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European Journal of Pharmacology 277 (1995) 159–164

ejp

Pharmacological studies of allergic cough in the guinea pig

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Received 14 July 1994; revised 23 January 1995; accepted 27 January 1995

Abstract

The pharmacological mechanisms of allergic cough in the guinea pig were studied. Actively sensitized guinea pigs were exposed to aerosols of antigen to elicit coughing. In separate experiments, naive guinea pigs were exposed to aerosols of capsaicin to elicit coughing. Both allergic and capsaicin-induced cough were inhibited by loratadine (0.3–10 mg kg^{-1} p.o.) and chlorpheniramine (0.1–3.0 mg kg^{-1} p.o.). Neither cimetidine (10 mg kg^{-1} s.c.), nor thioperamide (3–10 mg kg^{-1} s.c.), inhibited allergic or capsaicin-induced cough. Codeine (3–30 mg kg^{-1} p.o.), salbutamol (0.003–3.0 mg kg^{-1} s.c.) and ipratropium (0.03–1.0 mg kg^{-1} s.c.) inhibited both allergic and capsaicin-induced cough. Hexamethonium (10 and 30 mg kg^{-1} s.c.) inhibited allergic, but not capsaicin-induced cough. Allergic and capsaicin-induced cough were unaffected by phenidone (5.0 and 10.0 mg kg^{-1} s.c.). Indomethacin (5.0 and 10.0 mg kg^{-1} s.c.) had no effect on allergic cough but slightly inhibited capsaicin-induced cough. We conclude that allergic and capsaicin-induced cough are modulated by histamine H_1 receptor and cholinergic mechanisms. Histamine H_2 or histamine H_3 receptor mechanisms, and lipoxygenase and cyclooxygenase products of arachidonic acid metabolism do not influence allergic and capsaicin-induced cough. Ganglionic mechanisms play a minor role in the production of allergic cough and no role in capsaicin-induced cough.

Keywords: Cough; Antitussive; Antihistamine; (Guinea pig)

1. Introduction

Cough is the most common symptom of pulmonary disease (Choudry and Fuller, 1992). Prominent pulmonary diseases in which cough is an important symptom include asthma, chronic obstructive pulmonary disease, pulmonary infections, and upper respiratory tract infections (Braman and Corrao, 1987; O'Connell et al., 1991). In asthma, for example, the classical definition describes cough as one of a triad of symptoms which also includes wheeze and shortness of breath (McFadden, 1975). Although wheeze is generally considered the hallmark symptom of this disorder, chronic cough is present in at least 80% of asthmatics (Wynder et al., 1965). Furthermore, 5–6% of adult asthmatics have cough as their only symptom and are diagnosed with cough-variant asthma (Braman and Corrao, 1987; McFadden, 1975).

There are a variety of animal models of cough (Korpas and Tomori, 1979). Virtually all of them utilize electrical, mechanical, or chemical stimulation of sensory afferents or the CNS (central nervous system) to produce cough (Korpas and Tomori, 1979). For example, in recent years, the irritant capsaicin has been used with increasing frequency to study the cough reflex in the guinea pig (Bolser et al., 1991; Forsberg and Karlsson, 1986). Capsaicin elicits cough by activation of capsaicin-sensitive airway C-fibers (Forsberg and Karlsson, 1986).

Few studies have investigated the mechanisms of cough in allergic animals. Winter and Flataker (1955) showed that passively sensitized guinea pigs coughed in a dose-dependent manner in response to antigen aerosols. In this model, the histamine H_1 receptor antagonist pyrilamine inhibited cough (Winter and Flataker, 1955). Surprisingly, the antitussive agent, codeine, had no effect on allergic cough (Winter and Flataker, 1955). A recent study (Karlsson et al., 1992) showed that actively sensitized guinea pigs cough in response to inhalation of antigen aerosols. These inves-

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SSDI 0014-2999(95)00076-3

tigators showed that the cough responses of guinea pigs to inhaled irritants were not changed by sensitization, but they did not investigate the pharmacology of antigen-induced cough in sensitized guinea pigs. The purpose of this study was to investigate the pharmacological mechanisms of cough in actively sensitized guinea pigs exposed to antigen aerosols using standard antitussive and antiasthma drugs. From these studies, we could evaluate whether the pharmacological mechanisms of allergic cough are similar to the mechanisms of cough elicited by the irritant, capsaicin. A preliminary report of this work has been published (DeGennaro et al., 1993).

