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Bronchoconstrictor and Respiratory Effects of Neurokinin A in Dogs

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ABSTRACT

Neurokinin A (NKA) is the primary bronchoconstrictor tachykinin in the lungs of several species, including humans and has been implicated as an important mediator of inflammatory lung disorders, such as asthma. In this study, we investigated the effect of NKA on airway mechanics (lung resistance, dynamic lung compliance) and respiration (tidal volume, respiratory rate) in anesthetized, spontaneously breathing, male beagle dogs. The dogs were challenged with aerosolized NKA that was delivered from a jet nebulizer to the airways through an endotracheal tube. The challenge consisted of five separate inflations of 600 ml of air/inflation over a 1-min period. Challenge with aerosolized NKA (0.1–1%) produced a dose-dependent increase in lung resistance and a decrease in dynamic lung compliance. The bronchoconstriction induced by 1% NKA peaked at 0.5 min after challenge and had a duration of approximately 5 min. Challenge with 1% NKA also reduced tidal volume and increased respiratory rate. Pretreatment of dogs with the NK-$_2$ receptor antagonist, SR 48968 dose-dependently (1–10 mg/kg, p.o.) blocked the bronchoconstriction and respiratory responses to NKA challenge. Pretreatment with the NK_1-receptor antagonist, CP 99994 (1 mg/kg, i.v.) had no effect on the increase in lung resistance and the decrease in dynamic lung compliance due to NKA challenge, but blunted the respiratory response to NKA. Pretreatment of dogs with inhaled ipratropium bromide (0.01%) slightly, but significantly reduced the increase in lung resistance due to NKA challenge but had no effect on the decrease of dynamic lung compliance or on the respiratory responses to NKA. As expected, the bronchoconstrictor response to inhaled methacholine was completely blocked by inhaled ipratropium bromide (0.01%). In conclusion, we have identified an NK_2-receptor mediated bronchoconstrictor effect of NKA in dogs. Cholinergic reflexes play a small, but significant role in this response. Furthermore, both NK_1 and NK_2-receptors appear to be involved with the development of the rapid, shallow breathing response to NKA challenge. These results demonstrate an effect of tachykinins on airway mechanics and ventilatory reflexes in dogs.

NKA and SP are tachykinin neuropeptides that have a number of potentially important effects on airway function including airway smooth muscle contraction, vasodilatation, airway microvascular leakage, mucus hypersecretion and potentiation of cholinergic neurotransmission (Maggi *et al.*, 1995). Additionally, tachykinins have a number of proinflammatory effects and cause degranulation of mast cells and recruitment and activation of polymorphonuclear leukocytes and lymphocytes (Calvo *et al.,* 1992; Bost and Pascual, 1992; Kähler *et al.*, 1993; DeRose *et al.*, 1994; Joos *et al.*, 1994). These findings indicate that tachykinins may be involved in the pathogenesis of asthma.

Three tachykinin receptors have been pharmacologically identified (NK_1, NK_2, and NK_3 receptors) (Maggi *et al.*, 1995). Activation of both NK_1 and NK_2 receptors produces bronchoconstriction in guinea pigs (Regoli *et al.*, 1988; Ireland *et al.*, 1991; Maggi *et al.*, 1991; Ellis *et al.*, 1993) although in other species such as the hamster (Maggi *et al.*, 1989; Ellis *et al.*, 1993), rabbit (Sheldrick *et al.*, 1990) and humans (Ellis *et al.*, 1993; Sheldrick *et al.*, 1995) the contractile response to tachykinins is mediated predominantly by NK_2-receptor stimulation. NK_3 receptor activation increases excitability of the parasympathetic nervous system in guinea pigs (Myers and Undem, 1993) and this may contribute to augmented cholinergic hyperresponsiveness to tachykinins in this species. Tachykinins also constrict airway smooth muscle in dogs. Shioya *et al.* (1995) found that the dual NK_1/$NK_2$$NK_2$-receptor antagonist, FK 224, inhibited the contractile response of canine airway smooth muscle to SP and NKA. However, the functional role of NK_1 and NK_2 receptors on airway smooth muscle contractility cannot be ascertained from this study because selective NK_1 and NK_2 antagonists were not used. Tachykinins have a variety of effects on airway function in dogs and cause mucus gland hypersecretion (Coles *et al.*, 1984; Haxhiu *et al.*, 1991), stimulate tracheal ciliary beat frequency (Wong *et al.*, 1990, 1991), promote

