
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Gen. Pharmac. Vol. 30, No. 1, pp. 109–114, 1998



ISSN 0306-3623/98 $19.00 + .00
PII S0306-3623(97)00084-0


# A New Pharyngitis Model Using Capsaicin in Rats

*Mutsumi Yamabe,*[1]
*Tomokazu Hosokawa,*[2] *Teruro Taoka*[1] *and Miwa Misawa*[2]*
[1]Department of Research and Development,
Ryukakusan Co., Ltd., Katori-gun, Chiba 289-22, Japan;
and [2]Department of Pharmacology, School of Pharmacy, Hoshi University,
2-4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan [Tel: 81-3-5498-5784; Fax: 81-3-5498-5787]

**ABSTRACT. 1. Application of capsaicin solution onto the rat pharyngeal mucosa caused a well-reproducible increase in vascular permeability in the pharynx.**

**2. Capsaicin-induced pharyngeal inflammation was unaffected by a histamine $H_1$ blocker and non-steroidal anti-inflammatory agents, whereas dexamethasone was effective in its inhibition.**

**3. FK224, a dual antagonist of tachykinin $NK_1$ and $NK_2$ receptors, and FK888, a selective antagonist of $NK_1$ receptor, significantly inhibited capsaicin-induced plasma exudation in the pharynx.**

**4. In capsaicinized animals, the application of capsaicin solution in the pharyngeal mucosa did not induce pharyngitis.**

**5. These results suggest that the mechanism of the capsaicin-induced pharyngitis primarily involves tachykinins.** GEN PHARMAC 30;1:109–114, 1998. **© 1998 Elsevier Science Inc.**

**KEY WORDS.** Pharyngitis, capsaicin, anti-inflammatory drugs, tachykinin receptor, rat

## INTRODUCTION

The pharynx is always exposed to various external stimuli through nasal and oral cavities. Pharyngitis is a common inflammatory syndrome of the oropharynx which is attributed predominantly to infectious causes or, less commonly, to secondary involvement of systemic or noninfectious illness. The complaint of pharyngitis is frequently encountered and is responsible for an estimated 40 million outpatient visits in the United States (Vukmir, 1992). Irritation and sore throat accompanied by mild edema and erythema of the pharynx are present in about 80% of patients with the common cold syndrome due to viruses such as rhinoviruses, coronaviruses, influenza viruses, parainfluenza viruses and adenoviruses, and to bacteria including *Streptococcus pyogenes* (Lang and Singh, 1990; Peter, 1992). However, suitable animal models for studying pharyngitis and for development of effective drugs have not hitherto been reported.

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), the pungent algesic ingredient in red peppers, activates a population of primary afferent sensory neurons and causes the release of neuropeptides such as substance P (SP) and neurokinin A (NKA) (Buck and Burks, 1986). Capsaicin-sensitive nerves are recognized to distribute to a variety of tissues including the lower respiratory tract (Buchan and Adcock, 1992) and nasal mucosa (Tani *et al.*, 1990). Capsaicin has been used for inducing coughs in animals to assess antitussive drugs (Kamei *et al.*, 1994) and for inducing bronchoconstriction to study cholinergic and axon reflexes in the respiratory tract (Buchan and Adcock, 1992).

In the present article, a new animal model for the study of pharyngitis was used. Capsaicin was applied onto rat pharyngeal mucosa, and the effects of several drugs on the capsaicin-induced plasma exudation in the pharynx were investigated.

*To whom correspondence should be addressed.
Received 14 September 1996; revised 31 January 1997; accepted 17 February 1997.

## MATERIALS AND METHODS

### *Establishment of animal model of pharyngitis*

Male Wistar rats, weighing 260–360 g (Tokyo Laboratory Animal Co., Japan), were used. Animals were anesthetized with urethane (2 g/kg SC), placed in the supine position and given spontaneous respiration through a tracheal cannula. Experimental pharyngitis was induced by application of capsaicin solution with a moistened cotton-tipped applicator on the surface of pharyngeal mucosa. When the capsaicin solution was applied, the tongue was slightly pulled out with a forceps and the pharynx area was opened deep in the oral cavity with a small rib spreader. Capsaicin-soaked cotton was swabed each for about 3 sec gently three times at one time point (about 50 μl of the capsaicin solution left on the mucosa). Because capsaicin was dissolved in a mixture of 10% ethanol–10% Tween 80–80% distilled water, the rats in the control group were given vehicle alone. Capsaicin in concentrations of 0.1, 0.3 and 1.0 mM was used, and each concentration of the capsaicin solution was applied twice with a 30-min interval. In some experiments, capsaicin solution (0.3 mM) was applied only once, and a period of 30 or 60 min was allowed before evaluation of plasma exudation. For a quantitative evaluation of the capsaicin-induced plasma exudation in the rat pharyngeal mucosa, extravasation of Evans blue (EB) dye into the pharyngeal tissue was determined. Specifically, EB dye (30 mg/kg) was injected IV into the femoral vein 10 min prior to the first application of capsaicin. Thirty minutes (in some experiments, 60 min) after the last capsaicin application, exsanguination was done from the abdominal aorta. Each animal's head portion was perfused at a rate of 15 ml/min with 180 ml of citric acid buffer (5% of paraformaldehyde in 0.05 M sodium citrate solution adjusted to the pH 3.5 with 0.05 M citric acid solution) via the bilateral carotid arteries to expel the intravascular dye, with the perfused buffer being eliminated from an incision of the right atrium. Then, the bilateral musculus masseter of the rat was incised and the lower jaw was removed to enable extirpation of the pharynx. The pharynx was isolated by separation from the esophagus and trachea, and removal of the soft palate, tongue, larynx and nasal tissues; the isolated pharynx con-

