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Antitussive effect of K^+ channel openers

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Abstract

The effect of the K_{ATP} channel openers, pinacidil and cromakalim, on coughing was studied in guinea pigs exposed to a nebulized aqueous solution of citric acid (0.50 M). Both pinacidil and cromakalim, subcutaneously administered 45 min before the test, inhibited coughing. The D_{50} (95% CI) were 0.95 ± 0.90 mg/kg for cromakalim and 3.25 ± 0.92 mg/kg for pinacidil. Under our experimental conditions, the D_{50} (95% CI) of codeine was 1.74 ± 0.75 mg/kg. The combination of cromakalim and pinacidil with codeine produced an additive effect. An additive effect was also produced by the combination of pinacidil with the selective tachykinin NK_2 receptor antagonist MEN 10,627 = [cyclo(Met-Asp-Trp-Phe-Dap-Leu)cyclo(2β–5β)]. The antitussive effect of pinacidil and cromakalim was not a consequence of a bronchodilating effect, which was absent at these dose levels under our experimental conditions. These results indicate that K_{ATP} channel openers have an opioid-like antitussive effect and may suggest a novel approach to the symptomatic treatment of coughing.

Keywords: K_{ATP} channel opener; Pinacidil; Cromakalim; Codeine; Antitussive effect

1. Introduction

Several opioids, such as morphine and codeine, are considered to be the most potent and effective antitussive drugs. They are believed to inhibit coughing through both central and peripheral mechanisms. While it is well established that opioids suppress a cough center in the central nervous system (CNS) (Salem and Aviado, 1964; Eddy et al., 1969), airway actions of therapeutic doses of opioids are less extensively documented. However, (i) inhibitory opioid receptors have been demonstrated on vagal sensory neurons (Young et al., 1980; Laduron, 1984); (ii) in the anesthetized dog, close intra-airway injection of codeine and morphine inhibits coughing and tracheal constriction produced by electrical stimulation of the tracheal mucosa, and the opioids are thought to act locally in the airway (Yanaura et al., 1981); (iii) in conscious guinea pigs, codeine and morphine inhibit coughing and reflex bronchoconstriction produced by inhaled citric acid, and this effect is prevented by prior inhalation of a quaternary opioid receptor antagonist, suggesting that, in this case, the site of action of opioids must be located in the tracheobronchial tree (Karlsson et al., 1990).

The antitussive effect of opioids is primarily mediated by μ_2-opioid receptors (Kamei et al., 1993, 1996). Activation of μ-opioid receptors produces an increase in membrane permeability to K^+ (Mc Fadzean, 1988) with consequent hyperpolarization of target neurons. This may suggest that K^+ channel openers should have antitussive activity. Accordingly, the aim of our present work was to study the effect of two K_{ATP} channel opener drugs in a guinea-pig model of coughing.

2. Materials and methods

2.1. Animals

Adult guinea-pigs of both sexes (Morini, S. Polo d'Enza, Reggio nell'Emilia, Italy), weighing 500–550 g, were used in this study. They were maintained in climatized colony-rooms (temperature 21 ± 1°C; humidity 60%) fulfilling the requirements of the EEC ethical regulations for animal research (EEC Council 86/609; Italian D.L. 27/01/1992, no. 116), with food in pellets (enriched with fresh vegetables) and tap water freely available, on a natural light/dark

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cycle. They were allowed to adapt to our conditions for at least 1 week before use.

2.2. Experimental protocol

The method used has been described previously by Forsberg et al. (1988) and modified by Karlsson et al. (1990). Unrestrained animals were placed individually in a hermetically sealed transparent Perspex chamber (25 cm × 12 cm × 12 cm), and exposed to a nebulized aqueous solution of 0.50 M citric acid. An ultrasonic nebulizer was used (ALSA, Bologna, Italy) that produced an aerosol with particles having a mass median diameter of 1 μm. About 0.6 ml of solution was nebulized per minute. During the exposure, the animals were continuously watched by a trained observer unaware of the treatment. The coughs and the episodes of wheezing in 15 min were detected and counted by the observer at the same time as the altered airflow was recorded by means of a pressure transducer (P23Db, Statham, Oxnard, CA, USA), coupled to a polygraph (Battaglia Rangoni, Bologna, Italy). Coughs could easily be distinguished from bronchoconstriction episodes, both visually and instrumentally. Bronchoconstriction episodes were characterized by slow, laboured breathing with exaggerated abdominal movements; the change in breathing pattern was prompt but rather sustained (some seconds), well different from the abrupt burst of the cough episode.

2.3. Drugs and treatments

Codeine hydrochloride was purchased from Salars, Camerlata, Como, Italy. Cromakalim and pinacidil were obtained from Sigma (St. Louis, MO, USA) and RBI (Research Biochemicals International, Natick, MA, USA), respectively.

Citric acid was purchased from Carlo Erba, Milano, Italy.