2. Materials and methods

2.1. Sensitization of guinea pigs

Male Dunkin-Hartley guinea pigs (250–300 g) were actively sensitized to ovalbumin. Ovalbumin (200 μg ml^{-1}) was mixed with aluminum hydroxide (200 mg ml^{-1}) for 4 h before use. Animals were injected i.p. with 0.5 ml of this mixture for an approximate dose of 200 μg kg^{-1} ovalbumin and 200 mg kg^{-1} aluminum hydroxide. In addition, animals received 0.3 ml (i.p.) of 10×10^{10} heat-killed pertussis organisms. Sham-sensitized animals did not receive ovalbumin. The animals were used 28 days later when they weighed 400–600 g.

2.2. Measurement of allergic and capsaicin-induced cough

Unanesthetized sensitized guinea pigs were placed in a transparent plastic chamber and exposed to aerosols of ovalbumin (0.1–1%) at an airflow of 4 liter min^{-1} to elicit coughing. The dimensions of the chamber were $12'' \times 4''$. Unanesthetized non-sensitized guinea pigs were used for studies involving aerosolized capsaicin (Bolser et al., 1991). The ovalbumin or capsaicin (300 μM) aerosols were generated by a jet nebulizer (Puritan Bennett, Lenexa, KS, USA) and the volume of solution aerosolized was approximately 1.6 ml. When filled with water, the aerosol particle size generated by this type of nebulizer was 1.04–1.1 μm mass median aerodynamic diameter at an airflow of 6–8 liter min^{-1} (Puritan Bennett, Lenexa, KS, USA). No information is available regarding the aerosol particle size generated by this type of nebulizer at 4 liter min^{-1} or with solutions other than water.

Each animal was individually exposed only once to ovalbumin or capsaicin in an unpaired paradigm. On each experimental day, both control and drug-treated groups of animals were generated. The influence of drugs on the cough response of the animals was expressed as percentage inhibition of the average number of coughs observed in the control group. Coughs were detected by a microphone placed in the chamber and connected to an audio monitor and chart recorder. The number of coughs elicited during a 4 min exposure to ovalbumin or capsaicin was counted by visual inspection of the chart record. This is an established method for the measurement of cough (Bolser et al., 1991; Forsberg and Karlsson, 1986; Forsberg et al., 1988).

Animals were dosed with drug or vehicle 1 h (s.c.) or 2 h (p.o.) before challenge with ovalbumin or capsaicin aerosol. Vehicles for oral dosing were 0.4% methylcellulose in isotonic saline or isotonic saline itself. Saline was the vehicle for subcutaneous administration. All drugs were calculated as the free base.

2.3. Compounds

Compounds used in this study included codeine sulfate (Mallinkrodt, St. Louis, MO, USA), cimetidine, indomethacin, hexamethonium bromide, phenidone, and ipratropium bromide (Sigma Chemical Co., St. Louis, MO, USA). Salbutamol sulfate, loratadine, thioperamide maleate, and chlorpheniramine maleate were synthesized at Schering-Plough Research Institute.

