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# Ferret tracheal mucus rheology, clearability and volume following administration of substance P or methacholine

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*Ferret tracheal mucus rheology, clearability and volume following administration of substance P or methacholine. G.T. De Sanctis, B.K. Rubin, O. Ramirez, M. King.*
**ABSTRACT: We studied the effects of substances P administration on tracheal mucus viscoelasticity, water content, clearability and secretion rate. Six neutered adult male ferrets (weight 1.1.–1.5 kg) were studied, on four occasions each. They were anaesthetized with ketamine and xylazine, and intubated shallowly. Control mucus (pre- and post-Ringer instillation) was compared with the mucus obtained following instillation of 200 μl of $10^{-6}$, $10^{-5}$, and $10^{-4}$ M substance P (SP), and $10^{-4}$ M methacholine chloride as a reference. Tracheal mucus was collected by inserting a soft-bristled cytology brush to the level of the carina, and leaving it in contact with the mucosa for 30 min. After withdrawing the brush, the adherent mucus was quickly scraped off and layered with paraffin oil to prevent evaporation. The mucus was analysed for viscoelasticity by magnetic rheometry and solids content by evaporation to dryness. Mucus transportability was assessed by comparison with model gels, and also by means of the frog palate assay, which indicates how well mucus is cleared by normal ciliary action.**

**There was a dose-related increase in mucus volume, and a dose-related decrease in mucus viscosity and elasticity, with substance P administration. Mucus transportability increased with both substance P and methacholine. The effects of $10^{-4}$ SP and methacholine were comparable in terms of viscoelasticity and volume; with both methacholine and SP, there appeared to be an additive effect. The mucus solids content did not correlate with the viscoelastic changes for SP administration, whereas it did for methacholine.**

**These observations suggest that the hypersecretion induced by these two agents might involve different pathways.**
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The airways of the tracheobronchial tree are largely covered by a thin blanket of mucus. Mucus is a viscoelastic secretion that protects the underlying mucosa from dehydration, while trapping inhaled particles that come into contact with it. The mucociliary clearance mechanism is primarily responsible for the removal of mucus from the lungs. This is accomplished by the actions of airway cilia, which beat in a synchronized fashion. The movement of the cilia is characterized by a forward effective stroke, at which point the tips of the cilia contact and propel the mucus in a unidirectional fashion. The rate at which the mucus is cleared by the cilia is dependent not only on the action of the cilia, but to a large extent on the viscoelastic properties of the mucus [1]. An increase in mucus secretion, or an alteration in the mechanical properties of the mucus, will affect the efficiency of the mucociliary mechanism. In view of this, it is important to characterize the actions of airway secretagogues on mucus transportability and rheology in health and disease.

Although the nervous regulation of airway mucus secretion has been well studied, the actions of airway secretagogues on the viscoelastic properties of the mucus are poorly understood. In addition, very few studies have examined the relationship between changes in mucus rheology and transportability.

The epithelium of the tracheobronchial tree is innervated with sensory fibres. A population of these mucosal fibres has been found to contain various neuropeptides. Among these, substances P (SP) has been implicated in the control of mucus secretion. Several lines of evidence support the role of peptidergic nerves in the control of mucus secretion. Substance P has been shown to be a potent stimulant of airway mucus secretion in isolated canine, ferret and human airways [2–4]. In addition, baseline mucus secretion is not completely abolished after administration of alpha, beta and muscarinic antagonist [5–8]. Morphological studies have shown that unmyelinated fibres containing SP are localized between epithelial cells in the airways [9, 10], and irritation of the airway

mucosa is believed to initiate the release of SP from sensory collaterals. Whereas, numerous studies have confirmed the secretory actions of SP *in vitro*, its effect on the physical properties of mucus (airway surface liquid) has not been studied.

The present study reports experiments designed to evaluate the effects of increasing doses of substances P and a single dose of methacholine on airway secretion in anaesthetized ferrets. In particular, the effects of substance P on the physical properties of mucus were ascertained by magnetic rheometry and by measurement of frog palate transport rates.

