

Effects of airway inflammation on cough response in the guinea pig

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Xiang, Anbo, Yoshiyuki Uchida, Akihiro Nomura, Hiroaki Iijima, Fang Dong, Min-Jie Zhang, and Shizuo Hasegawa. Effects of airway inflammation on cough response in the guinea pig. *J. Appl. Physiol.* 85(5): 1847–1854, 1998.—We have developed a guinea pig model for cough related to allergic airway inflammation. Unanesthetized animals were exposed to capsaicin aerosols for 10 min, and cough frequency was counted during this period. The cough evaluation was performed by the following three methods: visual observation, acoustic analysis, and monitoring of pressure changes in the body chamber. These analyses clearly differentiated a cough from a sneeze. To elucidate the relationship between cough response and airway inflammation, animals were immunosensitized and multiple challenged. Sensitized guinea pigs presented no specific changes microscopically, but multiple-challenged animals showed an increased infiltration of inflammatory cells into the airway. Cough number in response to capsaicin increased significantly from 4.7 ± 1.4 coughs/10 min in normal animals to 10.6 ± 2.0 coughs/10 min in sensitized animals and further to 22.8 ± 1.3 coughs/10 min in multiple-challenged animals. This augmented cough frequency was significantly inhibited by the inhalation of tachykinin-receptor antagonists and by oral ingestion, but not inhalation, of codeine phosphate. The results suggest that airway inflammation potentiates an elevation of cough sensitivity in this model.

tachykinin; asthma; allergic inflammation; C fibers; capsaicin

COUGH IS THOUGHT TO BE ONE of the defense mechanisms of airways (33). This phenomenon is effective in cleaning accumulated secretions of foreign materials from the airway. However, an irritating, nonproductive cough is frequently encountered, especially after viral infection in the upper respiratory tract (5, 25). These cough responses can be caused by mechanical or chemical stimulation, including inflammatory reactions. Such nonproductive coughs serve no useful purpose, and there would be a clinical benefit from eliminating such a cough.

The cough response is thought to be regulated by a cough center in the brain stem that receives impulses from sensory receptors in the larynx or the lower respiratory tract and sends signals to such effector organs as the vocal cord, intracostal muscles, diaphragm, and abdominals (34). Suppressing agents of the central nervous system such as codeine are widely used clinically as antitussive agents to inhibit both persistent and dry cough caused by various pulmonary disorders (10). However, a cough sometimes is caused by airway inflammation like bronchitis or asthma (6, 9). It has recently been determined that tachykinins,

substance P and neurokinin (NK) A and B, are released from nonmyelinated C-fiber endings, playing an important pathophysiological role in airway diseases such as cough (1, 18). In this study, we investigated the relationship between cough response and airway inflammation and examined the effect of tachykinin-receptor antagonists in capsaicin-induced cough to elucidate the mechanism of dry cough.

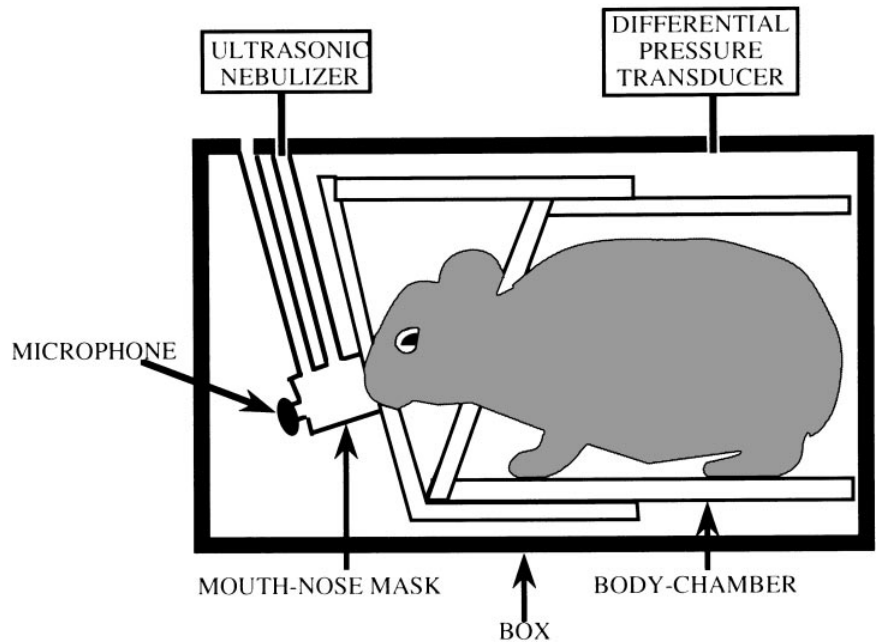
MATERIALS AND METHODS

Animals. Experiments using animals were performed in accordance with the institutional guidelines set forth by the University of Tsukuba Committee on the Use and Care of Animals. Female Hartley guinea pigs weighing 250–300 g (SLC farm, Shizuoka, Japan) were used for the following experiments.