tained the portion ranging from the caudal end of the soft palate to the epiglottis, just at the beginning of the larynx, and weighed 35–55 mg. EB dye in the tissue was extracted in formamide at 55°C for 24 hr and determined spectrophotometrically at 620 nm. The tissue dye content was expressed as micrograms of dye per gram of wet weight of tissue.

### *Effects of several drugs on pharyngeal plasma exudation induced by capsaicin*

To study the involvement of chemical mediators in capsaicin-induced pharyngeal plasma exudation, rats were injected iv with several agents into the femoral vein 5 min (or 30 min in the cases of dexamethasone and glycyrrhizinic acid) prior to capsaicin treatment. As pretreatment drugs, chlorpheniramine (1 mg/kg, histamine $H_1$ receptor antagonist), indomethacin (5 mg/kg), diclofenac (10 mg/kg; cyclooxygenase inhibitor), dexamethasone (0.3 mg/kg, steroidal anti-inflammatory drug), glycyrrhizinic acid (10 mg/kg), FK224 (1 and 3 mg/kg, tachykinin $NK_1$ and $NK_2$ receptor antagonist) and FK888 (1, 3 and 10 mg/kg, tachykinin $NK_1$ receptor antagonist) were given. Control rats received vehicle or saline instead of drugs.

### *Systemic capsaicinization*

In some experiments, rats were pretreated with systemic capsaicin to deplete sensory neuropeptides. Capsaicinization was done by using the method of Yonei *et al.* (1990) with a minor modification. Animals were administered subcutaneously with a total dose of 125 mg/kg capsaicin, in two increasing doses over 3 days, and used for the experiments 10 days after the last systemic capsaicin pretreatment. Each dose was made as follows: at day 1, 25 mg/kg; at days 2 and 3, 50 mg/kg. Capsaicin was dissolved in 10% ethanol–10% Tween 80–80% saline.

To counteract the respiratory impairment caused by systemic capsaicin, terbutaline (1 mg/kg SC, 60 min prior) and aminophylline (25 mg/kg IP, 30 min prior) were pretreated before each capsaicin treatment under anesthesia with ketamine (50 mg/kg SC) and thiopental sodium (40 mg/kg IP). The same dosage regimen was used in the control animals to deliver an equivalent volume of vehicle (10% ethanol and 10% Tween 80 in saline) without capsaicin. To check the effectiveness of the capsaicinization, a drop of a 0.1-mg/ml solution of capsaicin in saline was instilled into one eye of the rat. This test was carried out 3 hr before the experiment. The untreated or vehicle-pretreated rats responded instantly with wiping of the eye, whereas the capsaicin-pretreated rats did not.

### *Drugs*

The following drugs were used: capsaicin, citric acid, sodium citrate, sodium carbonate, Tween 80, dimethylsulfoxide and formamide (Wako Pure Chemical Industries, Osaka, Japan); Evans blue (EB; Merck); chlorpheniramine maleate, indomethacin, diclofenac sodium and urethane (Sigma); dexamethasone (dexamethasone sodium phosphate for intravenous infusion, Banyu Pharmacy Co.); glycyrrhizinic acid (glycyrrhizinate dipotassium, Tokiwa Plant Chemistry Research Co., Japan); and FK224 and FK888 (Fujisawa Pharmaceutical Co., Osaka). Indomethacin was dissolved in saline by adjusting to pH 7 with 1N sodium carbonate, and FK224 and FK888 were dissolved in dimethylsulfoxide (DMSO). The other drugs were dissolved and/or diluted in saline.

### *Statistical analyses*

All data are expressed as mean and SEM. Statistical significance was determined by Student's *t*-test for unpaired data, Cochran–Cox test or one-way analysis of variance (ANOVA). ANOVA was carried out with Tukey's test for multiple comparisons.

## RESULTS

### *Application timing and concentrations of capsaicin solution*

Single or double applications of capsaicin at 0.3 mM on the surface of pharyngeal mucosa were attempted as shown in Figure 1. The increase in EB exudation was assessed 30 or 60 min after local application of capsaicin. EB exudation in the pharynx was significantly increased at 60 min after the single application (group B) or at 30 min after double application of capsaicin at a 30-min interval (group C) ($P<0.01$ vs. vehicle) (Fig. 1). The maximal percentage increase in EB exudation was observed in group C; the increase percentage versus vehicle control was 82.8%. As for the concentration of capsaicin, 0.1, 0.3 and 1.0 mM of the agent (about 50 μl) was applied on the surface of pharynx (Fig. 2). Capsaicin elicited a concentration-dependent increase in EB extravasation in the rat pharyngeal tissue, and the increase was significant at 0.3 and 1.0 mM ($P<0.01$ vs. vehicle). Based on these findings, induction of pharyngeal inflammation was performed by two applications with a 30-min interval with the capsaicin concentration of 0.3 mM in subsequent experiments.

