Inhaled Pinacidil, an ATP-Sensitive K⁺ Channel Opener, and Moguisteine Have Potent Antitussive Effects in Guinea Pigs

Kayo Morita¹, Kenji Onodera² and Junzo Kamei¹,*

¹Department of Pathophysiology & Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, Tokyo 142-8501, Japan
²Department of Dental Pharmacology, Okayama University Graduate School of Medicine and Dentistry, Okayama 700-8525, Japan

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ABSTRACT—We investigated whether inhaled pinacidil and moguisteine inhibit capsaicin-induced coughs in guinea pigs. Inhaled pinacidil (15 – 60 µg/ml), an ATP-sensitive K⁺ channel opener, and moguisteine (15 – 60 µg/ml) each dose-dependently inhibited the number of capsaicin-induced coughs. The antitussive effects of pinacidil and moguisteine were significantly antagonized by pretreatment with glibenclamide (10 mg/kg, i.p.), an ATP-sensitive K⁺ channel blocker. However, pretreatment with naloxone methiodide (10 mg/kg, s.c.) had no significant effect on the antitussive effects of either pinacidil or moguisteine. On the other hand, inhaled dihydrocodeine (15 – 60 µg/ml) also dose-dependently suppressed the number of capsaicin-induced coughs. The antitussive effect of inhaled dihydrocodeine was significantly antagonized by pretreatment with naloxone methiodide (10 mg/kg, s.c.), but not by glibenclamide (10 mg/kg, i.p.). These results indicate that inhaled pinacidil and moguisteine both attenuate capsaicin-induced coughs. Pinacidil and moguisteine may exert their antitussive effects through the activation of ATP-sensitive K⁺ channels in the tracheobronchial tract. Furthermore, it is possible that ATP-sensitive K⁺ channels may be involved in the antitussive effects of peripherally acting non-narcotic antitussive drugs.

Keywords: Cough reflex, Antitussive effect, ATP-sensitive K⁺ channel, Moguisteine, Peripherally acting non-narcotic antitussive drug

Moguisteine ((R,S)-2-(2-methoxyphenoxy)-methyl-3-ethoxycarbonyl-acetyl-1,3-thiazolidine), a non-opioid compound, has been shown to be as active as codeine in reducing coughs induced in guinea pigs by chemical irritants, such as citric acid and capsaicin, or by mechanical or electrical stimulation of the trachea (1). Data from several researchers suggest that the antitussive action of moguisteine depends on a peripheral mechanism (2 – 4). Previously, we demonstrated that moguisteine dose-dependently inhibited the enhancement of capsaicin-induced coughs associated with angiotensin converting enzyme inhibitor (5). Furthermore, we suggested that moguisteine may be of a therapeutic benefit in reducing allergic coughs (6). Recently, we also reported that the systemic administration of moguisteine may have an antitussive effect through the activation of ATP-sensitive K⁺ channels (7). These results suggest that ATP-sensitive K⁺ channels may be involved in the antitussive effects of peripherally acting non-narcotic antitussive drugs.

In clinical studies in asthmatic patients, orally administered cromakalim has been shown to attenuate nocturnal bronchoconstriction (13). This finding, together with its plasma half-life of 22 h (14), appeared to give this drug considerable potential as an oral bronchodilator. However,
the oral dose may be limited by vasodilatation and a decrease in blood pressure (15). In view of the side effects of the systemic administration of cromakalim, it is unlikely to be beneficial in the treatment of asthma. However, K⁺ channel openers should be evaluated as inhaled agents. Previous studies in guinea pigs have shown that inhaled levromakalim inhibited bronchoconstriction induced by systemically administered histamine (16). It has also been shown that K⁺ channel openers given by inhalation could protect against induced bronchoconstriction and airway microvascular leakage (17).

