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Involvement of ATP-sensitive K^+ channels in the anti-tussive effect of moguisteine

Kayo Morita, Junzo Kamei *

Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-chome, Shinagawa-ku, Tokyo 142-8501, Japan

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Abstract

The effect of glibenclamide, an ATP-sensitive K^+ channel blocker, on the anti-tussive effect of moguisteine and of pinacidil, an ATP-sensitive K^+ channel opener in guinea pigs was studied. Pinacidil (1 and 5 mg/kg, subcutaneous (s.c.)) dose-dependently reduced the number of coughs. The anti-tussive effect of pinacidil was significantly and dose-dependently antagonized by pre-treatment with glibenclamide (3 and 10 mg/kg, i.p.). Moguisteine (1 and 5 mg/kg, s.c.) dose-dependently reduced the number of coughs. The anti-tussive effect of moguisteine was also reduced by pre-treatment with glibenclamide, in a dose-dependent manner. However, pre-treatment with glibenclamide had no effect on the anti-tussive effects of dihydrocodeine and dextromethorphan. Glibenclamide (10 mg/kg, i.p.), by itself, had no significant effect on the number of coughs. These results suggest that pinacidil and moguisteine may exert their anti-tussive effects through the activation of ATP-sensitive K^+ channels. Furthermore, it is possible that ATP-sensitive K^+ channels may be involved in the anti-tussive effect of peripherally acting non-narcotic anti-tussive drugs.

Keywords: Cough reflex; ATP-sensitive K^+ channel; Moguisteine; Anti-tussive drug

1. Introduction

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 (Gallico et al., 1994). Recently, we demonstrated that moguisteine dose-dependently inhibited the enhancement of capsaicin-induced coughs associated with angiotensin converting enzyme inhibitor (Kamei and Morita, 1996). Furthermore, we suggested that moguisteine may be of a therapeutic benefit in reducing allergic coughs (Kamei et al., 1998). Although the detailed mechanism of the anti-tussive effect of moguisteine is not clearly understood, data from several researchers suggest that the anti-tussive action of moguisteine depends on a peripheral mechanism (Gallico et al., 1994; Morikawa et al., 1997; Ishii et al., 1998; Sant'Ambrogio and Sant'Ambrogio, 1998).

ATP-sensitive K^+ channel openers are novel agents that relax smooth muscle and may have therapeutic potential in the treatment of asthma (Black and Barnes, 1990; Nielsen-Kudsk, 1996). Recently, Poggioli et al. (1999) demonstrated that pinacidil and cromakalim, which open ATP-sensitive K^+ channels, reduced citric acid-induced coughs in guinea pigs. They also reported that the anti-tussive effect of ATP-sensitive K^+ channel openers was not a consequence of a bronchodilating effect (Poggioli et al., 1999). Previously, we suggested that ATP-sensitive K^+ channels are not associated with the central mechanisms of anti-tussive drugs since the anti-tussive effect of morphine was not antagonized by pre-treatment with the intracerebroventricular (i.c.v.) administration of glibenclamide (Kamei et al., 1992, 1993). Thus, it is possible that ATP-sensitive K^+ channels may be involved in the peripheral mechanisms of these anti-tussive effects. To test this hypothesis, we investigated the effect of glibenclamide on the anti-tussive effect of moguisteine in guinea pigs.

* Corresponding author. Tel.: +81-3-5498-5030; fax: +81-3-5498-5029.

E-mail address: kamei@hoshi.ac.jp (J. Kamei).

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2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Tokyo Animal Laboratory, 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. 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, Science, Sports and Culture.

2.2. Anti-tussive assay

The cough reflex was induced as previously described (Kamei and Kasuya, 1992; Kamei et al., 1989). 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 mg/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 injection of anti-tussive drugs, to determine the frequency of control coughs (C_c). The animals were also exposed for 7 min to capsaicin 45 min after administration of the drugs. The number of coughs produced after anti-tussive drug injection (C_t) was compared with the number of control coughs (C_c). The anti-tussive effect was expressed as the % inhibition of the number of control coughs [$(C_c - C_t)/C_c \times 100$)].