### *Effects of several agents on capsaicin-induced pharyngeal plasma exudation*

The influences of various agents on the enhanced pharyngeal plasma exudation by twice application of 0.3 mM capsaicin are summarized in Table 1. Chlorpheniramine (1 mg/kg IV), histamine $H_1$ blocker, indomethacin (5 mg/kg IV), diclofenac (10 mg/kg IV), cyclooxygenase inhibitors and glycyrrhizinic acid (10 mg/kg IV), triterpenoid sapogenin from *Glycyrrhiza glabra L.* possessing an anti-inflammatory effect (Finney and Somers, 1958) were without effect on the capsaicin-induced increase in EB exudation. In contrast, dexamethasone (0.3 mg/kg IV), steroidal anti-inflammatory drug, significantly inhibited the increase in vascular permeability induced by capsaicin (45.7%, $P<0.05$ vs. control). FK224 (1 and 3 mg/kg IV), a tachykinin $NK_1$ and $NK_2$ receptor antagonist, significantly inhibited the capsaicin-induced increase in EB exudation in a dose-dependent manner (63.9% and 82.4% inhibition, respectively; $P<0.01$ vs. vehicle) (Fig. 3). FK888 (1, 3 and 10 mg/kg IV), a tachykinin $NK_1$ receptor antagonist, also significantly inhibited the increased plasma exudation at a dose of 10 mg/kg (66.4%, $P<0.05$ vs. vehicle) (Fig. 4).

### *Effect of sensory neuropeptides depletion on the capsaicin-induced pharyngeal plasma exudation*

To deplete sensory neuropeptides such as SP and NKA, systemic capsaicinization (total capsaicin dose of 125 mg/kg SC) was performed, as described in Materials and Methods. The capsaicin-induced increase in EB exudation was significantly attenuated by systemic capsaicinization ($P<0.01$ vs. sham treatment group) (Fig. 5).

## DISCUSSION

Pharyngitis is an inflammatory syndrome of the oropharynx derived predominantly from infectious causes or, less commonly, from secondary involvement of systemic or noninfectious illness. Cigarette smoking is also sometimes a cause of sore throat. The pathogenesis of infectious pharyngitis is infections from viruses such as rhinovirus, coronavirus and adenovirus, and of bacteria such as *Streptococcus pyrogenes*, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, and

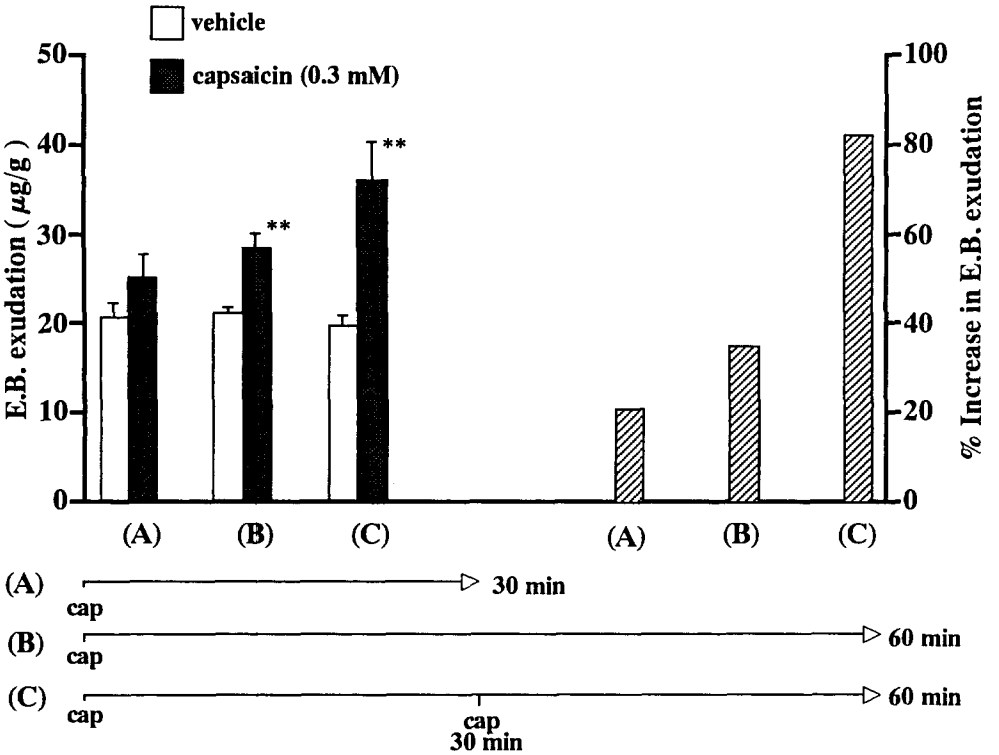


FIGURE 1. The effects of time schedule for capsaicin (cap) application onto the surface of rat pharyngeal mucosa on the amount of extravasated Evans blue dye (micrograms of EB/g wet weight tissue) in the pharynx. (A) EB exudation assessed 30 min after single application of 0.3 mM capsaicin; (B) assessed 60 min after single application of 0.3 mM capsaicin; and (C) assessed after twice applications of 0.3 mM capsaicin at a 30-min interval. White bars: Amount of EB exudation in vehicle control group; shaded bars: capsaicin-treated group. Each column represents the mean with SE from five or six animals. **$P<0.01$ vs. respective vehicle control. Hatched bars: EB exudation expressed as the increase percentage of that in respective vehicle control groups (calculated from the data of the left side of the figure).