The selective antagonist of tachykinin NK_2 receptors, MEN 10,627 = [cyclo(Met-Asp-Trp-Phe-Dap-Leu)cyclo-

Table 1
Inhibition of citric acid (0.50 M)-induced coughing in conscious guinea pigs by the combination of pinacidil or cromakalim with codeine

Treatment	Dose (mg/kg s.c.)	No. of coughs	% Inhibition
Saline +	–		
Saline	–	11.95 ± 1.25	
Saline +	–		
Codeine	0.5	7.50 ± 1.25	37.23
Saline +	–		
Cromakalim	1	5.00 ± 1.54[a]	58.15
Saline +	–		
Cromakalim	5	4.29 ± 1.48[a]	64.10
Cromakalim +	1		
Codeine	0.5	3.86 ± 1.40[a]	67.69
Cromakalim +	5		
Codeine	0.5	0.75 ± 0.48[ab]	93.72
Saline +			
Pinacidil	1	8.80 ± 3.43	26.35
Saline +			
Pinacidil	5	3.80 ± 0.85[a]	68.20
Pinacidil +	1		
Codeine	0.5	7.14 ± 1.74	40.25
Pinacidil +	5		
Codeine	0.5	2.00 ± 0.71[ab]	83.26

Drugs were s.c. injected 45 min before the test. Means ± S.E.M. for 7–8 animals per group. Fifteen minutes of observation.
[a] $P < 0.01$ vs. controls (saline + saline)(ANOVA, followed by the Student–Newman–Keuls test).
[b] $P < 0.002$ at least vs. codeine (ANOVA, followed by the Student–Newman–Keuls test).

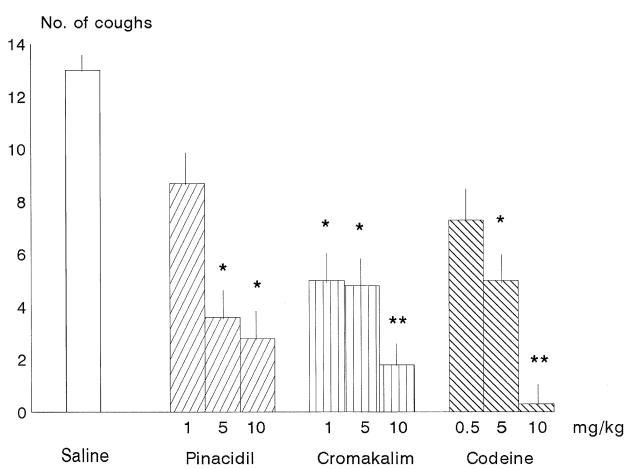

Fig. 1. Effect of pinacidil, cromakalim and codeine on the coughing induced in guinea-pigs during a 15-min exposure to a nebulized aqueous solution of 0.50 M citric acid. Means ± S.E.M.; 10–12 animals per group. * $P < 0.05$ and ** $P < 0.01$ vs. the saline-treated group (ANOVA followed by Student–Newman–Keuls test).

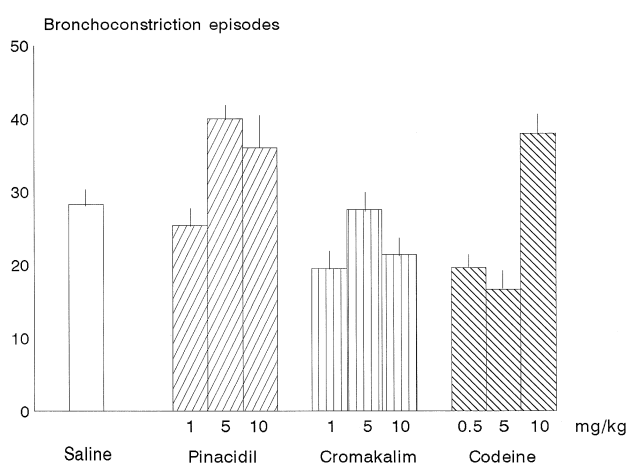

Fig. 2. Effect of pinacidil, cromakalim and codeine on the bronchoconstriction induced in guinea-pigs during a 15-min exposure to a nebulized aqueous solution of 0.50 M citric acid. Means ± S.E.M.; 10–12 animals per group.

(2β–5β)] (Maggi et al., 1994), was a kind gift of Dr. Sandro Giuliani, Menarini Pharmaceuticals, Firenze, Italy.

Drugs (or equivolume saline, in controls) were subcutaneously (s.c.) injected 45 min (codeine, cromakalim, and pinacidil) or 15 min (MEN 10,627) before the test, in a volume of 1 ml/kg. The doses of pinacidil and cromakalim were 1, 5 or 10 mg/kg s.c.; the doses of codeine were 0.5, 5 or 10 mg/kg s.c. The doses of MEN 10,627 were 36 and 76 μg/kg s.c. Combinations of 1 or 5 mg/kg of pinacidil or cromakalim with 0.5 mg/kg of codeine (injected separately in immediate succession, in two different s.c. sites), and of 1 mg/kg of pinacidil with 36 μg/kg of MEN 10,627 were also administered.

Each animal was used only once.

2.4. Statistical analysis

All data are given as means ± S.E.M. and were analyzed for statistical significance by analysis of variance (ANOVA) followed by the Student–Newman–Keuls test. D_{50} was calculated according to Tallarida and Jacob (1979).