Antigen sensitization and immunochallenge. Animals were sensitized as described previously (28). Briefly, guinea pigs were pretreated with an intraperitoneal (ip) injection of 30 mg/kg cyclophosphamide. Two days later, they were immunized with ovalbumin (OVA) and aluminum hydroxide (1 and 100 mg, respectively, ip). Three weeks after the primary immunization, a booster ip injection of 10 µg OVA together with 100 mg aluminum hydroxide was performed (2). The animals were used 6 wk after the primary immunization as sensitized guinea pigs. Moreover, sensitized guinea pigs were multiple immunochallenged with OVA. The animals were exposed to aerosols of OVA (4 mg/ml saline) for 4 min daily for 7 days through the use of an ultrasonic nebulizer (NEU12, Omron, Tokyo, Japan) at an airflow of 3 ml/min. Diphenhydramine (50 mg/kg ip) was injected 30 min before each OVA challenge to protect the animals from anaphylaxis. They were used 24 h after the last immunochallenge as multiple-challenged animals.

Measurement of cough responses. Animals were individually placed in an airtight box isolated from a body chamber and equipped with a mouth-nose mask and exposed to a nebulized aqueous solution of capsaicin for 10 min (Fig. 1). An ultrasonic nebulizer (NEU03, Omron) produced aerosols containing particles with an aerodynamic mass-median diameter of 5.0 µm (manufacturer's specification) at 0.25 ml/min. During the exposure, the animal was continuously observed by two trained observers, who counted the number of coughs. All movements of the guinea pigs were categorized on the basis of the observations: both observers confirmed that a movement was a cough; one observer confirmed that there was a cough, but the other did not; or both agreed that the movement was something other than a cough, such as a sneeze. To evaluate the cough response objectively, we further analyzed typical cough responses, as agreed on by two observers, by using two other methods: 1) pressure changes in the box were recorded with a chart recorder with a differential-pressure transducer (model TP-602T, Nihon Kohden, Tokyo, Japan) and 2) cough sounds were picked up concurrently and amplified via a microphone (EE-24, Fcz,

Fig. 1. System of cough monitoring.



Tokyo, Japan), which detected frequencies from 10 Hz to 12 kHz, placed in front of the mouth-nose mask. The sound waves were analyzed by a personal computer by using SoundEditTM16 2J software (Macromedia, Tokyo, Japan). The determinations of cough by both the acoustic-analysis method and box-pressure changes completely matched each other. Atypical cough responses, those for which the two observers disagreed in their determinations, were also analyzed by the acoustic analysis method and by box-pressure changes. In such cases, these methods also resulted in a complete match. Therefore, cough counting was performed by using the above-mentioned three methods in the following experiments.

Physiological evaluation of cough. The duration of exposure to capsaicin for the experiments was determined by the following observations. Most of the coughs in the 10 guinea pigs that were multiple challenged were observed within 10 min; $16.7 \pm 5.2\%$ of total coughs were observed within 2.5 min, $\sim 58.8 \pm 10.5\%$ were observed within 5.0 min, and $92.28 \pm 2.4\%$ were observed within 7.5 min. When animals were exposed to 10^{-5} M capsaicin for 30 min, most of the coughs were observed within 10 min. Therefore, an exposure time of 10 min was selected for the following experiments.

Unanesthetized guinea pigs were exposed to an aerosol of capsaicin solution from 10^{-11} to 10^{-4} M. Each guinea pig received only one concentration. In all experiments, once a guinea pig was exposed to capsaicin, it was never reused in subsequent experiments. The effects on capsaicin-induced cough of FK-888 and SR-140333, antagonists of the NK₁ receptor, FK-224, an antagonist of NK₁ and NK₂ receptors, and SR-48968, an antagonist of the NK₂ receptor, were examined. The animals were pretreated with a 5-min inhalation of a FK-888 or FK-224 solution for 30 min before the capsaicin treatment. SR-140333 and SR-48968 were administered ip to animals at 30 min before the capsaicin treatment. The effects of codeine, an antitussive agent, on capsaicin-induced cough responses were investigated. Codeine phosphate was administered ($5 \mu\text{mol/kg}$ by oral administration) 1 h before capsaicin exposure or by inhalation (2.5×10^{-4} M, 5 min) 10 min before the capsaicin challenge.