## Materials and Methods

All experiments were conducted in six neutered adult male ferrets, body weight 1.1–1.5 kg (Marshall Farms, North Rose, NY, USA). The animals were anaesthetized with an intramuscular injection of ketamine 4 mg and xylazine 2mg. Additional anaesthetic, at half of the induction dose, was administered at approximately 30 min intervals, as required. A topical anaesthetic, consisting of 100 µl of 1% xylocaine, was sprayed on the larynx, just before shallow intubation with a 2.5 mm *i.d.* endotracheal tube.

Five minutes after intubation, a 3 mm cytology brush (#151 Mill Rose, Mentor, OH, USA) was inserted, so that the brush rested in the lower half of the trachea beyond the tip of the endotracheal tube. The brush was left in contact with the airway for 30 min. When it was withdrawn, the end of the brush was immediately layered with light paraffin oil (#0-121 Fisher Scientific, Fair Lawn, NJ, USA) to minimize dehydration, and the adherent mucus was removed by scraping with a dulled scalpel blade. Because only small amounts of mucus could be collected from the unstimulated trachea, for most experiments the brush was cleaned and reinserted for a second 30 min control period. Following this, tracheal mucus was collected according to one of two experimental protocols.

In the first of these experiments, 200 µl of substance P dissolved in Ringer's solution (147.5 Na, 156 Cl, 4 K, 2.25 Ca mmol·$l^{-1}$) was delivered to the trachea *via* a catheter placed at the end of the endotracheal tube. Successive and increasing concentrations of $10^{-6}$, $10^{-5}$ and $10^{-4}$ M SP were delivered at 45 min intervals. Five min after each instillation of SP, a cytology brush was inserted and left in place for mucus collection for 30 min. This experiment was conducted up to three times in each animal on separate days.

On two other occasions, in order to compare the response to substance P with that to methacholine, mucus was also collected from each ferret after the administration of Ringer's solution (vehicle), followed sequentially at 45 min intervals by methacholine and substance P at the equimolar concentration of $10^{-4}$ M. Half hour mucus collections were carried out as described previously. Substance P (acetate salt) and methacholine chloride were obtained from Sigma Chemical, St. Louis, MO, USA.

The animals were extubated at the end of each experiment and allowed to awaken from anaesthetic under a warming lamp before being returned to their cages. In all, each ferret was anaesthetized for mucus collection on five separate days; they were rested for a minimum of one week between experiments. The studies were reviewed and approved by the University of Alberta Health Sciences Animal Welfare Committee.

### *Measuring mucus properties*

The collected mucus was preserved with a layer of light paraffin oil and stored at -80°C. Before rheological analysis, the samples of mucus were allowed to reach room temperature. Following this analysis, and before the other assays, they were cleansed of surface oil by a brief immersion in petroleum ether (#E-139B, Fisher Scientific, Fair Lawn, NJ, USA). Bloody samples, which were infrequent, were discarded.

*Viscoelasticity (rheological properties).* The viscoelasticity of mucus was determined by magnetic microrheometry. A steel ball, 70–150 µm in diameter was positioned in a 3–5 µl sample of mucus and oscillated by an electromagnet at frequencies of 1 and 100 rad·$s^{-1}$. The image of the ball was magnified and projected onto photocells, where the magnitude of displacement of the ball and its phase lag with respect to the driving force were used to calculate the viscoelastic properties of the mucus [11]. These measurements are reported here as log G* (mechanical impedance of mucus rigidity factor, expressed as a logarithm) and tan δ (loss tangent or mucus recoil factor). G* is the vector sum of elasticity and viscosity, and is a measure of the resistance to deformation or rigidity; tan δ is the ratio of viscous to elastic deformation. A material with high tan δ deforms permanently when subjected to a stress or force; a material with low tan δ recoils or snaps back after the stress is removed. The measurements of G* are logarithmically distributed, with a typical coefficient of within animal variation of *ca.* 0.15 log units [12].

*Mucociliary transportability.* A 1–3 µl mucus sample, free of oil, was placed on the mucus-depleted palate of the leopard frog and the average transport rate of this sample after several passes across the palate was normalized to the transport rate for collected endogenous frog mucus. This gives a measurement of the mucociliary transportability of the mucus - the normalized frog palate transport rate (NFPTR) - as described by Rubin *et al.* [13].