3. Results

As expected, codeine dose dependently (0.5–10 mg/kg s.c.) inhibited citric acid-induced coughs (Fig. 1). At the highest dose (10 mg/kg s.c.) coughing was inhibited by 94.97 ± 5.7% ($P < 0.001$), 45 min after treatment. D_{50} (95% CI) was 1.74 ± 0.75 mg/kg.

Citric acid-induced coughs were also dose dependently inhibited by the two K_{ATP} channel openers, pinacidil and cromakalim (Fig. 1). At the highest dose (10 mg/kg s.c., 45 min before the test) coughing was inhibited by 75.89 ± 4.5% in the case of pinacidil, and by 87.44 ± 5.2% in the case of cromakalim ($P < 0.001$ in both cases). Cromakalim was very effective even at the dose of 1 mg/kg (58.15 ± 3.6% inhibition of coughing). The D_{50} (95% CI) values were 0.95 ± 0.90 and 3.25 ± 0.92 mg/kg for cromakalim and pinacidil, respectively.

The combination of cromakalim and pinacidil with codeine produced an additive effect (Table 1).

On the other hand, neither codeine nor the two K_{ATP} channel openers had any significant effect on citric acid-induced bronchoconstriction (Fig. 2). Finally, the tachykinin NK_2 receptor antagonist, MEN 10,627, dose dependently reduced citric acid-induced coughing and bronchospasm. The combination with a per se inactive dose of pinacidil significantly increased its antitussive activity while having no influence on its antibronchospastic activity (Table 2).

Table 2
Inhibition of citric acid (0.50 M)-induced coughing and bronchoconstriction in conscious guinea pigs by MEN 10,627 and pinacidil with MEN 10,627

Treatment	Dose (mg/kg s.c.)	No of coughs	Bronchoconstriction episodes
Saline	1 ml/kg	7.83 ± 1.05	33.0 ± 5.6
MEN 10,627	0.036	8.0 ± 1.0	18.5 ± 1.5[a]
MEN 10,627	0.076	0.25 ± 0.25[a]	9.50 ± 2.18[a]
Pinacidil	1.0	8.8 ± 3.4	25.4 ± 6.5
Pinacidil + MEN 10,627	1.0 0.036	2.6 ± 1.08[ab]	19.80 ± 8.08

Drugs were s.c. injected 45 min before the test. Means ± S.E.M. for 7–8 animals per group. Fifteen minutes of observation.
[a] $P < 0.01$ vs. saline-treated (ANOVA, followed by the Student–Newman–Keuls test).
[b] $P < 0.001$ vs. MEN 10,627.

4. Discussion

In guinea-pigs, the inhalation of citric acid induces coughs by acting on capsaicin-sensitive sensory neurons (Forsberg and Karlsson, 1986; Forsberg et al., 1988). Independent data suggest that codeine may act locally in the airways to inhibit citric acid-induced coughing (Dragonetti et al., 1983; Karlsson et al., 1990), by activating opioid receptors within the tracheobronchial tree (Adcock et al., 1988).

Our present data, besides confirming the observation of Karlsson et al. (1990) on the effect of codeine in the citric acid model of cough, showed that a similar effect is obtainable with two typical K_{ATP} channel opener drugs, pinacidil and cromakalim.

The antitussive effect of pinacidil and cromakalim is not the consequence of a bronchodilating effect because, under our experimental conditions, these two drugs—at the doses effective as antitussive—had no influence on citric acid-induced bronchospasm, either alone or in combination with codeine.

Since citric acid induces coughing and reflex bronchoconstriction in the guinea pig by acting on capsaicin-sensitive sensory neurons (Forsberg and Karlsson, 1986; Forsberg et al., 1988), and since the effects of capsaicin on airways are in part dependent upon stimulation of tachykinin NK_2 receptors (Ballati et al., 1992), we wanted to ascertain whether tachykinin NK_2 receptors also were involved in the antitussive activity of K_{ATP} channel openers. Indeed, this seems to be the case, because the combination of per se inactive doses of pinacidil and the tachykinin NK_2 receptor antagonist, MEN 10,627, significantly inhibited citric acid-induced coughing. It seems justifiable to suggest that the opioid-like antitussive activity of pinacidil and cromakalim is linked to their mechanism of action, because the antitussive effect of opioids is primarily mediated by μ_2-opioid receptors (Kamei et al., 1993, 1996), and activation of μ-opioid receptors produces an increase in membrane permeability to K^+ (Mc Fadzean, 1988).

We have previously reported that K_{ATP} channel openers have an opioid-like antidiarrheal activity (Poggioli et al.,

1995), and this has been confirmed recently (Schirgi-Degen and Beubler, 1996). Now we show that another typical pharmacological effect of opioid drugs is mimicked by K_{ATP} channel opener drugs.

Our present data, if confirmed for other animal species and for humans, may suggest a novel approach to the symptomatic treatment of coughing; especially as K_{ATP} channel openers have practically no effect on the blood pressure of normotensive subjects (Singer et al., 1989), and as hyperglycemia is only produced by diazoxide, but not by other K_{ATP} channel openers (Garrino et al., 1989).

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