Measurement of specific airway conductance. Specific airway conductance (sGaw), as an index of airflow limitation,

was measured 5 min after exposure to each concentration of capsaicin by the method described previously (28). Briefly, the values of sGaw were determined as the slope in an x - y plot of airflow and box-volume change calculated from changes in the box pressure. An average of slopes in five respiratory cycles was used for the calculation. Airflow and box-pressure signals were monitored with a pneumotachograph (TV-241, Nihon Kohden) and a differential pressure transducer, respectively.

Evaluation of airway inflammation by light microscopy. Airway inflammation was assessed by histological examinations and an analysis of lavaged cells. For the preparation of tissues, guinea pigs were anesthetized with pentobarbital sodium (50 mg/kg ip). Multiple-immunochallenged guinea pigs were used 24 h after the last immunochallenge. Immediately after the trachea and lungs were removed en bloc, phosphate-buffered 10% Formalin (pH 7.4) was instilled into the lungs through the trachea at a pressure of 20 cmH₂O. After fixation for 48 h, tissues were mounted in paraffin and 2- μm sections were then stained with hematoxylin and eosin for light-microscopic examinations.

For tracheobronchial lavage, the trachea was cannulated with a 16-gauge tube, with the animal under anesthesia with pentobarbital sodium (50 mg/kg ip). One milliliter of ice-cold saline was instilled three times, and the recovered fluid was collected and centrifuged at 150 g for 10 min at 4°C. The pellet was resuspended in 1 ml of saline to count the total cell number with a hemocytometer. The rest of the fluid was applied to a Cytospin 3 (Shandon, Pittsburgh, PA). Cytospin preparations were stained with hematoxylin and eosin for cell differentials.

Preparation of drugs. Capsaicin (Sigma Chemical, St. Louis, MO) was dissolved in saline containing 10% ethanol and 10% Tween 80 to give stock solutions of 10^{-2} M, which were stored at 4°C. FK-888/FK-224 (kind gifts from Fujisawa Pharmaceutical, Osaka, Japan) were dissolved in DMSO to give a 0.1 M stock solution, which was stored at 4°C. SR-46968/SR-140333 (kind gifts of Sanofi Recherche, Cedex, France) were dissolved in ethanol to give a 20 mM stock solution, which was stored at 4°C. These stock solutions were diluted in saline as necessary for each experiment. All other

drugs were obtained from Wako Pure Chemicals (Osaka, Japan) and dissolved in saline.

Statistical analysis. Results are represented as means \pm SE. A statistical analysis was performed among three groups or between two groups. The data from three groups were analyzed by Bartlett test and then for statistical significance by one-way ANOVA and a post hoc Bonferroni test; the differences between the two groups were evaluated by an *F*-test and then by an unpaired Student's *t*-test. Significant differences were accepted when $P < 0.05$.

RESULTS

With regard to coughs and sneezes observed in multiple-challenged guinea pigs, an agreed rate for the two observers was 87.3 ± 1.1 (SE) % ($n = 10$). Typical traces of box-pressure changes and sound records of coughs and sneezes are shown in Fig. 2. Box-pressure changes in the cough response started with a small positive pressure, followed by a burst of negative pressure and then a small positive pressure. In the case of sneezing, pressure changes began with a large positive pressure, which then changed to a negative

pressure (Fig. 2A). In the sound records, the maximal sound pressure for both the coughs and sneezes was ~ 80 dB. For a cough, the sound pressure reached a maximal level immediately after the start of the cough and then diminished gradually. On the other hand, the sound of sneezing consisted of a two-part sound wave, which began as a crescendo wave and paused for ~ 0.03 s and then was followed by a second wave. The average duration of a cough was 0.12 s, whereas that of a sneeze was 0.20 s (Fig. 2B). The range of the frequency of a cough sound was from 0.5 to 8 kHz, and its peak was ~ 1.5 kHz (Fig. 2C). In contrast, a sneezing sound was distributed over a range of 0.01–10 kHz, and its peak was in the range of 3.5–6.5 kHz. These two responses could easily be differentiated by their characteristic waves patterns, and the observations of these monitorings were nearly matched by the visual observation.