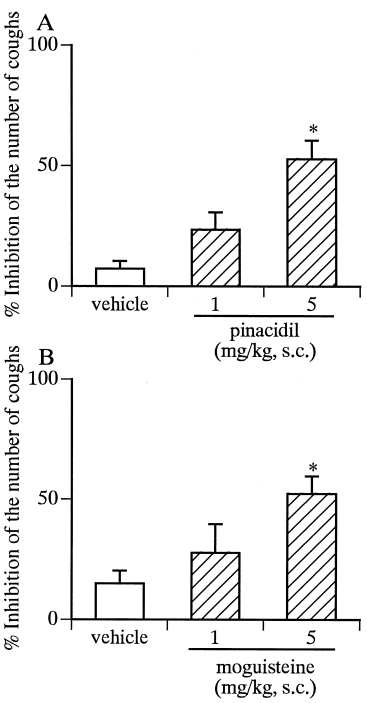

Fig. 1. Dose–response relationships of the anti-tussive effects of pinacidil (A) and moguisteine (B) in guinea pigs. The anti-tussive effects of pinacidil and moguisteine were assessed 45 min after the s.c. administration of each drug. The effects of pinacidil and moguisteine on the number of capsaicin-induced coughs were determined. Each column represents the mean with S.E. ($n = 5$–8). $^{*}P < 0.05$ vs. vehicle-treated group.

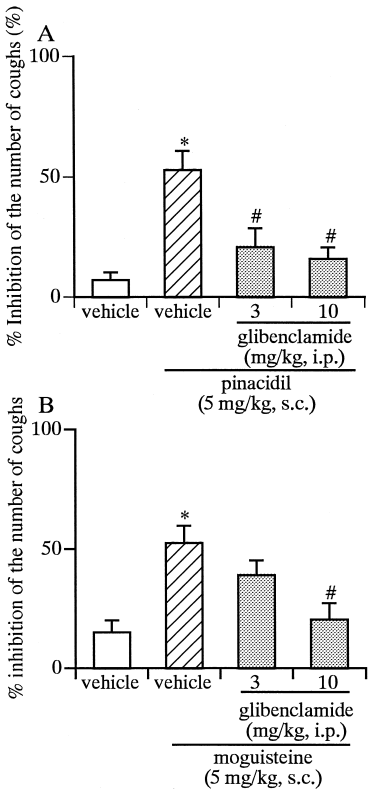

Fig. 2. Effects of glibenclamide on the anti-tussive effects of pinacidil (A) and moguisteine (B). Glibenclamide (3 and 10 mg/kg) was injected i.p. just before the administration of pinacidil or moguisteine. The anti-tussive effects of pinacidil (5 mg/kg) and moguisteine (5 mg/kg) were assessed 45 min after the s.c. administration of each drug. Each column represents the mean with S.E. ($n = 5$–8). $^{*}P < 0.05$ vs. vehicle alone-treated group (open column). $\#P < 0.05$ vs. respective vehicle-pretreated group (hatched column).

2.3. Drugs

Moguisteine was generously supplied by Boehringer Mannheim Italia. Dihydrocodeine hydrochloride was purchased from Sankyo (Tokyo, Japan). Pinacidil was purchased from Research Biochemical International (Natick, MA). Glibenclamide and dextromethorphan hydrobromide 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.

2.4. Statistics

Data are expressed as the means ± S.E. The statistical significance of differences was assessed by the Mann–

Whitney *U*-test to evaluate the anti-tussive effect. A level of probability of 0.05 or less was considered significant.

3. Results

3.1. Anti-tussive effects of pinacidil and moguisteine

Pinacidil, an ATP-sensitive K^+ channel opener, and moguisteine at doses of 1 and 5 mg/kg, s.c., dose-dependently inhibited the number of capsaicin-induced coughs when the anti-tussive effect was examined 45 min after administration (Fig. 1).

3.2. Effects of glibenclamide on the anti-tussive effects of pinacidil and moguisteine

The anti-tussive effect of pinacidil (5 mg/kg, s.c.) was significantly and dose-dependently antagonized by pre-treatment with glibenclamide (3 and 10 mg/kg, i.p.) (Fig. 2A). The anti-tussive effect of moguisteine (5 mg/kg, s.c.) was also reduced by pre-treatment with glibenclamide (3 and 10 mg/kg, i.p.), in a dose-dependent manner (Fig. 2B). However, glibenclamide (10 mg/kg, i.p.), 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, $n = 8$).

3.3. Effect of glibenclamide on the anti-tussive effects of centrally acting anti-tussive drugs

Dihydrocodeine (1 mg/kg, s.c.), a centrally acting narcotic anti-tussive drug, markedly inhibited the number of capsaicin-induced coughs. Dextromethorphan (5 mg/kg, s.c.), a centrally acting non-narcotic anti-tussive drug, also inhibited capsaicin-induced coughs. Pretreatment with glibenclamide (10 mg/kg, i.p.), had no significant effect on the anti-tussive effect of either dihydrocodeine or dextromethorphan (Fig. 3).