*Haemophilus influenza*. Virulence of *Streptococcus* infection is, for example, dependent on locally invasive cellular enzymes such as streptokinase and hyaluronidase (Vukmir, 1992). In our preliminary study, we attempted applications of capsaicin alone and hyaluronidase (400–1500 U/ml) plus capsaicin on the pharyngeal mucosa of the rat. As a result, no significant difference in inducing pharyngeal exudation was observed between groups. Therefore, we used capsaicin alone in the present study to induce experimental pharyngitis. Experimental pharyngitis in laboratory animals has not been reported as far as we know. The oropharynx consists of mucosal membrane layers with stratified squamous epithelium, which protects the mucosa from physical, chemical and thermal stimuli given by food, drink and inspired air. Such a robust oropharyngeal mucosa may be a reason why good models of pharyngitis have not been available so far.

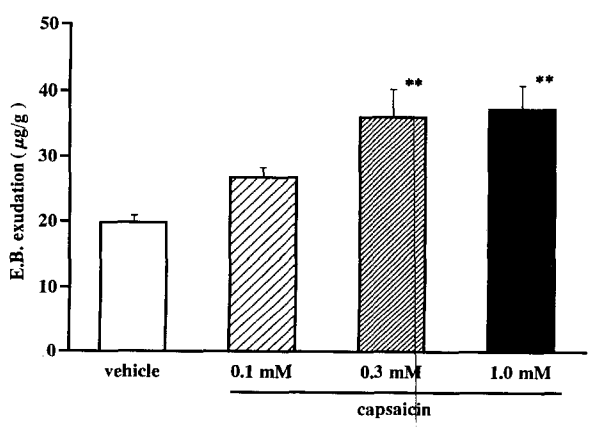


FIGURE 2. The concentration-related effect of capsaicin (0.1, 0.3 and 1.0 mM) on Evans blue (EB) exudation in the rat pharynx. Capsaicin was applied twice with a 30-min interval on the rat pharyngeal mucosa. Each column represents the mean with SE from six or seven animals. **$P<0.01$ vs. vehicle control.

Capsaicin is known to be the prototype of neurogenic irritants. The topical application of capsaicin to rat skin leads to excitation of afferent neurons (Kenins, 1982), increases in skin blood flow (Inoue *et al.*, 1995), and vasodilation (Lynn *et al.*, 1992). Neuropeptides such as SP and NKA, which are released by capsaicin from peripheral endings of afferent neurons, have been considered as chemical mediators of skin inflammation (Holzer, 1988, 1991; Maggi and Meli, 1988; Saria *et al.*, 1988). SP, a tachykinin found in the C-fiber nerve endings of the airways of a variety of species including humans, influences several airway functions; it increases mucus secretion, epithelial chloride secretion, and vascular permeability and stimulates airway smooth muscle contraction (Martling, 1987). In the present experiment, 0.3 or 1.0 mM capsaicin increased vascular permeability of the rat pharynx, reaching a significant effect at 60 min. It has been reported that ear edema developed rapidly and reached a maximum at 60 min after a topical capsaicin application on the surface of mouse ear (40 and 250 μg/ear of capsaicin) (Gabor and Razga, 1992; Inoue *et al.*, 1993). In our experiment, a concentration-dependent enhancement of plasma exudation was observed with increased concentrations of capsaicin (0.1–1.0 mM). The calculated amounts of capsaicin applied onto

**TABLE 1. Effects of several agents on the increase in rat pharyngeal plasma exudation induced by capsaicin**

| Drug | Dose (mg/kg IV) | EB exudation (μg/g tissue) | Inhibition (%) |
|---|---|---|---|
| Control | — | 37.5 ± 3.7 | — |
| Chlorpheniramine | 1 | 34.7 ± 1.7 | 15.8 |
| Indomethacin | 5 | 34.0 ± 1.4 | 19.8 |
| Diclofenac | 10 | 38.0 ± 2.5 | −2.8 |
| Control | — | 34.9 ± 3.0 | — |
| Dexamethasone | 0.3 | 27.4 ± 1.1[a] | 45.7 |
| Glycyrrhizinic acid | 10 | 35.1 ± 3.3 | −1.2 |

The drugs tested were administered intravenously 5 min (chlorpheniramine, indomethacin and diclofenac) and 30 min (dexamethasone and glycyrrhizinic acid) prior to the first capsaicin application on the surface of pharyngeal mucosa. Each value represents the mean ± SE from five to seven animals.
[a]$P < 0.05$ vs. control.

the pharyngeal mucosa were 4.6 and 15.1 μg/pharynx (50 μl of 0.3 and 1.0 mM capsaicin solution, respectively). Therefore, the effective dose needed to increase plasma exudation in the pharynx seems to be lower than that in the ear. The reason might be due to differences in capsaicin absorption from the application site between the mucosa and outer skin and/or to species difference.