To determine the concentration of capsaicin that induces cough, normal, sensitized, and multiple-challenged guinea pigs were exposed to an aerosol of capsaicin solution in concentrations from 10^{-11} to 10^{-4}

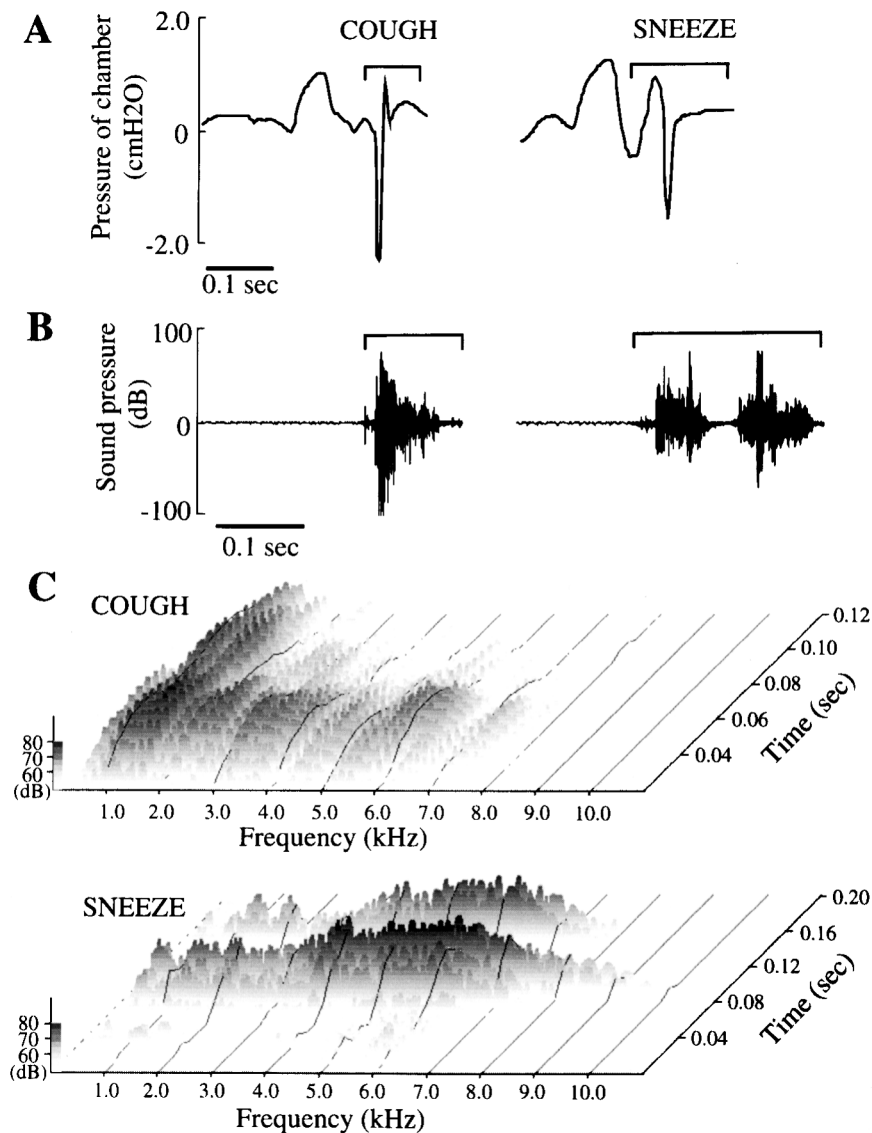
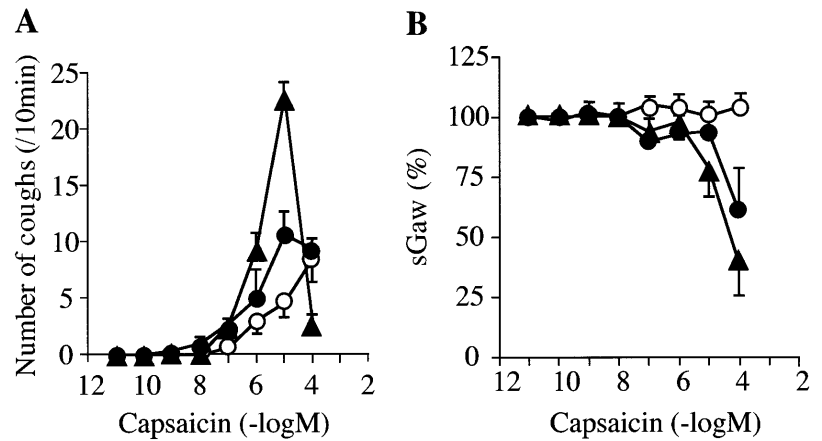


Fig. 2. Comparison between cough and sneeze induced by capsaicin in the guinea pig. *A*: changes in chamber pressure. *B*: changes in sound pressure. *C*: characteristic sound waves.