2.4. Statistics

Data are expressed as percent inhibition (mean ± S.E.M.) of cough relative to vehicle controls. Effective doses for 50% inhibition of cough (ED_{50}) were calculated by linear regression analysis of the dose-response relationship. Statistical analysis was performed using one-way analysis of variance, Student's t-test, or the Mann-Whitney test for nonparametric data; $P < 0.05$ was considered significant. One-way analysis of variance was used to assess significant differences between a vehicle-treated group and two or more drug-treated groups of animals. Student's t-test or the Mann-Whitney test were used to evaluate differences between a vehicle-treated group and one drug-treated group. The Mann-Whitney test was used only when the two groups had significantly different standard deviations.

3. Results

Fig. 1 shows the ability of inhaled ovalbumin to elicit cough in sensitized and sham-sensitized guinea pigs. Sensitized guinea pigs challenged with isotonic saline aerosol did not cough. Challenge of sensitized guinea pigs with ovalbumin aerosols resulted in a dose-related increase in coughing. At the highest dose of ovalbumin (1%), sensitized animals coughed approximately 10 times during the first 4 min following challenge. Animals that were sham-sensitized (alum and pertussis, no ovalbumin) exhibited negligible cough re-

Table 1
Antitussive effects of histamine antagonists on capsaicin and ovalbumin-induced cough in the guinea pig

Compound	Dose (mg kg^{-1})	Route [a]	% Inhibition of cough			
			Capsaicin	n	Ovalbumin	n
Chlorpheniramine	0.1	p.o.	29 ± 12	(12)	5 ± 20	(6)
	0.3		26 ± 9	(18)	39 ± 22	(6)
	1.0		43 ± 5 [b]	(18)	65 ± 8 [b]	(6)
	3.0		50 ± 10 [b]	(12)	86 ± 6 [b]	(6)
Loratadine	0.3	p.o	30 ± 6	(18)	0 ± 21	(5)
	1.0		34 ± 8	(24)	51 ± 24	(5)
	3.0		34 ± 8	(24)	89 ± 4 [b]	(5)
	10.0		62 ± 12 [b]	(12)	–	–
Cimetidine	10.0	s.c.	18 ± 13	(18)	0 ± 18	(15)
Thioperamide	3.0	s.c.	27 ± 9	(25)	29 ± 11	(6)
	10.0		−16 ± 12	(12)	–	–

[a] Animals were pretreated 2 h before capsaicin or ovalbumin challenge with chlorpheniramine or loratadine and 1 h before capsaicin or ovalbumin challenge with cimetidine or thioperamide. [b] $P < 0.05$ relative to control groups by one-way analysis of variance.

sponses to 1% ovalbumin aerosol. A dose of 1% ovalbumin elicited the most reproducible cough responses and was chosen to further investigate the pharmacology of allergic cough in sensitized animals.

In the pharmacology experiments, vehicle-treated animals ($n = 87$) that were challenged with ovalbumin averaged 13 ± 1 coughs. Among animals that were challenged with capsaicin, vehicle-treated groups ($n = 156$) averaged 9 ± 0.3 coughs per animal.

Table 1 shows the effects of histamine H_1 receptor antagonists (chlorpheniramine and loratadine), a histamine H_2 receptor antagonist (cimetidine) and a histamine H_3 receptor antagonist (thioperamide) on allergic and capsaicin-induced cough in the guinea pig. Both chlorpheniramine and loratadine significantly inhibited allergic cough in a dose-dependent manner. Each of these drugs produced maximum inhibition of allergic cough of approximately 90%. The oral ED_{50} values of these drugs for inhibiting allergic cough were 0.6 mg kg^{-1} for chlorpheniramine and 1.0 mg kg^{-1} for loratadine. Loratadine and chlorpheniramine also inhibited capsaicin-induced cough (Table 1). Under these conditions, the maximum observed inhibition produced by these compounds was 50–60%. The oral ED_{50} val-

Table 2
Antitussive effects of selected standards on capsaicin- and ovalbumin-induced cough in guinea pig

