. The number of coughs during 3 min of exposure to capsaicin was counted before (open column) and after exposure to roscovitine (hatched column). Each column represents the mean with S.E.M. of 10 mice in each group. *$P<0.05$ vs. respective vehicle-treated group.

activity may play an important role in C-fiber-mediated primary afferent signaling. Thus, it is reasonable to consider that Cdk5 may also play an important role in the modulation of cough sensitivity via the regulation of C-fiber activity.

To test this hypothesis, we investigated the effects of roscovitine, a selective Cdk5 inhibitor (Meijer et al., 1997; Pareek et al., 2006), on capsaicin- and citric acid-induced coughs in mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Tokyo Animal Laboratory Inc., Tokyo, Japan) weighing about 30 g were used. The animals had free access to food and water in animal room, which was maintained at 24±1 with a 12-h light–dark cycle. These studies were carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and 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, Culture, Sports, Science and Technology, Japan.

2.2. Antitussive assay

The cough reflex was induced as previously described (Kamei et al., 1993a,b; Morita and Kamei, 2003; Kamei et al., 2006). Briefly, animals were exposed to a nebulized solution of capsaicin (45 μM) or citric acid (0.25 M) under conscious and identical conditions using a body plethysmograph. The cough was analyzed on the basis of air-flow changes (sudden active expiratory effort following deep initial inspiration) measured by pneumotachograph (Nihon Kohden, TP-602T, Tokyo Japan) and recorded on polygraph (Nihon Kohden, RM-6100). Furthermore, the behavior of animals during the exposure period was observed carefully to exclude merely expiratory efforts other than cough reflex, such as sneezes from evaluation. 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 0.25 M in a saline solution. The mice were exposed for 3 min to capsaicin or citric acid 30 min before the inhalation of roscovitine to determine the frequency of control coughs. The mice were also exposed for 3 min to either capsaicin or citric acid 30 min after the inhalation of roscovitine. The number of coughs produced per 3-min period of exposure to capsaicin or citric acid was counted.

2.3. Desensitization of C-fibers by capsaicin pretreatment

A total dose of 300 mg/kg capsaicin was divided into three portions (50, 100, 150 mg/kg) and subcutaneously injected over 3 days. 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

Roscovitine and capsaicin were purchased from LKT Laboratories, Inc. (St. Paul, MN, USA) and Alpus Pharmaceutical Industries Co. (Gifu, Japan), respectively. Terbutaline sulfate and aminophylline hydrate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Sigma Chemical Co. (St. Luis, MO, USA), respectively. Roscovitine and capsaicin were dissolved in 10% dimethyl sulfoxide (DMSO). Terbutaline and aminophylline were dissolved in saline. Roscovitine was administered by inhalation as an aerosol for 2 min before the application of capsaicin.

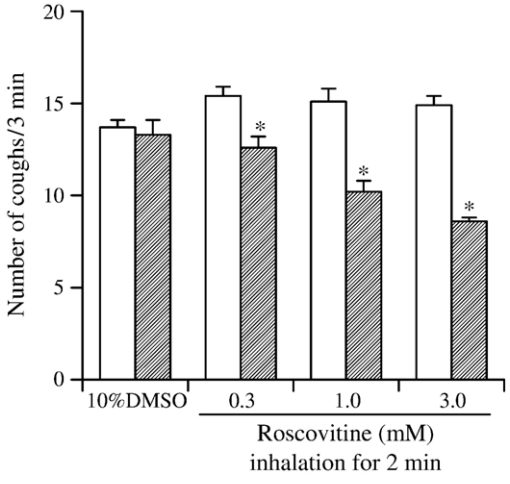

Fig. 2. Dose-response relationship of the effect of roscovitine on capsaicin-induced coughs in mice. Mice were exposed to roscovitine aerosol for 2 min during the 30 min preceding the inhalation of capsaicin. The number of coughs during 3 min of exposure to capsaicin was counted before (open column) and after exposure to vehicle or roscovitine (hatched column). Each column represents the mean with S.E.M of 10 mice in each group. *$P<0.05$ vs. vehicle-treated group.

2.5. Statistics

Data are expressed as means ± S.E.M. 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 roscovitine on the number of capsaicin-induced coughs

Fig. 1 shows the time course of the effect of inhaled roscovitine (3 mM) in naive mice. The effect reached its peak 30 min after the inhalation and then decreased. Thus, a time interval of 30 min after inhalation was chosen for experiments designed to quantify the antitussive effect of roscovitine. As shown in Fig. 2, exposure to roscovitine at concentrations of 0.3–3 mM for 2 min concentration-dependently and significantly reduced the number of citric acid-induced coughs.