Fig. 3. Capsaicin aerosol-induced cough responses (A) and specific airway conductance (sGaw) changes (B) in normal (○), sensitized (●), and multiple-challenged guinea pigs (▲). Values are means \pm SE; $n = 5$ guinea pigs were used at each capsaicin concentration.



M (Fig. 3). Animals in each group showed an increase in cough frequency in a dose-dependent manner from 10^{-10} to 10^{-5} M capsaicin, and a statistical difference ($P < 0.05$) among these groups was observed with the inhalation of 10^{-5} M capsaicin solution: 4.7 ± 1.4 in the normal group; 10.6 ± 2.0 in the sensitized group; and 22.8 ± 1.3 in the multiple-challenged group (Fig. 3A). However, in the case of the 10^{-4} M solution, multiple-challenged animals showed a significant decrease in the number of coughs, compared with those exposed to the 10^{-5} M solution. To evaluate the influence of bronchoconstriction induced by capsaicin, we monitored sGaw as an index of airflow limitation during capsaicin inhalation. Significant changes in sGaw were not observed from 10^{-11} to 10^{-5} M, but at 10^{-4} M capsaicin, the sGaw of multiple-challenged animals significantly decreased to $40 \pm 14\%$. A concentration of 10^{-5} M capsaicin also seemed to inhibit the sGaw in multiple-challenged guinea pigs, but there was no statistical difference (Fig. 3B). From these data, we could not determine whether slight airflow limitations potentiate cough responses, but the results did lead us to speculate that severe bronchoconstriction causes a decrease in the number of coughs. Moreover, from the data showing that the dose dependency of cough numbers shifted to the left compared with that of sGaw, it is possible that capsaicin can induce cough without severe bronchoconstriction at concentrations of 10^{-5} M. Therefore, capsaicin at a concentration of 10^{-5} M was used as an inducer of cough in further studies.

To elucidate the interaction of cough response and airway inflammation, guinea pigs were immunosensitized and multiple challenged by OVA inhalation. Microscopically sensitized animals did not show any specific changes, but multiple-challenged animals showed a distinct increased infiltration of inflammatory cells into the epithelium (Fig. 4). Moreover, analysis of the tracheobronchial lavage fluid revealed that in multiple-challenged animals inflammatory cells such as eosinophils were significantly increased compared with in normal and sensitized animals (Table 1). These results indicate that the multiple-challenged guinea pig is a suitable model for airway inflammation.

On the other hand, oral administration of codeine phosphate, which has been used widely and clinically

as an antitussive agent acting on the central nervous system, suppressed cough numbers in both sensitized and multiple-challenged groups, but inhalation of this compound did not influence cough responses (Fig. 5). Thus it appears that codeine cannot act locally in the airway.

We examined the effects of the tachykinin-receptor antagonists FK-888 and SR-140333 (NK_1 antagonists), FK-224 (an NK_1 - and NK_2 -receptor antagonist), and SR-48968 (an NK_2 antagonist) on capsaicin-induced cough (Table 2). Pharmacological characteristics of those compounds are listed in Table 3. All antagonists inhibited capsaicin-induced coughs in a dose-dependent manner. Notably, SR-48968 inhibited coughs in all guinea pigs more than did SR-140333, indicating that capsaicin-induced cough is mediated via an NK_2 receptor. Otherwise, both FK-888, as an NK_1 -receptor antagonist, and FK-224, as an NK_1 - and NK_2 -receptor antagonist, inhibited the increase in cough frequency in the sensitized and the multiple-challenged groups. These

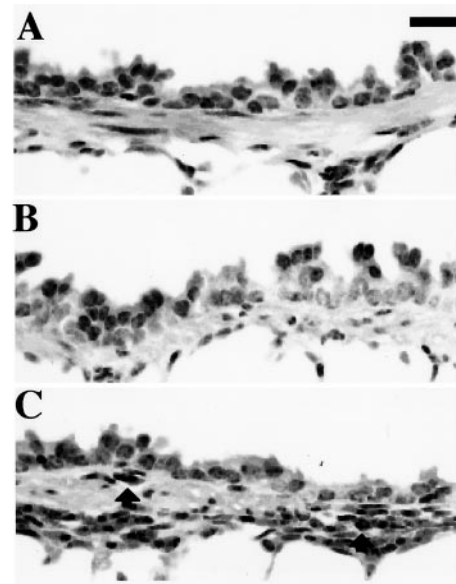


Fig. 4. Hematoxylin and eosin staining of bronchial wall in normal (A), sensitized (B), and multiple-challenged guinea pigs (C). Arrows in C show a distinct increase in infiltration of eosinophils into the epithelium. Scale bar = 20 μ m.