Compound	Dose (mg kg^{-1})	Route [a]	% Inhibition of cough			
			Capsaicin	n	Ovalbumin	n
Codeine	3.0	p.o	28 ± 9	(6)	–	–
	10.0		22 ± 8	(6)	8 ± 11	(6)
	30.0		50 ± 9 [b]	(6)	54 ± 14 [b]	(6)
Salbutamol	0.0003	s.c.	23 ± 21	(6)	–	–
	0.001		44 ± 8 [b]	(12)	–	–
	0.003		40 ± 7 [b]	(12)	–	–
	0.01		59 ± 6 [b]	(18)	–	–
	0.03		70 ± 6 [b]	(12)	–	–
	0.3		–	–	11 ± 24	(6)
	1.0		–	–	65 ± 13 [b]	(12)
	3.0		–	–	55 ± 12 [b]	(11)
Ipratropium	0.03	s.c	−10 ± 20	(18)	–	–
	0.1		37 ± 14	(18)	33 ± 9	(11)
	0.3		73 ± 11 [b]	(12)	59 ± 14 [b]	(12)
	1.0		50 ± 11 [b]	(12)	–	–
Hexamethonium	10.0	s.c.	−3 ± 13	(8)	13 ± 23	(6)
	30.0		21 ± 10	(8)	38 ± 6 [b]	(12)
Phenidone	5.0	s.c.	−32 ± 27	(12)	39 ± 12	(6)
	10.0		–	–	13 ± 17	(6)
Indomethacin	5.0	s.c.	33 ± 9 [b]	(24)	29 ± 11	(6)
	10.0		26 ± 8 [b]	(12)	−8 ± 19	(6)

[a] Animals were pretreated p.o. with codeine 2 h before capsaicin or ovalbumin challenge. The pretreatment time for the other drugs given s.c. was 1 h. [b] $P < 0.05$ relative to control groups by one-way analysis of variance.

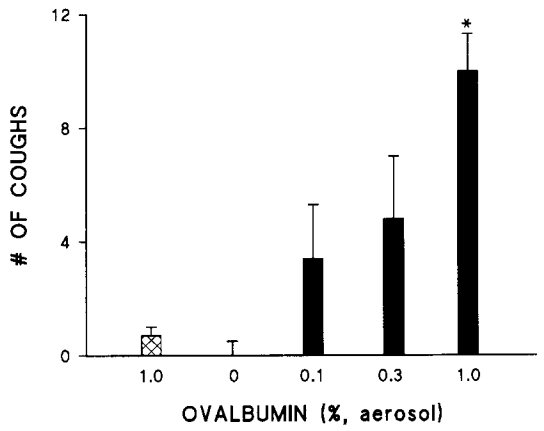

Fig. 1. Dose-dependent effect of ovalbumin aerosol on cough in sensitized and sham-sensitized guinea pigs. Hatched bar: sham-sensitized guinea pigs (pertussis, alum, no ovalbumin), filled bars: sensitized (pertussis, alum, ovalbumin) guinea pigs. $n = 6$–12 per group, * $P < 0.05$ relative 0% ovalbumin group by one-way analysis of variance.

ues of these drugs for inhibiting capsaicin-induced cough were 2.6 mg kg^{-1} for chlorpheniramine and 5.8 mg kg^{-1} for loratadine. Neither allergic cough nor capsaicin-induced cough were significantly inhibited by the histamine H_2 receptor antagonist, cimetidine, or the histamine H_3 receptor antagonist, thioperamide (Table 1).

The antitussive drug, codeine, was equally active against allergic and capsaicin-induced cough (Table 2). The maximum inhibition was seen at 30 mg kg^{-1} and this dose inhibited cough by approximately 50%.

Salbutamol inhibited both allergic and capsaicin-induced cough (Table 2). Maximum inhibition by salbutamol of allergic cough of 65% was seen at 1.0 mg kg^{-1} and no greater inhibition was seen at 3.0 mg kg^{-1}. The subcutaneous ED_{50} for the antitussive effect of salbutamol in the allergic cough model was 1.3 mg kg^{-1}. Salbutamol was considerably more potent against capsaicin-induced cough with an ED_{50} of 0.004 mg kg^{-1}.