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ABBREVIATIONS: NK, neurokinin; NKA, neurokinin A; SP, substance P; RL, lung resistance; C_{Dyn}, dynamic lung compliance; V_T, tidal volume; f, respiratory rate; $\dot{V}$, pulmonary airflow; Ptp, transpulmonary pressure.

chloride flux and modulate transmucosal potential difference across tracheal epithelium (Al-Bazzaz *et al.*, 1985; Rangachari *et al.*, 1987) and cause vasodilation of bronchial and pulmonary arteries (McCormack *et al.*, 1989). Surprisingly, the *in vivo* effect of tachykinins on bronchomotor tone in dogs has not been previously studied.

In our study, we investigated the effect of NKA on airway mechanics and respiration in spontaneously breathing, anesthetized male beagle dogs. We also performed studies with the NK_1-receptor antagonist, CP 99994 (McLean *et al.*, 1993) and the NK_2-receptor antagonist, SR 48968 (Emonds-Alt *et al.*, 1992; Advenier *et al.*, 1992) to determine the role of these tachykinin receptors on responses to NKA. Furthermore, we measured responses to NKA in dogs that were treated with the anticholinergic drug, ipratropium bromide (Pakes *et al.*, 1980), to evaluate the role of cholinergic reflexes.

Materials and Methods

Animal preparation. Studies were performed on spontaneously breathing, male beagle dogs ranging in weight from 10 to 15 kg. The dogs were fasted overnight but given water ad libitum. The front paw was shaved and a 22 gauge Surflo catheter (Terumo Medical Corp., Elkton, MD) was inserted into the cephalic vein and secured in place with adhesive tape. A luer-lock Surflo injection plug (Terumo Medical Corp., Elkton, MD) was connected to the i.v. catheter to facilitate the administration of drugs. An i.v. drip of isotonic saline (0.9%, pH 5.6) was maintained throughout the experiments. Anesthesia was induced by the i.v. injection of sodium thiopental (25 mg/kg). Occasionally, a supplemental bolus of sodium thiopental (5 mg/kg, i.v.) was given just before the start of the experiment.

Pulmonary measurements. A cuffed endotracheal tube (Rüsch AG, Waiblingen, Germany; size 7.0 mm) was inserted into the trachea with the aid of a laryngoscope. The endotracheal tube was connected to a heated pneumotachograph (Hans Rudolph Inc., Kansas City, MO; model 3719, Flow 0–100 liter/min) and the pressure drop across the pneumotachograph was measured with a differential pressure transducer (Validyne, Northridge, CA; model MP 45-14-871, range ± 2 cm H_2O) and used to derive the measurement of $\dot{V}$. The airflow signal was converted to an electrical signal proportional to the V_T with an integrator circuit (Buxco Electronics Inc., Sharon, CT, model 6). A balloon-tipped esophageal catheter was placed into the esophagus and positioned at the point where recorded inspiratory pressure was greatest. Ptp was measured with a differential pressure transducer (Validyne, Northridge, CA; model MP 45-24-87, range ± 20 cm H_2O) connected to the esophageal balloon and to an air port in front of the endotracheal tube.