An antihistamine, chlorpheniramine, had a negligible effect on the capsaicin-induced rat pharyngeal plasma exudation. The dose of chlorpheniramine (1 mg/kg IV) used presently is a dose sufficient to inhibit the bronchoconstriction induced by 0.2% histamine inhalation in dogs (Yamatake *et al.*, 1977). It has been reported that the neuropeptides liberated by capsaicin through activation of sensory nerves are able to release histamine from mast cells (Gabor and Razga, 1992; Inoue *et al.*, 1993; Wang *et al.*, 1994). However, in the present study, that may not be the case. Our result might be consistent with the results of other studies in *in vivo* experiments concerning the trachea, esophagus, conjunctiva, paw skin, urinary bladder and nasal mucosa (Saria *et al.*, 1983) and lower respiratory tract of the rat (Lundberg and Saria, 1983). These groups reported that enough high local SP concentrations are necessary to activate mast cells and that SP in lower concentrations acts mainly via a mechanism whereby mast cells are not involved.

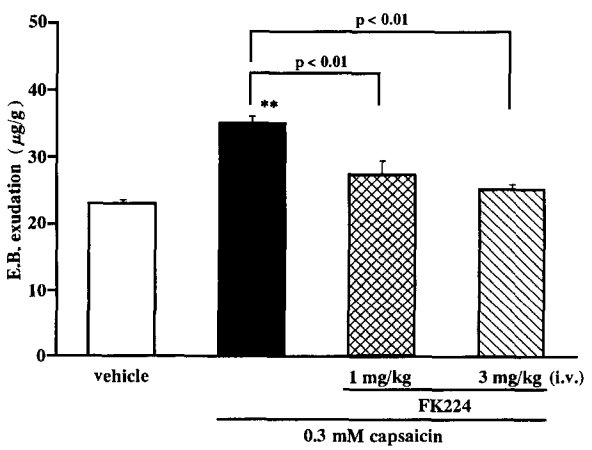


**FIGURE 3. Effect of FK224, $NK_1$- and $NK_2$-receptor antagonist, on Evans blue (EB) exudation in the rat pharynx induced by the local capsaicin treatment (two applications of 0.3 mM solution with a 30-min interval). FK224 was administered intravenously 5 min before the first capsaicin application. Each column represents the mean with SE from six or seven animals. **$P < 0.01$ vs. vehicle control.**

Cyclooxygenase inhibitors, indomethacin and diclofenac, had no effect on capsaicin-induced pharyngeal plasma exudation. The dose of indomethacin (5 mg/kg IV) completely blocked the decrease in arterial blood pressure produced by IV injection of 1 mg of arachidonic acid in the dog (Roberts *et al.*, 1985), and has also been often used as a typical dose for complete blockade of cyclooxygenase in other *in vivo* experiments (Hong *et al.*, 1995; Kuroiwa *et al.*, 1996; Yoshihara *et al.*, 1995, 1996). IV administration of diclofenac sodium (3 mg/kg) prevented EB leakage induced by electrical stimulation in the rat dental pulp (Kerezoudis *et al.*, 1993). In addition, preadministered diclofenac (9 mg/kg IV) inhibited the carrageenan-induced inflammation of the rat ankle and paw (Buritova *et al.*, 1995). A diclofenac dose of 10 mg/kg IV seems sufficient for an anti-inflammatory effect, although presently it was without effect. From the present results with cyclooxygenase inhibitors, it is conceivable that the role of prostaglandins would be minimal, if any, in our pharyngitis model. This was in accordance with the results of Inoue *et al.* (1993) that indomethacin (10 mg/kg orally) and aspirin (200 mg/kg orally) did not suppress capsaicin-induced mouse ear edema. They suggested that prostaglandins did not primarily mediate the development of the capsaicin-induced ear edema. Furthermore, Wang *et al.* (1994) reported that pretreatment with indomethacin (10 mg/kg IP) had no effect on SP-induced ear edema in mice. Also, glycyrrhizinic acid (10 mg/kg IV) did not reduce the rat pharyngeal plasma exudation induced by capsaicin. The IV dose of glycyrrhizinic acid inhibited the passive anaphylaxis reaction in the rat conjunctiva (Iso *et al.*, 1980). Glycyrrhizin is reported to have a similar action as steroidal anti-inflammatory drugs although the effect is much weaker than steroids (Finney and Somers, 1958). On the other hand, a steroidal anti-inflammatory agent, dexamethasone (.3 mg/kg IV), was effective in suppressing the capsaicin-induced increase in plasma exudation in the present study. Inoue *et al.* (1993) applied capsaicin on the mouse ear to cause an edema on which dexamethasone (0.1 mg/kg, applied topically) was greatly effective (76% inhibition). Sheppard *et al.*, (1992) reported that dexamethasone, in a dose of 0.25 mg/kg IV, significantly reduced edema by laser exposure in rat skin. The mechanism of the antiexudative action of the steroid on the pharyngeal mucosa is now unexplainable, but could possibly be due to phospholipase $A_2$ inhibition leading to inhibition of generation of 5-lipoxygenase products, but not to inhibition of generation of cyclooxygenase products, because indomethacin and diclofenac were without effect in our model.