Table 1. Total lavage cells and macrophages, eosinophils, lymphocytes, and neutrophils in tracheobronchial lavage fluid of normal, sensitized, and multiple-challenged guinea pigs

	Normal	Sensitized	Multiple Challenged
Total cells	10.09 ± 1.41	18.73 ± 1.55	76.23 ± 11.28 ^{c,f}
Macrophages	8.79 ± 1.54	13.53 ± 1.64	19.46 ± 3.34 ^a
Eosinophils	1.19 ± 0.50	5.04 ± 1.30	55.80 ± 8.02 ^{c,f}
Lymphocytes	0.07 ± 0.02	0.11 ± 0.04	0.45 ± 0.07 ^{b,e}
Neutrophils	0.04 ± 0.01	0.05 ± 0.01	0.52 ± 0.15 ^{b,d}

Values are means ± SE expressed in $\times 10^5$; $n = 5$ guinea pigs/group. ^a $P < 0.01$, ^b $P < 0.005$, and ^c $P < 0.0001$ vs. normal group. ^d $P < 0.005$, ^e $P < 0.0005$, and ^f $P < 0.0001$ vs. sensitized group.

two antagonists were administered by inhalation, whereas SR compounds were intraperitoneally given. Because of these differences in the routes of administration, it has not yet been determined which tachykinin receptor is responsible for capsaicin-induced cough. However, these observations indicate that the induction of sensitization or immunochallenge could induce tachykinin release in the airway and consequently implicate the tachykinins responsible for dry cough.

DISCUSSION

It is difficult to accurately quantify the number of coughs in a conscious guinea pig because atypical coughs are not easily distinguishable from sneezes. It is therefore necessary to establish a method of scientific analysis and quantification. Forsberg et al. (12) has reported that the sound of coughs can be differentiated from that of sneezes by a microphone, tape recorder, and loudspeaker system. Korpas et al. (23) and Thorpe et al. (27) have performed a sound analysis of coughs in cats and in humans. However, differences in the sound waves of coughs and sneezes in the guinea pig have not yet been reported. In the present study, we have devised a novel method for the qualitative and quantitative monitoring of cough in guinea pigs. We have obtained the objective characteristics of a cough and

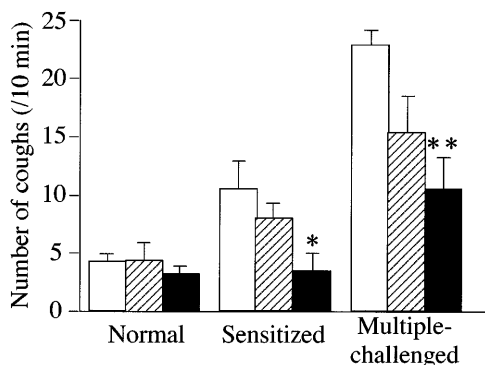


Fig. 5. Effect of codeine on capsaicin-induced cough in normal, sensitized, and multiple-challenged guinea pigs. Codeine phosphate was administered by inhalation (hatched bars, 2.5×10^{-4} M, 5 min) 10 min before 10^{-5} M capsaicin challenge or by oral administration (solid bars, 5 μ mol/kg) 1 h before 10^{-5} M capsaicin exposure. Open bars, vehicle. Values are means ± SE; $n = 4$ and $n = 6$ in vehicle and others, respectively. * $P < 0.05$ and ** $P < 0.01$ vs. corresponding vehicle.

sneeze by acoustic and box-pressure recordings. Inside the box, an inhalation produces a positive pressure, and an exhalation, a negative pressure. In a cough, a box-pressure change is characterized by a sudden, deep negative pressure followed by a small positive pressure, and sound recordings showed a decrescendo-shaped wave. These recordings reflected the quick expiration and the maximal cough-sound pressure at the glottal opening (37). These changes agree with reports that the entry of foreign material into the laryngeal approaches might trigger an immediate cough without previous inspiration (24, 37). In contrast, on the basis of box-pressure measurements, a sneeze is characterized by a strong positive pressure and then a large negative pressure. In sound-pressure recordings, a sneeze consists of two-part sound waves, reflecting the deep inspiration and powerful expiration of a sneeze (32). Therefore, we considered that both acoustic analysis and box-pressure changes could be used for the detection of real coughs. Among the three methods, there was no disagreement in the results for typical coughs. For atypical coughs for which we could not judge except by observation, both acoustic recording and the monitoring of box-pressure changes were helpful in making a final determination.