The purpose of this study was to determine if moguisteine and pinacidil given by inhalation could protect against capsaicin-induced coughs. We also investigated the effects of glibenclamide and naloxone methiodide on the antitussive effects of moguisteine and pinacidil in guinea pigs.

MATERIALS AND METHODS

Animals
Male Hartley guinea pigs (Tokyo Animal Laboratory Inc., Tokyo) weighing about 300 – 350 g at the beginning of the experiments 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. This study was 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 care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Antitussive assay
The cough reflex was induced as described previously (18, 19). Briefly, animals were exposed to a nebulized solution of capsaicin (30 μM) under conscious and identical conditions using a body-plethysmograph. Capsaicin was dissolved to a concentration of 30 μg/ml in a 10% ethanol and 10% Tween 80 saline solution. The solution was diluted with saline. The animals were exposed for 7 min to capsaicin 60 min before the inhalation of antitussive drugs to determine the number of control coughs. The animals inhaled antitussive drugs for 2 min. The animals were also exposed for 7 min to capsaicin 5 min after the inhalation of antitussive drugs. The number of coughs produced per 7-min period of exposure to capsaicin was counted. The number of coughs produced after antitussive drug treatment (Ct) was compared with the number of control coughs (Cc). The antitussive effect was expressed as the % inhibition of the number of control coughs (((Cc – Ct) / Cc) × 100).

Drugs
Moguisteine was generously supplied by Boehringer Mannheim Italia (Monza, Italy). Dihydrocodeine hydrochloride was purchased from Sankyo (Tokyo). Pinacidil was purchased from Research Biochemical International (Natick, MA, USA). Glibenclamide and naloxone methiodide were purchased from Sigma (St. Louis, MO, USA). Moguisteine was suspended in 0.5% sodium carboxymethyl cellulose. Glibenclamide was dissolved in 2.5% Tween 80 solution. Pinacidil was dissolved in 10% dimethylsulphoxide in saline. All other drugs were dissolved in saline. Glibenclamide (10 mg/kg, i.p.) and naloxone methiodide (10 mg/kg, s.c.) were injected 30 min before the inhalation of each antitussive drug.

Statistics
Data are expressed as the means ± S.E.M. The statistical significance of differences was assessed by the Mann-Whitney U-test to evaluate the antitussive effect. A level of probability of 0.05 or less was considered significant.

RESULTS

Effects of inhaled pinacidil, moguisteine and dihydrocodeine on capsaicin-induced cough
Pinacidil, an ATP-sensitive K⁺ channel opener, and moguisteine at doses of 15, 30 and 60 μg/ml, dose-dependently inhibited the number of capsaicin-induced coughs when the antitussive effect was examined 5 min after inhalation (Fig. 1: A and B). Inhaled dihydrocodeine, a centrally acting narcotic antitussive drug, at doses of 15, 30 and 60 μg/ml, also dose-dependently inhibited the number of capsaicin-induced coughs (Fig. 1C).

Effects of glibenclamide and naloxone methiodide on the antitussive effects of pinacidil, moguisteine and dihydrocodeine
The antitussive effects of inhaled pinacidil (60 μg/ml) and moguisteine (60 μg/ml) were each significantly antagonized by pretreatment with glibenclamide (10 mg/kg, i.p.). However, pretreatment with naloxone methiodide (10 mg/kg, s.c.) had no significant effect on the antitussive effects of either pinacidil or moguisteine (Fig. 2: A and B). The antitussive effect of inhaled dihydrocodeine (60 μg/ml) was significantly antagonized by pretreatment with naloxone methiodide (10 mg/kg, s.c.). However, pretreatment with glibenclamide (10 mg/kg, i.p.) had no significant effect on the antitussive effect of inhaled dihydrocodeine (Fig. 2C).

Glibenclamide, by itself, had no significant effect on the number of capsaicin-induced coughs (before glibenclamide, 21.8 ± 2.5 coughs/7 min; after glibenclamide, 19.8 ± 2.9 coughs/7 min, n = 8).