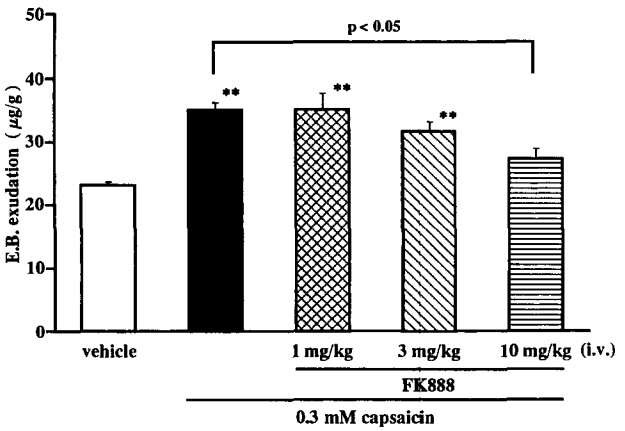


**FIGURE 4. Effect of FK888 ($NK_1$-receptor antagonist) on Evans blue (EB) exudation in the rat pharynx induced by local capsaicin treatment (two applications of 0.3 mM solution with a 30-min interval). FK888 was administered intravenously 5 min before the first capsaicin application. Each column represents the mean with SE from six or seven animals. **$P<0.01$ vs. vehicle control.**

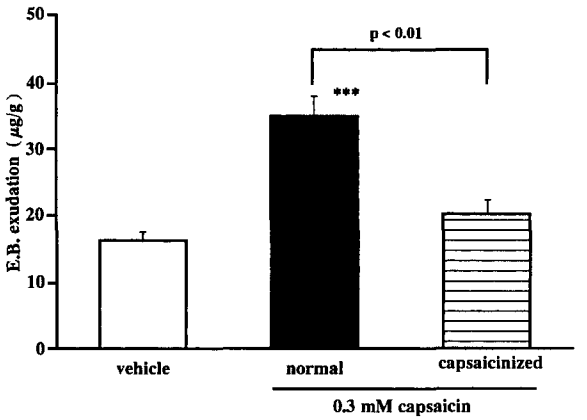


**FIGURE 5. Effect of systemic capsaicinization on Evans blue (EB) exudation in the rat pharynx induced by local capsaicin treatment (two applications of 0.3 mM solution with a 30-min interval). Other explanations are as in Figure 3. ***$P<0.001$ vs. vehicle control.**

FK224 and FK888 effectively and dose-dependently suppressed the capsaicin-induced increase in pharyngeal plasma exudation. Because FK224 is a dual antagonist for both $NK_1$ and $NK_2$ receptors (Morimoto *et al.*, 1992; Murai *et al.*, 1992) and FK888 is a selective antagonist for $NK_1$ receptor (Fujii *et al.*, 1992), sensory neuropeptides such as SP and NKA are considered to be involved in our pharyngitis model. It is known that capsaicin stimulates sensory C-fibers, causing a release of SP and NKA from nerve endings (Ingenito *et al.*, 1991; Szallasi *et al.*, 1993), and that neurogenic plasma exudation induced by capsaicin is mediated via tachykinins released from capsaicin-sensitive sensory nerves (Lundberg and Saria, 1982; Murai *et al.*, 1992). Edema resulting from increased microvascular permeability and vasodilation in the airway of the guinea pig is reportedly induced by SP more potently than by NKA (Murai *et al.*, 1992; Rogers *et al.*, 1988), suggesting that $NK_1$ receptor is more important than $NK_2$ receptor in airway edema induced by SP, NKA and capsaicin. In the present experiment, however, the inhibitory action of FK224 seemed to be stronger than that of FK888. Neurogenic plasma protein exudation in the rat paw induced by mustard oil, SP, antidromic stimulation of the saphenous nerve and vagal stimulation was inhibited by an $NK_1$ receptor antagonist, CP-96345, although not completely (Lembeck *et al.*, 1992). Capsaicin-induced plasma exudation in the guinea pig bronchi was also inhibited partly (not completely) by CP-96345 (Lei *et al.*, 1992). Therefore, it is likely that capsaicin-induced pharyngeal plasma exudation in the present study involves both $NK_1$ and $NK_2$ receptors, because FK224 is an antagonist for both receptors with a similar potency (Murai *et al.*, 1992). In addition, it is possible that the relatively weak effect of FK888 in the present study is due to the existence of species difference in potency of FK888. FK888 inhibited [$^3$H]-SP binding to guinea pig lung membrane with an approximately 100-fold higher affinity than that to rat brain cortical membranes (Fujii, 1995), although another $NK_1$ antagonist, RP67580, has been reported to be a potent inhibitor of [$^3$H]-SP binding to rat brain membrane preparations ($K_i$=4.16 nM). Nevertheless, the effect of RP67580 on SP-induced guinea pig ileum contraction is relatively weak ($pA_2$=7.16) (Garret *et al.*, 1991). Therefore, the relatively weaker effect of FK888 on rat tissues might be derived from the characteristics of FK888 itself, and probably not from the characteristics of $NK_1$ receptor in rat tissues. The participation of subtypes of neurokinin receptors in rat pharyngitis induced by capsaicin is necessary to be clearly demonstrated in the future.