Exposure to an aerosol of capsaicin, which is a powerful tussigenic agent in humans and other species, has been extensively used as a standard method for eliciting cough (34). Capsaicin can act on both rapid-adapting receptors and C-fiber receptors, with the release of tachykinins from the latter that, in turn, further stimulates rapid-adapting receptors, which causes a cough response (34). In this study, we used capsaicin as a tussigenic agent to determine the sensitivity of the peripheral cough receptor in the guinea pig. The inhalation of capsaicin solution brought about an increase in cough number in a dose-dependent manner, but, at the highest concentration of 10^{-4} M capsaicin, the cough responses were reduced and bronchoconstriction could be detected. Karlsson et al. (20) have determined that cough and bronchoconstriction can be produced individually and separated pharmacologically, and in guinea pigs destruction of airway C-fiber receptors by large doses of capsaicin abolishes the cough reflex due to capsaicin (19). Therefore, we speculated that doses of capsaicin lower than 10^{-4} M may only elicit cough but not bronchoconstriction, and high doses of 10^{-4} M capsaicin may destroy the airway C-fiber receptor so that decreases in the cough reflex are due to capsaicin without any bronchoconstrictive effects.

Tachykinins are believed to be neurotransmitters in the peripheral and central nervous system (16). They are released from peripheral endings of capsaicin-sensitive C fibers in the airway and have a variety of inflammatory effects in the airway (13). Recently, antagonists of NK₁, NK₂, and NK₃ receptors have been extensively reported to be effective against cough (1, 4, 17, 29), but the sites of their antitussive action are still debatable. Our results indicate that the inhalation of FK-888 and FK-224 (antagonists of NK₁ and NK₁/NK₂ receptors, respectively) inhibit capsaicin-induced coughs

Table 2. Effects of tachykinin antagonists on capsaicin-induced cough in guinea pigs

Drug/Concentration or Dose	Route of Administration	Cough, no./10 min		
		Normal	Sensitized	Multiple challenged
Vehicle	Inhalation	3.75 ± 1.71 (4)	11.00 ± 1.47 (4)	20.25 ± 1.47 (4)
FK-888				
10 ⁻⁵ M	Inhalation	3.83 ± 1.50 (6)	4.66 ± 1.15 ^a (9)	8.33 ± 1.18 ^b (6)
10 ⁻⁶ M	Inhalation		6.75 ± 2.02 (4)	
FK-224				
10 ⁻⁵ M	Inhalation	3.67 ± 0.71 (6)	4.13 ± 1.79 ^a (8)	8.89 ± 1.30 ^b (9)
10 ⁻⁶ M	Inhalation		6.50 ± 1.19 (4)	
Vehicle	ip	4.52 ± 1.41 (4)	10.37 ± 2.66 (4)	22.65 ± 1.31 (4)
SR-140333				
1.30 µmol/kg	ip	2.67 ± 0.49 (6)	6.17 ± 0.70 (6)	16.00 ± 0.91 ^d (6)
0.13 µmol/kg	ip		7.67 ± 2.72 (3)	
SR-48968				
0.50 µmol/kg	ip	0.83 ± 0.31 ^c (6)	3.83 ± 1.11 ^c (6)	10.20 ± 1.77 ^e (5)
0.17 µmol/kg	ip		5.33 ± 1.20 (3)	
0.05 µmol/kg	ip		6.00 ± 1.53 (3)	
0.017 µmol/kg	ip		7.00 ± 2.08 (3)	

Values are means ± SE with no. of animals in parentheses. ^a $P < 0.01$, ^b $P < 0.001$ vs. corresponding vehicle control inhalation. ^c $P < 0.05$, ^d $P < 0.01$, and ^e $P < 0.001$ vs. corresponding ip vehicle.