The $\dot{V}$, V_T and Ptp signals were monitored by means of a pulmonary computer (Buxco Electronics, Inc., model 6) and displayed on a chart recorder. RL was calculated from the simultaneous measurement of Ptp and $\dot{V}$, which were sampled at isovolumetric points during inspiration and expiration (Amdur and Mead, 1958) and provided a measure of combined inspiratory and expiratory airflow resistance. C_{Dyn} was calculated from measurements of Ptp and V_T measured at the start and end of an inspiration (Amdur and Mead, 1958). The parameters of RL, C_{Dyn}, V_T and f were measured for three consecutive breaths before and at different times after the aerosol challenge.

Aerosol challenge. A three-way breathing valve was interposed between the pneumotachograph and the endotracheal tube to facilitate the pulmonary delivery of aerosols. A Raindrop jet nebulizer (Puritan Bennett, Lenexa, KS) was used to generate aerosols that were delivered with 40 psi of compressed air at a flow of 150 ml/sec. Each challenge with the aerosolized drug consisted of five separate forced inflations of 4-sec duration per inflation (600 ml of air/inflation) that was given over a 1-min period. During this period a one-way breathing valve (Hans Rudolph Inc., model 140) was connected to the end of the pneumotachograph and the exhaled gas was collected in a Douglas bag for disposal. Doses were altered by varying the concentrations of the solution in the nebulizer.

Experimental studies. Initially, to determine the dose-response and temporal effects of NKA challenge on lung mechanics, RL and C_{Dyn} were measured immediately before and 0.5, 1, 3, 5 and 10 min after challenge with NKA (0.1 and 1%). Comparisons were made in the same dogs after challenge with aerosolized saline. In all subsequent studies we used a 1% solution of NKA for the challenge. In one such study, lung mechanics (RL and C_{Dyn}) and ventilatory parameters (V_T and f) were measured immediately before and 0.5 min after challenge with NKA. This time was selected in this, and in subsequent experiments, to measure the peak bronchoconstrictor and ventilatory response to the challenge (see "Results").

To evaluate the role of NK_2 receptors on the response to NKA, dogs were treated with the NK_2-antagonist, SR 48968 (1–10 mg/kg, p.o.) or sham control (oral capsule minus SR 48968) given 2 hr before challenge with NKA. To evaluate the role of NK_1 receptors on the response to NKA, dogs were treated with the NK_1-antagonist, CP 99994 (1 mg/kg, i.v.) or saline given 10 min before challenge with NKA. The NK_1-antagonist activity of this dose of CP 99994 was confirmed by blocking the hypotension caused by the i.v. injection of 100 ng/kg of SP.

To determine the role of cholinergic reflexes on the response to NKA, studies were performed in dogs pretreated with aerosolized ipratropium bromide (0.01%) or aerosolized saline given 10 min before challenge with NKA. The dose of ipratropium bromide was selected from results of experiments that showed complete blockade of the bronchoconstrictor response to inhaled methacholine ($n = 12$).

Statistics. Statistical significance of treatment effects was assessed by repeated measures analysis of variance on log-transformed data. Pair-wise comparisons between treated and control groups were performed using t tests based on model-estimated S.E. Comparisons with $P < .05$ were considered to be evidence of significant treatment effects.

Drugs. Sodium thiopental was purchased from Abbott Labs. (Chicago, IL), NKA from Peninsula Labs. (Belmont, CA), ipratropium bromide from Sigma Chemical Co. (St. Louis, MO) and methacholine chloride from Aldrich Chemical Co. (Milwaukee, WI). SR 48968 and CP 99994 were synthesized at Schering-Plough Research Institute (Kenilworth, NJ).

Animal care and use. These experiments were performed with prior approval of the Animal Care and Use Committee of Schering-Plough Research Institute which is a facility accredited by the American Association for the Accreditation of Laboratory Animal Care.