The present rat pharyngitis model is the first one capable of quantitative determination of induced pharyngeal inflammation, requires only a simple technique and provides well-reproducible results. However, throat inflammation in the common cold syndrome can be somewhat treated clinically by nonsteroidal anti-inflammatory drugs, which were without effect in this study, so the present model requires improvement.

*We wish to thank Dr. Yoshihiko Chiba, Ms. Tomoko Ishikawa, and Mr. Masatoshi Ichiba for helpful discussion.*

## References

Buchan P. and Adcock J. J. (1992) Capsaicin-induced bronchoconstriction in the guinea-pig: contribution of vagal cholinergic reflexes, local axon reflexes and their modulation by BW443c81. *Br. J. Pharmac.* **105,** 448–452.

Buck S. H. and Burks T. F. (1986) The neuropharmacology of capsaicin: review of some recent observations. *Pharmac. Rev.* **38,** 179–226.

Buritova J., Honore P., Chapman V. and Besson J. M. (1995) Concurrent reduction of inflammation and spinal Fos-LI neurons by systemic diclofenac in the rat. *Neurosci. Lett.* **188,** 175–178.

Finney R. S. H. and Somers G. F. (1958) The anti-inflammatory activity of glycyrrhetinic acid and derivatives. *J. Pharm. Pharmacol.* **10,** 613–620.

Fujii T., Murai M., Morimoto H., Maeda Y., Yamaoka M., Hagiwara D., Miyake H., Ikari N. and Matsuo M. (1992) Pharmacological profile of a high affinity dipeptide NK1 receptor antagonist, FK888. *Br. J. Pharmac.* **107,** 785–789.

Fujii T. (1995) Discovery and pharmacological properties of selective neurokinin-receptor antagonists, FK224 and FK888. *Folia Pharmacol. Jpn.* **106,** 193–204.

Gabor M. and Razga Z. (1992) Development and inhibition of mouse ear oedema induced with capsaicin. *Agents Actions* **36,** 83–86.

Garret C., Carruette A., Fardin V., Moussaoui S., Peyronel J. F., Blanchard J. C. and Laduron P. M. (1991) Pharmacological properties of a potent and selective nonpeptide substance P antagonist. *Proc. Natl. Acad. Sci. USA* **88,** 10208–10212.

Holzer P. (1988) Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience* **24,** 739–768.

Holzer P. (1991) Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmac. Rev.* **43,** 143–201.

Hong J.-L., Rodger I. W. and Lee L.-Y. (1995) Cigarette smoke-induced

bronchoconstriction; cholinergic mechanisms, tachykinins, and cyclooxygenase products. *J. Appl. Physiol.* **78,** 2260–2266.

Ingenito E. P., Pliss L. B., Martins M. A. and Ingram R. H. Jr. (1991) Effects of capsaicin on mechanical, cellular, and mediator responses to antigen in sensitized guinea pigs. *Am. Rev. Respir. Dis.* **143,** 572–577.

Inoue H., Nagata N. and Koshihara Y. (1993) Profile of capsaicin-induced mouse ear oedema as neurogenic inflammatory model: comparison with arachidonic acid-induced ear oedema. *Br. J. Pharmac.* **110,** 1614–1620.

Inoue H., Nagata N. and Koshihara Y. (1995) Involvement of substance P as a mediator in capsaicin-induced mouse ear oedema. *Inflammation Res.* **44,** 470–474.

Iso T., Nakajima N., Suda H., Yamauchi H. and Uda K. (1980) Passive anaphylaxis in rat conjunctiva and topical effects of antiallergic agents. *Ophthal. Res.* **12,** 9–15.

Kamei J., Iwamoto Y., Suzuki T., Misawa M., Nagase H. and Kasuya Y. (1994) Involvement of $\delta_1$-opioid receptor antagonism in the antitussive effect of δ-opioid receptor antagonists. *Eur. J. Pharmac.* **251,** 291–294.

Kenins P. (1982) Responses of single nerve fibers to capsaicin applied to the skin. *Neurosci. Lett.* **29,** 83–88.

Kerezoudis N. P., Olgart L. and Edwall L. (1993) Evans blue extravasation in rat dental pulp and oral tissues induced by electrical stimulation of the inferior alveolar nerve. *Arch. Oral. Biol.* **38,** 893–901.

Kuroiwa C., Umeno E., Nogami H., Kano S., Hirose T. and Nishima S. (1996) Role of tachykinins in bronchoconstriction induced by intravenous administration of bradykinin in guinea-pigs. *Eur. Respir. J.* **9,** 741–746.

Lang S. D. R. and Singh K. (1990) The sore throat: when to investigate and when to prescribe. *Drugs* **40,** 854–862.

Lei Y.-H., Barnes P. J. and Rogers D. F. (1992) Inhibition of neurogenic plasma exudation in guinea pig airways by CP-96,345, a new non-peptide $NK_1$ receptor antagonist. *Br. J. Pharmac.* **105,** 261–262.

Lembeck F., Donnerer J., Tsuchiya M. and Nagahisa A. (1992) The non-peptide tachykinin antagonist, CP-96,345, is a potent inhibitor of neurogenic inflammation. *Br. J. Pharmac.* **105,** 527–530.