in OVA-sensitized and multiple-challenged guinea pigs, and both antagonists have the same antitussive activity. These data regarding the effects of FK-888 and FK-224 are in agreement with the results obtained in guinea pigs by others (1, 17, 29), who have shown that FK-888 and SR-48968 inhibit coughs induced by citric acid. Their results suggest that tachykinin-receptor antagonists do not cross the blood-brain barrier and that the site of their antitussive action is restricted to the periphery. Our results, however, are in conflict with reports of Bolser et al. (4), who have shown that tachykinin-receptor antagonists inhibit cough induced by mechanical stimulation in cats via an effect in the central nervous system. On the basis of their results, they have suggested that central action of tachykinin-receptor antagonists cannot be excluded, at least in areas not protected by the blood-brain barrier. It must be noted that these antagonists have significant effects in the central nervous system only when administered by either intravenous or intravertebral arterial routes. As such, our data showing that SR-48968 is more potent than SR-140333 in inhibiting capsaicin-induced coughs are interesting. Because both compounds were administered via an intraperitoneal route, it was therefore not possible to determine that NK₂ was the peripheral tachykinin receptor responsible for the capsaicin-

induced cough. We can conclude, however, that the site of action for the NK₁ and NK₂ tachykinin-receptor antagonists in our model is in the airway and that the observed cough responses are mediated by tachykinins released as a result of capsaicin exposure. Furthermore, treatment with sensitization and immunochallenge potentiates the release of tachykinins into the airway. These observations correspond with a previous report suggesting that antigenic stimulation potentiates tachykinin-release from afferent fibers in the guinea pig isolated airway (31). Although FK-888, FK-224, and SR-140333 could not significantly inhibit capsaicin-induced coughs in normal animals, SR-48968 could suppress the responses, indicating that even a few coughs observed in normal animals could be related to tachykinins.

In this study, the correlation between cough response and airway inflammation was examined. The multiple-immunochallenged guinea pig exhibited chronic airway inflammation, which was observed as an infiltration of inflammatory cells into the epithelium and submucosa (11, 22) and as an increase in the inflammatory cells in bronchoalveolar lavage fluid. Chronic inflammation leads to hyperesthesia and to a lowering of the threshold of activation of the sensory nerve and an increase in the number of substance P-immunoreactive nerves (3). In addition, inflammatory cells can produce a number of chemical mediators, such as thromboxanes and prostaglandins, which contribute to airway inflammation (8, 35), in which condition the relationship between thromboxane and angiotensin-converting enzyme inhibitor-induced cough has been studied (30). Our results for OVA-challenged animals having a hyperreactive cough reflex demonstrate the correlation between cough response and airway inflammation.

Interestingly, in the present study an increase in the number of coughs occurred not only in the multiple-immunochallenged guinea pigs but also in the sensitized guinea pigs. The reason for this phenomenon is unclear. From the capsaicin dose-response curve, it

Table 3. Comparison of IC₅₀ of NK-receptor antagonists

Compound	IC ₅₀ , nM*		Specificity	Ref. No.	Assay Method
	NK ₁	NK ₂			
FK-224	1,690	1,860	NK ₁ and NK ₂ antagonist	26	Binding assay
FK-888	0.64	11,000	NK ₁ antagonist	14	Bioassay
SR-48968	1,100	0.23	NK ₂ antagonist	21	Binding assay
SR-140333	0.82	>10,000	NK ₁ antagonist	36	Binding assay

NK₁ and NK₂, neurokinin receptors. *Values were obtained from experiments of receptor-binding assay and bioassay as shown in respective references.

seems likely that sensitization lowered the threshold of the sensory nerve to capsaicin. On the other hand, although there were no significant changes in the total cell number and the eosinophils in sensitized and normal animals, we could observe a tendency toward elevation of these numbers in sensitized animals. Several studies have reported that OVA sensitization can selectively alter the coupling of β -adrenergic and vasoactive intestinal peptide receptors to adenylate cyclase in the lung membrane (15) and alter the intestinal mucosa to produce an immediate secretory response (7). Therefore, we speculate that immunosensitization may facilitate the release of tachykinins from C-fiber endings by single stimulation of capsaicin. Tachykinins then stimulate rapid-adapting receptors, causing a strong cough response. This could also explain why FK-888 and SR-48968 block this increase in the number of coughs in sensitized animals.

In conclusion, we have established a novel method for accurately quantifying the number of coughs, excluding sneezes, and have developed a cough model. In this model, the site of action of the tachykinin-receptor antagonists is in the airway. Our study suggests that the capsaicin-induced cough is potentiated by airway inflammation. Further study is necessary to determine how neurokinins are released in normal, sensitized, and multiple-challenged guinea pigs.

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