*Mucus hydration (% solids composition)* The mucus samples were then weighed in a microbalance on a previously weighed glass slide. This slide was dried in a microwave oven (650 W for 30 min) and allowed to cool. The dried sample was then reweighed in

order to calculate the percentage solids composition (SC).

*Data analysis*

Computations were performed using the Statview II statistical package (Abacus Concepts, Berkeley, CA, USA) for the Macintosh. Repeated measures analysis of variance was applied to test for time and treatment effects. Paired t-tests were used to determine the significance of changes from baseline after either substance P or methacholine administration. Results are expressed as mean±standard deviation, unless otherwise stated, and were considered statistically significant at the $p<0.05$ level.

## Results

*Dose-response to substance P*

Sequentially increasing concentration of substance P, 200 µl volume each, were instilled intratracheally and, following a 5 min rest period, mucus was collected for 30 min. The samples of mucus were analysed for their rheological and transport properties, and changes were compared with the properties of the mucus from one or two control collection periods. Repeated measures analysis of variance was used to test for both time and dose effects. This analysis revealed no significant time effect over the three experiments, and significant dose effects for the following parameters: log G* at 1 and 100 rad·s$^{-1}$, NFPTR, collection rate and % solids. Specific dose effects were then tested using paired t-tests adjusted for multiple comparisons.

The logarithm of G* at 1 rad·s$^{-1}$ (a measure of rigidity) decreased significantly after administration of $10^{-5}$ and $10^{-4}$ M but not $10^{-6}$ M SP, when compared to control mucus samples ($p<0.05$). The maximal decrease in G* (0.42 log units) is equivalent to a 2.6 fold decrease in rigidity. The relationship between tracheal viscoelasticity at 1 rad·s$^{-1}$ and the dose of substances P is illustrated in figure 1. The changes in viscoelasticity at 100 rad·s$^{-1}$ followed a similar pattern to that demonstrated at 1 rad·s$^{-1}$, but a significant difference from control was only found for $10^{-4}$ M SP. No significant changes from control were noted with regard to tan δ at either frequency. On several occasions, the samples of mucus were too small for rheological analysis, particularly from animals that had received no drug or only low doses of SP.

The effect of substance P administration on mucus transportability, as indicated by the frog palate assay, is shown in figure 2. It is evident that there was a progressive and significant increase in ciliary transportability of the collected mucus with increasing dose of substance P. Mean values for NFPTR prior to and after administration of SP were 0.46±0.14, 0.63±0.22, 0.65±0.25, and 0.71±0.25 for control, $10^{-6}$, $10^{-5}$ and $10^{-4}$ M SP, respectively, ($p<0.05$ with respect to control for $10^{-6}$ and $10^{-4}$ M). These increases in NFPTR seem to mirror the changes in mucus viscoelasticity at each dose of SP.

Examination of the total weight of mucus collected (in 30 min) revealed an initial increase at the lowest dose of SP, followed by a decrease at the intermediate dose, and finally another increase in total weight at the highest dose of SP. The volume of mucus collected at the highest dose of SP was significantly elevated over control (total weight in 30 min 9.7±7.9 mg for SP $10^{-4}$ M *vs.* 3.5±3.5 mg for control; $p<0.05$).

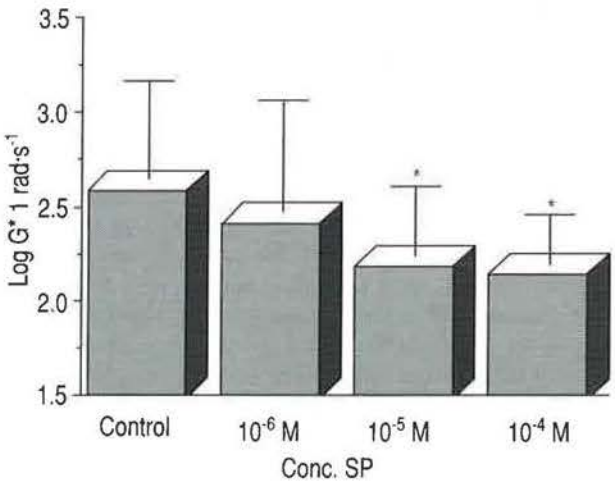


Fig. 1. – Mucus rigidity factor (log G* measured at 1 rad·s$^{-1}$) for tracheal mucus, collected from ferrets treated intratracheally with saline or substance P (SP) at the concentrations indicated. Mean±SEM shown; *: significantly different from control. Note that Y axis does not extend to zero.