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The antitussive effect of inhaled dihydrocodeine (60 μg/ml) was significantly antagonized by pretreatment with naloxone methiodide (10 mg/kg, s.c.). However, pretreatment with glibenclamide (10 mg/kg, i.p.) had no significant effect on the antitussive effect of inhaled dihydrocodeine (Fig. 2C).

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Fig. 1. Dose-dependent inhibition of capsaicin-induced cough in conscious guinea pigs by nebulized pinacidil (A), moguisteine (B) and dihydrocodeine (C). The antitussive effect was assessed 5 min after inhalation of each drug. Each column represents the mean with S.E.M. (n = 6–8). *P<0.05 vs the vehicle-treated group (open column).

Fig. 2. Effects of glibenclamide and naloxone methiodide on the antitussive effects of pinacidil (A), moguisteine (B) and dihydrocodeine (C). Glibenclamide (10 mg/kg, i.p.) and naloxone methiodide (10 mg/kg, s.c.) were injected 30 min before the inhalation of each antitussive drug. The antitussive effect was assessed 5 min after inhalation of each drug. Each column represents the mean with S.E.M. (n = 6–8). *P<0.05 vs the vehicle of each antitussive drug alone-treated group (open column). #P<0.05 vs the respective vehicle of each antitussive drug-pretreated group (hatched column).
DISCUSSION

In the present study, we found that the inhaled pinacidil had a marked and dose-dependent antitussive effect in guinea pigs. Furthermore, while this antitussive effect of inhaled pinacidil was antagonized by pretreatment with glibenclamide, an ATP-sensitive $K^+$ channel blocker, pretreatment with naloxone methiodide, an opioid antagonist that does not cross the blood-brain barrier, had no significant effect on the antitussive effect of pinacidil. These results indicate that the antitussive effect of inhaled pinacidil is mediated via the activation of ATP-sensitive $K^+$ channels, but not peripheral opioid receptors. Furthermore, while the antitussive effect of inhaled moguisteine, a peripherally acting antitussive drug, was significantly antagonized by pretreatment with glibenclamide, pretreatment with naloxone methiodide had no significant effect. Previously, we demonstrated that the antitussive effect of cromakalim, an ATP-sensitive $K^+$ channel opener, was not antagonized by pretreatment with i.c.v. glibenclamide (12). We also reported that the antitussive effect of morphine was not antagonized by i.c.v. pretreatment with glibenclamide (11). Recently, we demonstrated that pinacidil and moguisteine may exert their antitussive effects through the activation of ATP-sensitive $K^+$ channels (7). Based on these results, it is possible that ATP-sensitive $K^+$ channels may be involved in the neuronal mechanisms of inhaled peripherally acting antitussive drugs.

Although cough and bronchoconstriction are believed to have separate afferent neural pathways, they often occur simultaneously and have been considered to be closely related (20). Several pieces of evidence indicate that promoting $K^+$ efflux through ATP-sensitive $K^+$ channels may inhibit bronchoconstriction (e.g., 9, 17). However, Poggioli et al. (10) demonstrated that pinacidil and cromakalim at doses that produced antitussive effects had no influence on tussive stimuli-induced bronchospasm. Therefore, the antitussive effects of inhaled pinacidil and moguisteine are not the consequence of a bronchodilating effect.