Lundberg J. M. and Saria A. (1982) Capsaicin-sensitive vagal neurons involved in control of vascular permeability in rat. *Acta Physiol. Scand.* **115,** 521–523.

Lundberg J. M. and Saria A. (1983) Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical irritants. *Nature* (Lond.) **302,** 251–253.

Lynn B., Ye W. and Cotsell B. (1992) The actions of capsaicin applied topically to the skin of the rat on C-fiber afferents, antidromic vasodilation and substance P levels. *Br. J. Pharmac.* **107,** 400–406.

Maggi C. A. and Meli A. (1988) The sensory-efferent function of capsaicin-sensitive sensory neurons. *Gen. Pharmac.* **19,** 1–43.

Martling C. R. (1987) Sensory nerves containing tachykinins and CGRP in the lower airways. *Acta Physiol. Scand.* **563**(suppl.), 1–57.

Morimoto H., Murai M., Maeda Y., Yamaoka M., Nishikawa M., Kiyotoh S. and Fujii T. (1992) FK224, a novel cyclopeptide substance P antagonist with NK1 and NK2 receptor selectivity. *J. Pharmac. Exp. Ther.* **262,** 398–402.

Murai M., Morimoto H., Maeda Y., Kiyotoh S., Nishikawa M. and Fujii T. (1992) Effects of FK224, a novel compound NK1 and NK2 receptor antagonist, on airway constriction and airway edema induced by neurokinins and sensory nerve stimulation in guinea pigs. *J. Pharmac. Exp. Ther.* **262,** 403–408.

Peter G. (1992) Streptococcal pharyngitis: current therapy and criteria for evaluation of new agents. *Clin. Infect. Dis.* **14**(suppl.), S218–S223.

Roberts A. M., Schultz H. D., Green J. F., Armstrong D. J., Kaufman M. P., Coleridge H. M. and Coleridge J. C. G. (1985) Reflex tracheal contraction evoked in dogs by bronchodilator prostaglandins $E_2$ and $I_2$. *J. Appl. Physiol.* **58,** 1823–1831.

Rogers D. F., Belvisi M. G., Aursudkig B., Evans T. W. and Barnes P. (1988) Effects and interactions of sensory neuropeptides on airway microvascular leakage in guinea-pigs. *Br. J. Pharmac.* **95,** 1109–1116.

Saria A., Lundberg J. M., Skofitsch G. and Lembeck F. (1983) Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, serotonin, histamine and by antigen challenge. *Naunyn-Schmiedeberg's Arch. Pharmac.* **324,** 212–218.

Saria A., Martling C. R., Yan Z., Theodorsson-Norheim E., Gamse R. and Lundberg J. M. (1988) Release of multiple tachykinins from capsaicin-sensitive sensory nerves in the lung by bradykinin, histamine, dimethylphenyl piperazinium, and vagal nerve stimulation. *Am. Rev. Respir. Dis.* **137,** 1330–1335.

Sheppard L. M., Werkhaven J., Mickelson S., Crissman J. D., Peterson E. and Jacobsen G. (1992) Effect of steroids or tissue precooling on edema and tissue thermal coagulation after $CO_2$ laser impact. *Lasers Surg. Med.* **8,** 108–118.

Szallasi A. and Blumberg P. M. (1993) Mechanisms and therapeutic potential of vanilloids (capsaicin-like molecules). *Adv. Pharmac.* **24,** 123–155.

Tani E., Senba E., Kokumai S., Masuyama K., Ishikawa T. and Tohyama M. (1990) Histamine application to the nasal mucosa induces release of calcitonin gene-related peptide and substance P from peripheral terminals of trigeminal ganglion: a morphological study in the guinea pig. *Neurosci. Lett.* **112,** 1–6.

Vukmir R. B. (1992) Adult and pediatric pharyngitis: a review. *J. Emerg. Med.* **10,** 607–616.

Wang J. P., Raung S. L., Lin C. N. and Teng C. M. (1994) Inhibitory effect of norathyriol, a xanthone from *Tripterospermum lanceolatum*, on cutaneous plasma extravasation. *Eur. J. Pharmac.* **251,** 35–42.

Yamatake Y., Sasagawa S., Yanaura S. and Kobayashi N. (1977) Involvement of histamine $H_1$- and $H_2$-receptors in induced asthmas in dogs. *Jpn. J. Pharmac.* **27,** 791–797.

Yonei Y., Holzer P. and Guth P. H. (1990) Laparotomy-induced gastric protection against ethanol injury is mediated by capsaicin-sensitive sensory neurons. *Gastroenterology* **99,** 3–9.

Yoshihara S., Chan B., Yamawaki I., Geppetti P., Ricciardolo F. L. M., Massion P. P. and Nadel J. A. (1995) Plasma extravasation in the rat trachea induced by cold air is mediated by tachykinin release from sensory nerves. *Am. J. Respir. Crit. Care Med.* **151,** 1011–1017.

Yoshihara S., Geppetti P., Hara M., Linden A., Ricciardolo F. L. M., Chan B. and Nadel J. A. (1996) Cold air-induced bronchoconstriction is mediated by tachykinin and kinin release in guinea pigs. *Eur. J. Pharmac.* **296,** 291–296.