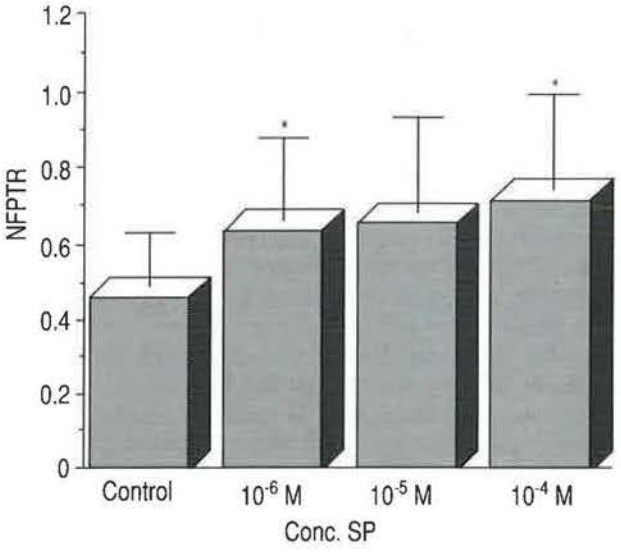


Fig 2. – Normalized frog palate transport rate (NFPTR), an index of mucociliary transportability, for tracheal mucus, collected from ferrets treated with saline or with substance P (SP) at increasing concentrations. Mean±SEM shown; *: significantly different from control.

The percentage solids content of the mucus passed through a maximum at intermediate SP concentration (significant for $10^{-5}$ M SP). All the dose-response data, including the results of the other rheological measurements, are summarized in table 1. Although the experiments were conducted over a period of time, the results obtained did not vary appreciably from week to week, nor was there a significant difference between the control measurements for the two experiments.

*Response to methacholine and substance P*

In a separate series of experiments, the effect of equimolar concentrations of methacholine and substance P ($10^{-4}$ M) on the rate of mucus secretion and the rheological and transport properties of the mucus was measured in each ferret and compared with control. Ferrets were challenged with 200 µl of methacholine ($10^{-4}$ M), followed by SP ($10^{-4}$ M) 45 min later. Both solutions were made up in the vehicle, *i.e.* Ringer's solution. Administration of the vehicle alone produced no apparent changes in either the mechanical properties of the mucus or the amount of mucus collected, when compared with non-vehicle controls of the first set of experiments. Also in preliminary experiments, an additional dose of Ringer's solution resulted in no apparent increase in secretion volume above baseline.

The changes in the mechanical properties and transportability of tracheal mucus with methacholine and SP are presented in table 2. When compared to control, methacholine induced a change in mucus viscoelasticity (a decrease of 0.34 log units) that, while nonsignificant, was very similar to the one seen with the equivalent dose of SP. At the same time, the increase in volume collected in 30 min with methacholine (2.13 × control value) was very similar to the increase in volume after $10^{-4}$ M SP reported above (2.35 × control value). Methacholine caused a decrease in mean mucus solids content, when compared with control (8.1±4.3 *vs.* 11.5±6.0%).

When substance P was administered subsequent to methacholine, as indicated in table 2, there was a further decrease in mean viscoelasticity, and a further increase in volume collected, but no further decrease in solids content (in fact a slight increase). Frog palate transportability increase by comparable degrees with both methacholine ($10^{-4}$M) and substance P ($10^{-4}$ M). The total change in viscoelasticity with $10^{-4}$ M SP in experiments No. 2 was 0.42 log units, identical to the mean change observed in experiment No. 1.