In the present study, nebulized dihydrocodeine produced a dose-dependent antitussive effect in guinea pigs. Furthermore, the antitussive effect of dihydrocodeine was antagonized by pretreatment with naloxone methiodide. These results suggest that inhibition of cough can be produced by dihydrocodeine, acting on opioid receptors in the tracheobronchial tract. Opioids, such as dihydrocodeine, are generally considered to be the most potent and effective antitussive drugs available and are believed to inhibit cough through suppression of a “cough center” in the CNS (21, 22). However, some experimental data indicate that opioids may interact with the peripheral nervous system. In the anesthetized dog, close i.a. injection of codeine and morphine inhibited cough and tracheal constriction produced by electrical stimulation of the tracheal mucosa, and opioids were thought to act locally in the airway (23). It has also been reported that nebulized codeine and morphine dose-dependently inhibited coughs and bronchoconstriction (24). Furthermore, prior inhalation of a quaternary opioid receptor antagonist, levalorphan methyl iodide, completely inhibited the antitussive and anti-bronchoconstrictor effects of inhaled codeine (24). These data indicate the presence of functional opioid receptors within the tracheobronchial tract. Furthermore, it seems likely that inhaled dihydrocodeine may exert its antitussive effect through the activation of opioid receptors in the tracheobronchial tract.

Morikawa et al. (2) and Sant’Ambrogio and Sant’Ambrogio (4) indicated that moguisteine inhibits the excitatory response of rapidly adapting irritant receptors to tussive stimuli, and this effect could account for the antitussive effect. It has been proposed that the nerves that are traditionally thought to play an important role in the cough reflex are myelinated afferent $\alpha\delta$ fibers, which are also known as rapidly adapting irritant receptors (e.g., 25). The opening of ATP-sensitive $K^+$ channels leads to $K^+$ efflux, and cell membrane hyperpolarization reduces excitability. In this regard, Fox et al. (26) reported that the efflux of $K^+$ through large-conductance calcium-activated $K^+$ channels, another type of $K^+$ channel, inhibited the firing of $\alpha\delta$ fibers. Thus, it is possible that inhaled moguisteine exerts its antitussive effect through inhibition of the excitability of rapidly adapting irritant receptors to tussive stimuli via the modulation of ATP-sensitive $K^+$ channels in the tracheobronchial tract.

In summary, the present results suggest that ATP-sensitive $K^+$ channels in the tracheobronchial tract may play an important role in the antitussive effect of inhaled moguisteine. Furthermore, the present results also provide a basis for the potential use of inhaled ATP-sensitive $K^+$ openers as peripherally acting non-narcotic antitussive drugs.