Results

Response to NKA. The results of figure 1 illustrate the dose-response and temporal effects of NKA challenge on RL and C_{Dyn}. After challenge with NKA (0.1 and 1%) there was a dose-dependent increase in RL and decrease in C_{Dyn} that peaked at 0.5 min after the challenge. This effect lasted approximately 3 to 5 min after challenge with 1% NKA. By 10 min after the NKA challenge, the RL and C_{Dyn} had returned to baseline values. Upon challenge with aerosolized saline there was a transient (0.5–1 min duration) increase in C_{Dyn} with no change in RL over the 10-min period (fig. 1). Similar effects on C_{Dyn} were also seen after challenge with compressed air indicating that this response is a function of the lung hyperinflation (600 ml of air/inflation) induced by the challenge procedure.

The peak changes in pulmonary mechanics and respiration were measured in twelve dogs after challenge with a 1% solution of aerosolized NKA (table 1). After challenge with

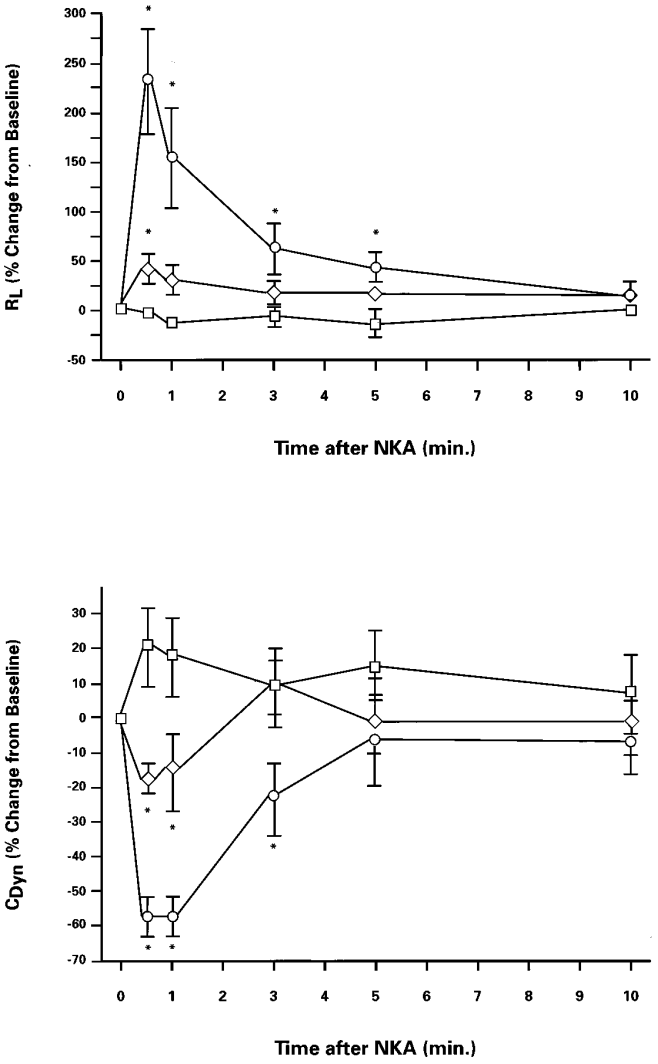

Fig. 1. Dose-response and temporal effects of NKA challenge on pulmonary mechanics. The percentage change in RL and C_{Dyn} were measured for 10 min after challenge with aerosolized NKA (0.1%) ◇, (1%) 0, or saline, ❐. Values represent mean ± S.E.M. (n = 6 for 1% NKA and saline, n = 3 for 0.1% NKA). * $P < .05$ compared to aerosol saline.

TABLE 1
Effect of NKA on airway mechanics and respiration

Parameter	Baseline[a]	NKA[a,b]
RL (cmH_2O/L/S)	3.6 ± 0.4	14.0[c] ± 3.4
C_{Dyn} (ml/cmH_2O)	66 ± 6	25[c] ± 6
V_T (ml)	148 ± 7	111[c] ± 16
f (breaths/min)	16 ± 1	62[c] ± 16

[a] Values represent mean ± S.E.M. (n = 12).
[b] Measurements at 0.5 min after challenge with NKA (1%).
[c] $P < .05$ compared to baseline.