The anticholinergic agent, ipratropium bromide, significantly inhibited both allergic and capsaicin-induced cough (Table 2). The maximum inhibition of allergic and capsaicin-induced cough by ipratropium was 60–70% at a dose of 0.3 mg kg^{-1}. Also, the ganglionic blocking agent, hexamethonium, significantly reduced allergic cough by 38% at the highest dose (30 mg kg^{-1}) but had no significant effect on capsaicin-induced cough (Table 2).

The 5-lipoxygenase inhibitor, phenidone, had no significant effect on allergic or capsacin-induced cough (Table 2). Likewise, the cyclooxygenase inhibitor, indomethacin, had no effect on allergic cough (Table 2). However, indomethacin had a small, but significant, effect on capsaicin-induced cough that did not increase in magnitude when the dose of this drug was doubled.

4. Discussion

The major findings of this study are that sensitized guinea pigs will cough reproducibly in response to antigen aerosols and that histamine H_1, cholinergic, and ganglionic mechanisms participate in the production of allergic cough in actively sensitized animals in this species. Furthermore, histamine H_1 and cholinergic mechanisms play a role in the production of capsaicin-induced cough. Lipoxygenase and cyclooxygenase products of arachidonic acid metabolism do not appear to play an important role in the production of allergic or capsaicin-induced cough.

Winter and Flataker (1955) showed that codeine had no effect on allergic cough in passively sensitized guinea pigs. Our results show that allergic cough in actively sensitized animals can be inhibited by 30 mg kg^{-1} codeine. The reason for this difference in results is probably related to the dosage of codeine used in their study (10 mg kg^{-1}). Both allergic and capsaicin-induced cough were inhibited with equal efficacy and potency by codeine. This observation is consistent with the idea that codeine influences the cough reflex in the two models by a similar mechanism. The extent to which this effect of codeine involves central (Wang et al., 1977) or peripheral (Karlsson et al., 1990) segments of the reflex pathway for cough is unknown.

The anticholinergic drug, ipratropium, and the ganglionic blocker, hexamethonium, also had antitussive activity in the allergic cough model. These results suggest that reflex cholinergic mechanisms, such as mucus secretion, contribute to the production of allergic cough in the guinea pig. Conversely, ipratropium, but not hexamethonium, had antitussive activity in the capsaicin-induced cough model. These results are consistent with the idea that capsaicin elicits cough by cholinergic, non-reflex mechanisms in the guinea pig. For example, capsaicin may elicit the release of acetylcholine locally in the airways to produce mucus secretion. It is also possible that acetylcholine may directly stimulate sensory afferents that elicit cough. Sensory C-fibers in other organ systems can be activated by acetylcholine receptor agonists (Steen and Reeh, 1993). Indeed, inhalation of acetylcholine aerosols will elicit cough in the cat (Gardiner et al., 1978).

The histamine H_1 receptor antagonists, loratadine and chlorpheniramine, were effective inhibitors of allergic cough in the guinea pig. These results are consistent with a previous report that the histamine H_1 receptor antagonist, pyrilamine, inhibited allergic cough in guinea pigs that were passively sensitized with antigen (Winter and Flataker, 1955). Therefore, it is likely that the histamine H_1 receptor antagonist activity of both drugs contributed significantly to their antitussive effects. This finding is consistent with clinical reports that preparations containing histamine H_1 receptor

antagonists inhibit chronic cough in humans (Ciprandi et al., 1992; Irwin et al., 1990; Rafferty et al., 1990). Conversely, neither the histamine H_2 receptor antagonist, cimetidine, nor the histamine H_3 receptor antagonist, thioperamide, inhibited allergic cough. Likewise, histamine H_1, but not histamine H_2 or H_3 receptor antagonists inhibited capsaicin-induced cough.