3.2. Effect of roscovitine on the number of citric acid-induced coughs in capsaicin-pretreated mice

Exposure to capsaicin (45 μM) for 3 min produced 11.6 ± 1.1 coughs ($n = 10$) in vehicle-treated naive mice. On the other hand, the number of coughs (5.6 ± 0.7 coughs, $n = 10$) was significantly ($P < 0.05$) reduced when capsaicin-pretreated mice were exposed to capsaicin (45 μM) for 3 min. The number of citric acid-induced coughs was slightly but significantly reduced in capsaicin-pretreated mice as compared with that in naive mice. As shown in Fig. 3, although the inhalation of roscovitine at a concentration of 3 mM significantly reduced the number of citric acid-induced coughs in naive mice to the level observed in capsaicin-pretreated mice, roscovitine had no effect on the number of citric acid-induced coughs in capsaicin-pretreated mice.

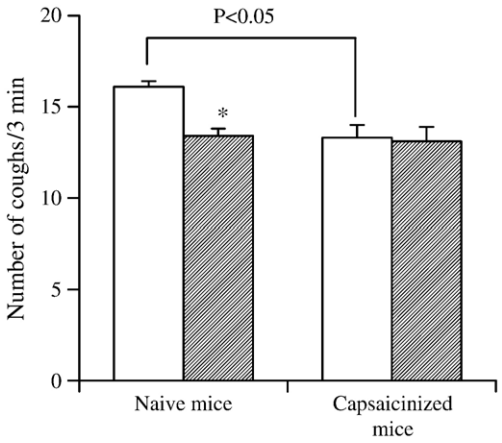

Fig. 3. Effect of the roscovitine on citric acid-induced coughs in naive and capsaicinized mice. Mice were exposed to roscovitine aerosol for 2 min during the 30 min preceding the inhalation of citric acid. The number of coughs during 3 min of exposure to citric acid was counted after exposure to vehicle or roscovitine (hatched column). Each column represents the mean with S.E.M of 10 mice in each group. *$P < 0.05$ vs. vehicle-treated group.

4. Discussion

In the present study, roscovitine, a selective Cdk5 inhibitor, administered by inhalation concentration-dependently and significantly reduced the number of capsaicin-induced coughs. This result suggests that Cdk5 may play an important role in the control of cough reflex.

The sensory afferent nerves within the respiratory tract are thought to consist of two types: rapidly adapting receptors which respond to mechanical stimuli and mucus within the airways and C-fiber receptors which respond to chemical stimulation and react to agents such as capsaicin (e.g., Harrison, 2004). Activation of C-fibers is thought to result in the antidromic release of neuropeptides such as substance P and neurokinin A, which in turn activate 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). In the present study, we observed that roscovitine slightly but significantly reduced the number of citric acid-induced coughs. While the inhalation of citric acid stimulates both C-fibers and rapidly adapting receptors, capsaicin appears to stimulate mainly 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). Thus, it is necessary to clarify whether Cdk5 affects C-fibers or rapidly adapting receptors to modulate the cough reflex. 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 observed that roscovitine reduced the number of citric acid-induced coughs in naive mice to the level that observed in C-fiber-desensitized mice. This result suggests that citric acid-induced coughs are partially mediated via the activation of C-fiber-dependent pathways. Thus, it is possible that the antitussive effect of roscovitine on the citric acid-induced cough reflex might account for the inhibitory effect of roscovitine on the activation of C-fiber-dependent pathways by citric acid. Furthermore, we also demonstrated that both capsaicin-induced and citric acid-induced coughs were inhibited by the desensitization of C-fibers. However, the roscovitine-induced reduction in the number of citric acid-induced coughs was abolished in C-fiber-desensitized mice. These findings suggest that Cdk5 may be involved in C-fiber-dependent pathways to modulate the cough reflex. Pareek et al. (2007) recently suggested that Cdk5-mediated TRPV1 phosphorylation is important in the C-fiber-mediated pain signaling. Thus, it seems likely that phosphorylation of TRPV1 by Cdk5 may play an important role in the signal transduction mechanisms in the afferent C-fibers that are responsible for the modulation of cough transmission.

In conclusion, Cdk5 may play an important role in the modulation of cough sensitivity via the regulation of C-fiber activities, namely TRPV1 receptor activities.

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