NKA there was an increase in RL with individual values ranging from 48 to 850% increase over baseline. There was also a reduction in C_{Dyn}, with individual values ranging from 11 to 96% decrease over baseline. In most dogs challenge with 1% NKA produced a decrease in V_T and an increase in f (table 1).

Effect of tachykinin antagonists. Pretreatment of dogs with oral SR 48968 (1–10 mg/kg, p.o.) dose-dependently inhibited the increase in RL and decrease in C_{Dyn} due to challenge with NKA (fig. 2). There was also a dose-dependent inhibition by oral SR 48968 of the decrease in V_T and increase in f due to NKA challenge (table 2). At doses of 3 and 10 mg/kg, V_T increased after the NKA challenge (table 2). This response is a function of the lung hyperinflation induced by the challenge procedure because an increase in V_T is also seen after challenge with compressed air. There was no change in baseline RL, C_{Dyn}, V_T or f after treatment with SR 48968.

Pretreatment of dogs with CP 99994 (1 mg/kg, i.v.) had no significant effect on the increase in RL and C_{Dyn} due to challenge with NKA (table 3). However, the reduction of V_T and increase in f due to NKA challenge was significantly attenuated after treatment with CP 99994 (table 3). There

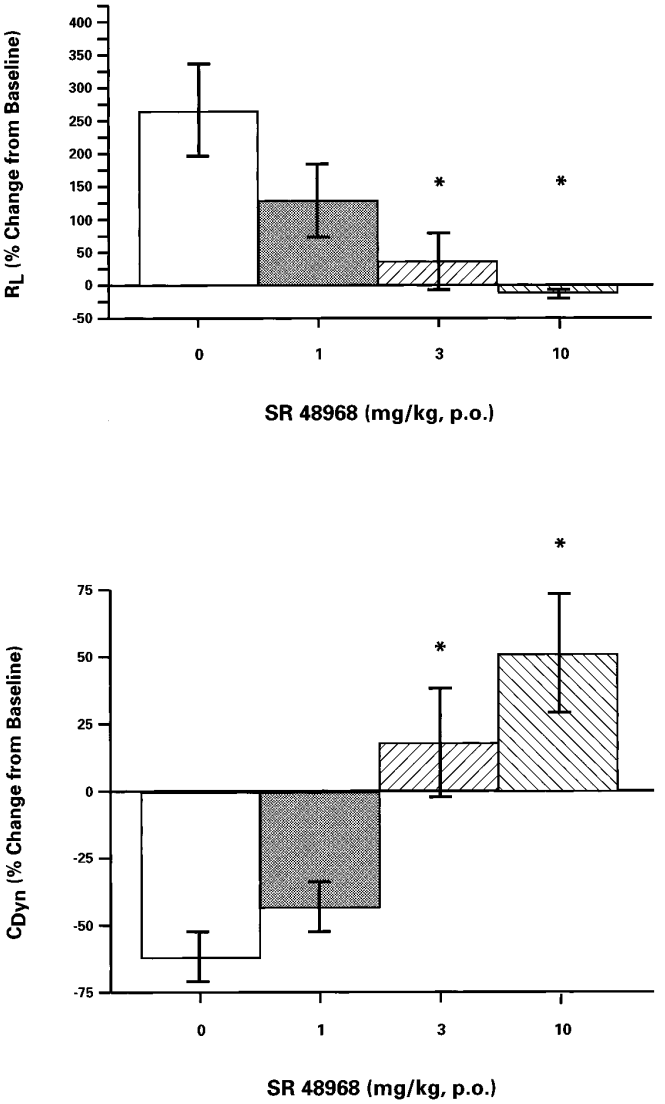

Fig. 2. Effect of oral SR 48968 on the bronchoconstrictor response to challenge with NKA. Dogs were treated with SR 48968 2 hr before challenge with NKA (1%). Values represent the mean ± S.E.M. (n = 11 per dose). * $P < .05$ compared to vehicle control.