It is possible that the antitussive effects of histamine H_1 receptor antagonists are due to other activities of these drugs, such as cholinergic antagonism. Loratadine shows anticholinergic activity in the guinea pig (Kreutner et al., 1987), but at doses (10 mg kg^{-1} p.o.) much higher than those necessary to inhibit responses to histamine ($ED_{50} = 0.2$ mg kg^{-1}) or allergic cough. The dose of loratadine necessary to inhibit capsaicin-induced cough was in the range of the anticholinergic activity of this compound. However, chlorpheniramine has no anticholinergic activity in the guinea pig at high doses (Tozzi et al., 1974), but this histamine H_1 receptor antagonist inhibited both allergic and capsaicin-induced cough. Therefore, our findings are unlikely to be due to anticholinergic activity of the histamine H_1 receptor antagonists. These results indicate that histamine H_1 receptor mechanisms are important in the generation of both allergic and capsaicin-induced cough in the guinea pig.

The mechanism that is traditionally cited for the antitussive effect of β_2-adrenoceptor agonists is bronchodilation (Braman and Corrao, 1987). However, a large amount of recent work has demonstrated that the underlying mechanisms of cough and bronchospasm are different (Forsberg et al., 1992; Karlsson et al., 1988,1992). Furthermore, clinical studies have shown that cough is unaffected by doses of salbutamol that have significant bronchodilating effects (Smith et al., 1991). This is an important finding, because it refutes the notion that bronchodilatation and antitussive activity of β_2-adrenoceptor agonists have a cause and effect relationship. The most likely explanation for the antitussive effects of salbutamol is inhibition of mediator release (Butchers et al., 1980; Karlsson et al., 1988) and/or direct inhibitory effects on sensory afferents responsible for cough (Smith et al., 1991).

The antitussive activity of salbutamol in the present study is also consistent with the known antitussive effect of β_2-adrenoceptor agonists in a model of citric acid-induced cough in the guinea pig (Clay and Thompson, 1985) and in asthmatic patients (Braman and Corrao, 1987; Irwin et al., 1990). The large difference in potency of salbutamol in allergic and capsaicin-induced cough is suggestive of different mechanisms of action of the drug in each model. Salbutamol may inhibit allergic cough by reducing release of pro-tussive mediators from surface mucosal mast cells (Butchers et al., 1980). Conversely, salbutamol may inhibit capsaicin-induced cough by reducing the responsiveness of C-fibers to capsaicin (Engelstad et al., 1992). The extent to which these different mechanisms account for our observations regarding the potency of salbutamol is unknown.

Neither the 5-lipoxygenase inhibitor, phenidone (Blackwell and Flower, 1978), nor the cyclooxygenase inhibitor, indomethacin (Vane, 1971), inhibited allergic cough. Therefore, these mechanisms probably do not contribute to the production of allergic cough. Similarly, phenidone had no effect on capsaicin-induced cough, so 5-lipoxygenase products of arachidonic acid metabolism probably do not contribute to the production of cough in this model. However, indomethacin did slightly inhibit capsaicin-induced cough. The small magnitude and the fact that the response to indomethacin did not increase when the dose of this drug was doubled suggest that this drug may have nonspecific effects on the cough reflex that do not include inhibition of cyclooxygenase products.

In summary, these results show that sensitized guinea pigs exhibit reproducible cough reponses to inhalation of antigen aerosols. Histamine H_1 receptor, but not histamine H_2 or H_3 receptor mechanisms can influence allergic and capsaicin-induced cough in the guinea pig. Cholinergic mechanisms, but not lipoxygenase or cyclooxygenase products of arachidonic acid metabolism, play important roles in the production of allergic and capsaicin-induced cough in the guinea pig. Conversely, ganglionic mechanisms appear to play a role in the production of allergic, but not capsaicin-induced cough.

Acknowledgements

We thank Robert W. Egan and William Kreutner for their thoughtful comments on the manuscript. We also thank Carol Battle for typing the manuscript.

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