Table 1. – Mucus rheology, transportability and volume with increasing doses of substance P

| Parameter | Control | SP $10^{-6}$ M | SP $10^{-5}$ M | SP $10^{-4}$ M |
|---|---|---|---|---|
| log G* 1 rad·s$^{-1}$ | 2.58±0.54 | 2.42±0.59 | †2.19±0.37 | †2.16±0.26 |
| tan δ 1 rad·s$^{-1}$ | 0.25±0.05 | 0.25±0.07 | 0.26±0.05 | 0.26±0.05 |
| log G* 100 rad·s$^{-1}$ | 2.82±0.55 | 2.74±0.61 | 2.52±0.40 | 2.51±0.23 |
| tan δ 100 rad·s$^{-1}$ | 0.52±0.09 | 0.48±0.11 | 0.60±0.20 | 0.58±0.13 |
| NFPTR | 0.46±0.14 | †0.63±0.22 | 0.65±0.25 | †0.71±0.25 |
| Wt coll in 30min mg | 3.5±3.5 | 7..2±7.1 | 3.6±2.9 | †9.7±7.9 |
| % solids | 11.5±4.8 | 13.1±3.1 | †16.9±5.5 | 13.5±5.7 |
| CCI | 0.89±0.46 | 0.98±0.61 | †1.26±0.38 | †1.27±0.30 |

All values are means±SD. †:$p<0.05$ by paired t-test. CCI = 3.44 - 1.07 × log G* 100 + 0.89 × tan δ 100 [14]. SP: substance P; NFPTR: normalized frog palate transport rate; CCI: cough clearability index; Wt coll.: weight collected.

Table 2. – Mucus rheology, transportability and volume after sequential doses of methacholine and substance P $10^{-4}$ M

| Parameter | Control | Met $10^{-4}$ M | SP $10^{-4}$ M |
|---|---|---|---|
| log G* 1 rad·s$^{-1}$ | 2.65±0.51 | 2.31±0.54 | 2.23±0.44 |
| tan δ 1 rad·s$^{-1}$ | 0.25±0.13 | 0.20±0.05 | 0.21±0.05 |
| log G* 100 rad·s$^{-1}$ | 2.93±0.54 | 2.58±0.52 | 2.48±0.47 |
| tad δ 100 rad·s$^{-1}$ | 0.57±0.31 | 0.47±0.12 | 0.47±0.12 |
| NFPTR | 0.76±0.29 | 0.86±0.22 | 0.83±0.23 |
| Wt coll. in 30 min mg | 5.3±4.3 | 13.5±14.7 | †18.2±14.7 |
| % solids | 11.5±6.0 | 8.1±4.3 | 8.8±4.1 |
| CCI | 0.82±0.46 | 1.10±0.63 | 1.20±0.53 |

All values are mean±SD. †:$p<0.05$ by paired t-test. CCI = 3.44 - 1.07 × log G* 100 + 0.89 × tan δ 100 [14]. Met: methacholine. For further abbreviations see legend to table 1.

## Discussion

SP immunoreactivity has been demonstrated within the airway epithelium of several species, including man and ferret [15–19]. Substance P is synthesized in the nodose ganglia of the vagus nerve and transported down to peripheral branches [20]. Stimulation of sensory C-fibres within the epithelium by noxious agents or inflammatory mediators may release neuropeptides, such as SP, a neuropeptide with pro-inflammatory actions in the airways.

Substance P is a potent stimulant of airway secretion in isolated canine, ferret and human airways [2–4, 21–24]. Recent work by SHIMURA *et al.* [25] has shown that administration of SP to an *in vitro* cat tracheal preparation produces an increase in submucosal gland secretion by glandular contraction and macromolecular release. Their findings indicate that SP-induced glandular contraction in the airway is mediated by a peripheral cholinergic mechanism. Results reported by COLES *et al.* [26] also suggest that substance P appears to induce an increase in submucosal glandular output. However, they concluded that substance P evokes secretion by a direct action on a stereospecific receptor, rather than by inducing release of other endogenous secretagogues. The role of nonadrenergic noncholinergic innervation on the reflex control of airway secretions has been clearly demonstrated in cats. Pathogen–free cats, pretreated with atropine and propranolol, secreted mucus into the upper trachea after breathing dust into the lower trachea and lungs [5]. The inhaled dust presumably activated a reflex, which resulted in the release of neuropeptides (possibly SP) from within the epithelium. This may constitute an important pulmonary defence mechanism against inhaled airway irritants.