TABLE 2
Effect of SR 48968 on the respiratory response to NKA

SR 48968[a] (mg/kg, p.o.)	Percent Change Due to NKA[b,c]	
	V_T	f
0	−26 ±11	+370 ±159
1	+9 ±15	+139 ±58
3	+22[d] ±11	+98 ±49
10	+44[d] ±10	+45[d] ±21

[a] SR 48968 given orally 2 hr before challenge with NKA (1%).
[b] Values represent mean ± S.E.M. (n = 11).
[c] Measurements at 0.5 min after challenge with NKA.
[d] $P < .05$ compared to zero dose.

TABLE 3
Effect of CP 99994 on the bronchoconstrictor and respiratory responses to NKA

CP 99994[a] (mg/kg, i.v.)	Percent Change Due to NKA[b,c]			
	RL	C_{Dyn}	VT	f
0	±691 ±298	−84 ±5	−55 ±9	+436 ±177
1	+592 ±249	−67 ±9	−15[d] ±12	+83[d] ±40

[a] CP 99994 given i.v. 10 min before challenge with NKA (1%).
[b] Values represent mean ± S.E.M. (n = 6).
[c] Measurements at 0.5 min after challenge with NKA.
[d] $P < .05$ compared to zero dose.

was no change in baseline RL, C_{Dyn}, V_T or f after treatment with CP 99994.

Effect of ipratropium bromide. When dogs were treated with aerosolized ipratropium bromide (0.01%) and challenged with NKA (1%), there was a partial reduction of the increase in RL after the NKA challenge (fig. 3). Statistically significant effects with ipratropium bromide were observed at 1 and 3 min after the NKA challenge. However, the reduction of C_{Dyn} due to NKA was not significantly changed by treatment with ipratropium bromide (fig. 3). Ipratropium bromide had no effect on the reduction of V_T and increase in f after NKA challenge (data not shown). Furthermore, ipratropium bromide alone had no effect on baseline RL, C_{Dyn}, V_T and f when assessed before the NKA challenge.

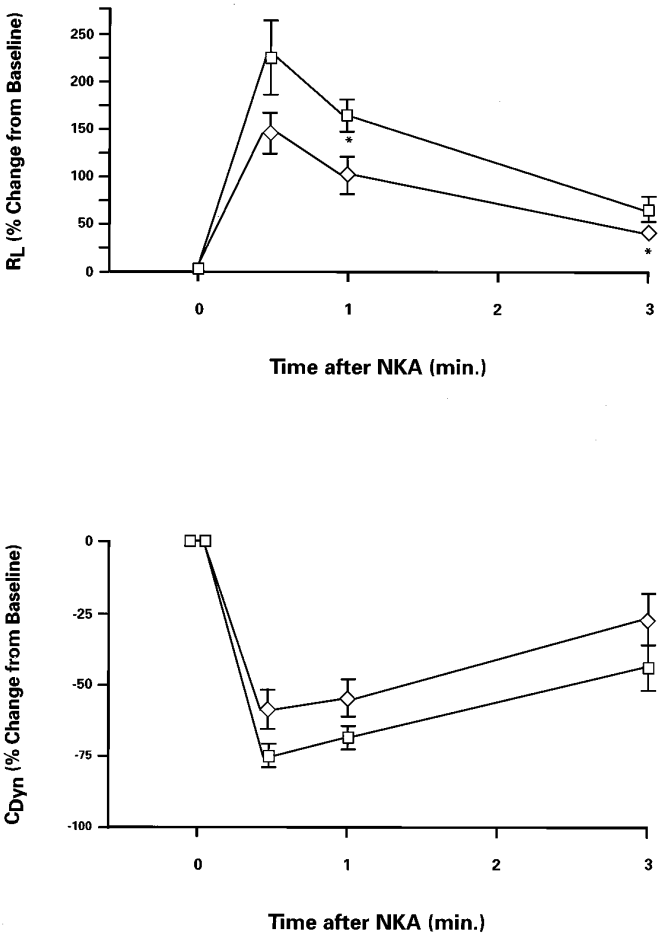

Fig. 3. Effect of ipratropium bromide on the bronchoconstrictor response to challenge with NKA. Dogs were treated with aerosolized ipratropium bromide (0.01%), ◇ or aerosolized saline, ❒ 10 min before challenge with NKA (1%). Values represent the mean ± S.E.M. (n = 8 per dose). * $P < .05$ compared to aerosolized saline.