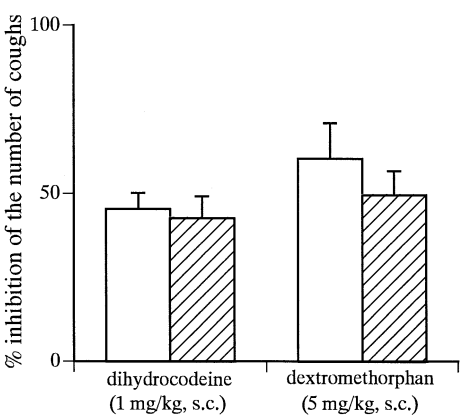

Fig. 3. Effects of glibenclamide on the anti-tussive effects of dihydrocodeine (1 mg/kg, s.c.) and dextromethorphan (5 mg/kg, s.c.) in guinea pigs. Glibenclamide (10 mg/kg, hatched column) was injected i.p. just before the administration of dihydrocodeine and dextromethorphan. The anti-tussive effects of dihydrocodeine (1 mg/kg) and dextromethorphan (5 mg/kg) were assessed 45 min after the s.c. administration of each drug. Each column represents the mean with S.E. ($n = 5–8$).

4. Discussion

In the present study, we found that the s.c. administration of pinacidil produced a marked and dose-dependent anti-tussive effect in guinea pigs. Furthermore, the anti-tussive effect of pinacidil was antagonized by pre-treatment with glibenclamide, an ATP-sensitive K^+ channel blocker. These results indicate that the anti-tussive effect of pinacidil is mediated via the activation of ATP-sensitive K^+ channels. Furthermore, we observed that the anti-tussive effect of moguisteine, a peripherally acting anti-tussive drug, was dose-dependently and significantly antagonized by pre-treatment with glibenclamide. Previously, we demonstrated that the i.p. administration of cromakalim, an ATP-sensitive K^+ channel opener, also dose-dependently and significantly suppressed the number of coughs (Kamei et al., 1993). However, the anti-tussive effect of cromakalim was not antagonized by pre-treatment with the i.c.v. administration of glibenclamide (Kamei et al., 1993). Moreover, we also reported that the anti-tussive effect of morphine was not antagonized by pre-treatment with the i.c.v. administration of glibenclamide (Kamei et al., 1992). In the present study, the systemic administration of glibenclamide had no significant effect on the anti-tussive effects of dihydrocodeine and dextromethorphan, typical centrally acting anti-tussive drugs. Based on these results, it is possible that the ATP-sensitive K^+ channels may be involved in the neuronal mechanisms of peripherally acting anti-tussive drugs, but not of centrally acting anti-tussive drugs.

Coughing is caused by several pathophysiological conditions of the airways (Salem and Aviad, 1964; Widdicombe, 1977; Karlsson et al., 1988). Although cough and bronchoconstriction are believed to have separate afferent neural pathways, cough and bronchoconstriction often occur simultaneously and have been considered to be closely related (Karlsson et al., 1988). Several pieces of evidence indicate that promoting K^+ efflux through ATP-sensitive K^+ channels may inhibit bronchoconstriction (e.g., Nielsen-Kudsk, 1996; Kidney et al., 1999). It is possible that the anti-tussive effects of ATP-sensitive K^+ channel openers are the consequence of a bronchodilating effect. However, this possibility was negated by the findings of Poggioli et al. (1999), who demonstrated that pinacidil and cromakalim (1–10 mg/kg, s.c.), at doses that gave anti-tussive effects (1–10 mg/kg, s.c.), had no influence on tussive stimuli-induced bronchospasm.

Moguisteine is a novel peripherally acting non-narcotic anti-tussive drug. Morikawa et al. (1997) and Sant'Am-

brogio and Sant'Ambrogio (1998) indicated that moguisteine has an inhibitory effect on the excitatory response of rapidly adapting irritant receptors to tussive stimuli, and this effect could account for the anti-tussigenic effect of moguisteine. It has been proposed that the nerves that are traditionally thought to play an important role in the cough reflex are myelinated afferent Aδ fibers, which are also known as rapidly adapting irritant receptors (e.g., Widdicombe, 1996). The opening of ATP-sensitive K^+ channels leads to K^+ efflux, and cell membrane hyperpolarization reduces excitability. In this regard, Fox et al. (1997) reported that the efflux of K^+ through large-conductance calcium-activated K^+ channels, another type of K^+ channel, inhibited the firing of Aδ fibers. Thus, it is possible that moguisteine exerts its anti-tussive effect through inhibition of the excitability of rapidly adapting irritant receptors to tussive stimuli as a result of modulation of ATP-sensitive K^+ channels.

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

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