The present experiments report important new information describing the effects of substance P on airway secretions and rheology in an *in vivo* ferret model. As illustrated in figures 1 and 2, SP caused a dose-dependent decrease in mucus viscoelasticity (G* or rigidity) and a dose-dependent increase in mucus clearability, as reflected in an augmented frog palate transport rate. In line with the observations of previous investigators, SP administration also caused an increase in secretion volume output, an indication of the capacity of SP to act as a secretagogue.

A cough clearability index (CCI), expressed as mm displacement per normalized cough, can be derived from model studies of mucus clearance using a simulated cough machine [27]. It relates negatively with the rigidity factor, log G* 100, but positively with the recoil factory tan $\delta$ 100. In other words, cough clearability is impaired by mucus with high rigidity or resistance to deformation (high G*); it is also disfavoured when the mucus deformation is elastic rather than viscous, *i.e.* when the ratio of viscosity to elasticity (tan $\delta$) is low. In the latter case, mucus appears to recoil after the cough is completed [14]. In the present experiments, the changes in viscoelastic properties, thus, also predict and increased cough clearability in the mucus obtained after SP administration at both $10^{-5}$ and $10^{-4}$ M (table 1). It should be noted, that these considerations are based only on mucus bulk viscoelasticity, and do not consider any additional effects due to surface properties [28] or airflow dynamics, that could also lead to altered cough clearance.

An increase in mucus secretion might confer added protection to the tracheobronchial epithelium, either by impeding passage of inhaled agents or by diluting them. In support of this line of reasoning, cigarette smoke–induced mucus hypersecretion has been shown to decrease bronchial reactivity to aerosolized methacholine in dogs [29]. This reflexly mediated increase in mucus secretion constitutes an important safety mechanism for protecting the underlying epithelium.

Although the response to substance P was similar in many respects to that elicited by methacholine, there was an important difference, *i.e.* in the water content of the mucus. Methacholine, when given alone (*i.e.* prior to SP in experiment No. 2), caused an increase in volume output, a decrease in solids content and a decrease in viscoelasticity. This constitutes the classic secretagogue response *i.e* an outputting of a large volume of watery mucus, as described in earlier investigation [12, 30, 31]. WEBBER and WIDDICOMBE [32] reported similar results but added that methacholine is a potent stimulator of serous but not mucous cell secretions, while LEIKAUF *et al.* [31], in a different species, reported an effect on both serous and mucous cells. Substance P, when given alone (the dose-response experiment), caused first an increase in solids content, then ultimately a return to baseline, despite causing a significant decrease in viscoelasticity ($\gamma^*$); it also led to a significant increase in volume output. In experiment No. 2, when substance P was compared to methacholine, there was a greater decrease in mucus viscoelasticity and volume output (similar to that achieved in experiment No. 1), although with a slightly higher solids content.

The fact that the fall in viscoelasticity or rigidity associated with substance P was not accompanied by a fall in solids content, as was the case with methacholine, suggests that the administration of substance P led to the production of a mucus with decreased crosslinking capacity. This finding suggests that a different type of mucin macromolecule was secreted in response to the substance P, but it would also be consistent with the dilution of the mucus by serum exudate (adding solids - serum proteins - but not crosslinking) rather than by water, as seems to be the case with methacholine. It has been shown that serum proteins do not contribute strongly to the crosslinking of mucus [33]. This finding would also be consistent with the secretion of excess albumin [34]. The disparity of response, in terms of mucus water content, suggests that the secretagogue activity of methacholine and substance P involve different pathways.

The alterations in mucus rheology and volume with substance P may have arisen *via* three mechanisms.