Discussion

NKA is a potent constrictor of airway smooth muscle and causes bronchospasm in rats (Joos *et al.*, 1988; Joos and Pauwels, 1990), guinea pigs (Hua *et al.*, 1984), monkeys (Mauser *et al.*, 1997) and human asthmatics (Evans *et al.*, 1988; Crimi *et al.*, 1992; Joos *et al.*, 1996). NKA is the preferred ligand for NK_2-receptors which is the predominant receptor subtype producing bronchoconstriction in most species, including humans (Maggi *et al.*, 1995). In our study in dogs, we demonstrated that inhaled NKA produced bronchoconstriction that was blocked by NK_2-receptor antagonist SR 48968, but not by the NK_1-receptor antagonist CP 99994. These results identify the NK_2-receptor as the functionally relevant receptor subtype producing bronchospasm to NKA in dogs. We also performed a few experiments with aerosolized SP but found no bronchoconstrictor response after challenge. Only coughing was observed in some of the dogs. These findings suggest that the NK_1-receptor is not functionally important for producing bronchoconstriction in dogs.

Challenge with NKA produced an increase in lung resistance and a decrease in dynamic lung compliance. This change in pulmonary mechanics is typically seen with other bronchoconstrictor agents, such as methacholine chloride. The bronchoconstrictor response to NKA peaked at 0.5 min after challenge and had a duration of only 3 to 5 min. Tachykinins are rapidly metabolized by a variety of endopeptidases present in the lungs (Lilly *et al.*, 1993) which would explain the relatively transient nature of the bronchoconstrictor response. Most of the dogs studied responded with a bronchospasm but there was a wide range in bronchial reactivity to this spasmogen. In some of the more reactive dogs, coughing was occasionally seen immediately after the challenge. It is important to note that our studies were performed in normal, healthy dogs that had no evidence of pulmonary inflammation or pulmonary dysfunction. In humans, bronchoconstrictor responses to NKA are greater in asthmatics compared to normals (Joos *et al.*, 1987), suggesting that bronchoconstric-

tor responses to this spasmogen in dogs would be augmented in the presence of pulmonary inflammation.

Both NK_1 and NK_2 receptors are found in the lungs and in some species, like the guinea pig, both NK_1 and NK_2 receptor stimulation produce bronchoconstriction (Regoli *et al.*, 1988; Ireland *et al.*, 1991; Maggi *et al.*, 1991; Ellis *et al.*, 1993). In other species such as hamster (Maggi *et al.*, 1989; Ellis *et al.*, 1993), rabbit (Sheldrick *et al.*, 1990) and humans (Ellis *et al.*, 1993; Sheldrick *et al.*, 1995), only NK_2-receptor stimulation mediates airway smooth muscle contraction. Tachykinins constrict airway smooth muscle in dogs and Shioya *et al.* (1995) found that the dual NK_1/NK_2-receptor antagonist, FK 224, inhibited the contractile response of canine airway smooth muscle to SP and NKA. The results from our study identify the NK_2-receptor as the functionally important receptor subtype. We found that pretreatment with SR 48968, a selective NK_2-receptor antagonist (Emonds-Alt *et al.*, 1992; Advenier *et al.*, 1992) blocked the increase in RL and decrease in C_{Dyn} in response to NKA challenge whereas CP 99994, a selective NK_1-receptor antagonist (McLean *et al.*, 1993) had no effect. We used a dose of CP 99994 that completely blocked the hypotensive response to i.v. SP. This physiological response is an established pharmacological procedure for evaluating NK_1-receptor antagonists in dogs (McLean *et al.*, 1996).