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REFERENCES

1 Gallicco L, Borghi PJ, Dalla Rosa C, Ceserani R and Tognella S: Moguisteine: a novel peripheral non-narcotic antitussive drug. Br J Pharmacol 112, 795 – 800 (1994)
2 Morikawa T, Gallicco L and Widdicombe JG: Actions of moguisteine on cough and pulmonary rapidly adapting receptor activity in the guinea pig. Pharmacol Res 35, 113 – 118 (1997)
3 Ishii R, Furuta M, Hashimoto M, Naruse T, Gallicco L and Ceserani R: Effects of moguisteine on the cough reflex induced by afferent electrical stimulation of the superior laryngeal nerve in guinea pigs. Eur J Pharmacol 362, 207 – 212 (1998)
4 Sant’Ambrogio G and Sant’Ambrogio FB: Action of moguisteine on the activity of tracheobronchial rapidly adapting receptors in the dog. Eur Resp J 11, 339 – 344 (1998)
5 Kamei J and Morita K: Antitussive effect of moguisteine on the enhanced coughing associated with enalapril. Eur J Pharmacol 312, 235 – 239 (1996)
6 Kamei J, Morita K, Kashiwazaki T and Ohsawa M: Antitussive effect of moguisteine on allergic coughs in the guinea pig. Eur J Pharmacol 347, 253 – 255 (1998)
7 Morita K and Kamei J: Involvement of ATP-sensitive K+ channels in the antitussive effect of moguisteine. Eur J Pharmacol 395, 161 – 164 (2000)
8 Black JL and Barnes PJ: Potassium channels and airway function: new therapeutic prospects. Thorax 45, 213 – 218 (1990)
9 Nielsen-Kudsk JE: Potassium channel modulation: a new drug principle for regulation of smooth muscle contractility. Dan Med Bull 43, 429 – 447 (1996)
10 Poggioli R, Benelli A, Arletti R, Cavazzuti E and Bertolini A: Antitussive effect of K+ channel openers. Eur J Pharmacol 371, 39 – 42 (1999)
11 Kamei J, Iwamoto Y, Misawa M and Kasuya Y: The antitussive effect of morphine is insensitive to ATP-sensitive potassium channel blocker. Res Commun Subst Abuse 13, 341 – 344 (1992)
12 Kamei J, Iwamoto Y, Narita M, Suzuki T, Misawa M and Kasuya Y: The antitussive effect of cromakalim in rats is not associated with adenosine triphosphate sensitive K+ channels. Res Commun Chem Pathol Pharmacol 80, 201 – 210 (1993)
13 Williams AJ, Lee TH, Cochrane GM, Hopkirk A, Vyse T, Chiew F, Lavender E, Richards DH, Owen S, Stone P, Church S and Woodcock AA: Attenuation of nocturnal asthma by Cromakalim. Lancet 336, 334 – 336 (1990)
14 Davies BE, Dierdorf HD, Eckl KM, Greb WH, Mellow G and Thomsen T: The pharmacokinetics of cromakalim, BRL 34915, a new antihypertensive agent in healthy male subjects. Br J Clin Pharmacol 25, 136P (1988)
15 Weston AH: Smooth muscle K+ channel openers; their pharmacology and clinical potential. Pflugers Arch 414, 899 (1989)
16 Englert HC, Wirth K, Gehring D, Furst DU, Albus U, Schols W, Rosenkranz B and Scholken BA: Airway pharmacology of the potassium channel opener, HOE 234, in guinea pigs: in vitro and in vivo studies. Eur J Pharmacol 210, 69 – 75 (1992)
17 Kidney JC, Lotvall JO, Lei Y, Chung KF and Barnes PJ: The effect of inhaled K+ channel openers on bronchoconstriction and airway microvascular in anaesthetized guinea pigs. Eur J Pharmacol 296, 81 – 87 (1996)
18 Kamei J, Tanihara H, Igarashi H and Kasuya Y: Effects of N-methyl-D-aspartate antagonists on the cough reflex. Eur J Pharmacol 168, 153 – 158 (1989)
19 Kamei J and Kasuya Y: The effect of hydrochlorothiazide on the enhanced coughing associated with treatment with enalapril. Eur J Pharmacol 213, 137 – 139 (1992)
20 Karlsson JA, Sant’Ambrogio G and Widdicombe JG: Afferent neural pathways in cough and reflex bronchoconstriction. J Appl Physiol 65, 1007 – 1023 (1988)
21 Salem H and Aviado DM: Antitussive drugs. With special reference to a new theory for the initiation of the cough reflex and the influence of bronchodilators. Am J Med Sci 247, 585 – 600 (1964)
22 Eddy NB, Friefel H, Hahn KJ and Halbach H: Codeine and its alternates for pain and cough relief. 3. The antitussive action of codeine-mechanisms, methodology and evaluation. Bull WHO 40, 425 – 454 (1969)
23 Yanaura S, Hosokawa T, Kitagawa H and Misawa M: Peripheral effects of morphine and codeine on the cough reflex. Jpn J Pharmacol 31, 529 – 536 (1981)
24 Karlsson JA, Lanner AS and Persson CGA: Airway opioid receptors mediate inhibition of cough and reflex bronchoconstriction in guinea pigs. J Pharmacol Exp Ther 252, 863 – 868 (1990)
25 Widdicombe JG: Sensory mechanisms. Pulm Pharmacol 9, 383 – 387 (1996)
26 Fox AJ, Barnes PJ, Venkatesan P and Belvisi MG: Activation of large conductance potassium channels inhibits the afferent and efferent function of airway sensory nerves in the guinea pig. J Clin Invest 99, 513 – 519 (1997)