Tachykinin receptors and tachykinin-containing immunoreactive nerve fibers are widely distributed in the pulmonary system of dogs (Hisa *et al.*, 1985; Rangachari *et al.*, 1987; McCormack *et al.*, 1989; Nohr and Weihe, 1991). In several species, such as the guinea pig (Watson *et al.*, 1993; Hey *et al.*, 1996), rabbit (Tanaka and Grunstein, 1984, 1986) and sheep (Corcoran and Haigh, 1992), tachykinin receptors are located on airway parasympathetic nerves and augment the release of acetylcholine from postganglionic nerve terminals causing exaggerated cholinergic bronchoconstrictor response. In our study, we found that the bronchoconstrictor response to NKA was partially blocked by ipratropium bromide. This result identifies a cholinergic component to the bronchoconstrictor response to NKA in dogs. It is interesting to note that the predominant effect of ipratropium bromide was on the increase in RL. These results imply that the cholinergic component of the NKA-induced bronchospasm in dogs involves effects on airway caliber or possibly on the tissue viscance of the lungs because both these elements contribute to the derivation of pulmonary resistance in dogs (Ludwig *et al.*, 1989). In this regard, the parasympathetic innervation of the pulmonary system in dogs is predominantly in the central conducting airways of the trachea, bronchi and bronchioles (Richardson, 1979) which makes it likely that the cholinergic component of the NKA-induced bronchospasm was at this location of the tracheobronchial tree.

In addition to their effects on airway smooth muscle contractility, tachykinins also stimulate a variety of airway sensory nerves such as lung irritant receptors (Prabhakar *et al.*, 1987), pulmonary "C" fibers (Prabhakar *et al.*, 1987; Widdicombe 1995) and carotid bodies (Prabhakar *et al.*, 1989; Cragg *et al.*, 1994). Therefore, the ventilatory response to NKA seen in dogs likely involves a complex interplay between the direct effect of NKA on airway caliber-producing airflow obstruction and an indirect, reflex effect from airway sensory nerve stimulation. Our results suggest that both NK_1 and NK_2 receptors are involved in this response because the respiratory response to NKA challenge was inhibited by SR 48968 and CP 99994. It is likely that the NK_2-receptor component involves effects on airway caliber-producing airflow obstruction that in turn would activate the lung irritant receptors producing rapid, shallow breathing (Widdicombe, 1995). The NK_1-receptor component does not involve airway smooth muscle contraction and may stimulate pulmonary reflexes directly. Indeed, from our studies in dogs (unpublished observations J. E. Sherwood and R. W. Chapman), we have found that intravenous SP (100 ng/kg), has a profound effect on respiration, *i.e.*, produced an increase in respiratory rate, a reduction in V_T and an increase in minute volume with no concomitant change in lung mechanics. In this study we also found that the respiratory response to SP was completely blocked by CP 99994 indicating that it is produced by activation of the NK_1-receptor. Although SR 48968 and CP 99994 are capable of inhibiting pulmonary reflexes by acting at the level of the central nervous system (Bolser *et al.*, 1997), we consider this to be an unlikely scenario in our study because neither SR 48968 nor CP 99994 had an effect on baseline ventilation and neither drug affected the respiratory response to inhaled methacholine challenge in our dogs (J. E. Sherwood and R. W. Chapman, unpublished observations).

In conclusion, we have identified an NK_2-receptor-mediated bronchoconstrictor effect of NKA in dogs. Cholinergic reflexes play a small, but significant role in this response. Furthermore, both NK_1- and NK_2-receptors appear to be involved in the respiratory response to NKA challenge. These results demonstrate an effect of tachykinins on airway mechanics and ventilatory reflexes in dogs.

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