Table 3. – Between-species comparison of control mucus from distal trachea obtained using a bronchoscopy brush

| Species | Collection time | n | Sample wt mg | log G* 1 rad·s$^{-1}$ | log G* 100 rad·s$^{-1}$ | tan δ 1 rad·s$^{-1}$ | tan δ 100 rad·s$^{-1}$ |
|---|---|---|---|---|---|---|---|
| Man | 30s | 20 | 2.06 | 2.19±0.39 | 2.58±0.40 | 0.30±0.07 | 0.99±0.26 |
| Dog | 10 min | 96 | 5.62 | 2.25±0.38 | 2.63±0.38 | 0.31±0.05 | 0.67±0.16 |
| Ferret | 30 min | 34 | 4.56 | 2.57±0.49 | 2.84±0.49 | 0.25±0.09 | 0.53±0.19 |

| Species | n | Mucociliary transport (NFPTR) | % SC |
|---|---|---|---|
| Dog | 96 | 0.71±0.24 | 12.3±4.7 |
| Ferret | 34 | 0.58±0.25 | 11.4±5.6 |

Results are expressed as mean±SD. NFPTR: normalized frog palate transport rate; SC: solids content. Data on Man from [37], Dog [38, 39], Ferret from present study.

Substance P may have diffused across the mucus layer acting directly on the seromucous glands. Alternatively, SP may have acted to reflexly increase mucus secretion *via* an afferent-efferent parasympathetic reflex arc, involving activation of muscarinic receptors on seromucous glands. In the third scenario, activation of sensory C-fibres by SP may have initiated local axon reflexes, liberating substance P in the vicinity of seromucous glands, and thereby increasing mucus secretion. It should be noted that, while the concentrations of SP in the present study are apparently nonphysiological, the effective concentrations are presumably much lower, due to dilution by airway surface fluid and rapid degradation by encephalinase present in the epithelium.

Whatever the mechanism, the final secretory response to substance P may also have involved the contributions of other peptides or mediators of inflammation, which would help to account for the divergence of the secretagogue response in terms of the mucus solids content. Substance P has been demonstrated to release inflammatory mediators from mast cells [35], and may play a role in mediating the wheal and flare skin response [36]. Co-release of other neuropeptides, such as calcitonin gene-related peptide (CGRP), may modify cholinergic-induced mucus secretion. Inhibition of methacholine-induced submucosal secretions by CGRP has recently been reported in a ferret *in vitro* tracheal preparation [34]. It is even possible that SP could evoke the parallel stimulation of degradative enzymes, which could contribute in part to the reduction in viscoelasticity. Thus, while substance P has been shown to be a potent secretogogue, the resulting changes in mucus rheology and volume in the present study may involve the interactions of other inflammatory mediators.

As this is the first report of the viscoelasticity and transportability of ferret tracheal mucus, we show in table 3 a comparison of the viscoelastic and transport properties of the mucus collected as control samples (=34) in the course of this study and compare these with the same measurements made on control samples of mucus collected from the distal trachea of man and beagle dogs [37–39]. Although the numerous species differences in airways size, type of anaesthesia used, and collection times, preclude a thorough statistical treatment of these data, for the most part all values are within one standard deviation of those reported for the other species. The degrees of similarity with mucus from other species, including man, indicate that the ferret is a useful model for clinically relevant studies of mucus rheology.

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## References

1. King M. – Mucus, mucociliary clearance and coughing. *In*: Respiratory Function in Disease. 3rd Ed. D.V. Bates, Saunders, 1989; pp. 69–78.
2. Coles SJ, Neill KH, Reid LM. – Potent stimulation of glycoprotein secretion in canine trachea by substance P. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1984; 57: 1323–1327.
3. Rogers DF, Awvdkij B. Barnes PJ. – Effects of tachykinins of mucus secretion in human bronchi *in vitro*. *Eur J Pharmacol*, 1989; 174: 283–286.
4. Hahn HL, Hummer B, *et al.* – Stimulation of mucous glands of the respiratory tract by neuropeptides: comparison of exogenous administration and endogenous liberation. *Pneumologie*, 1990; 44: 526–528.
5. Peatfield AC, Richardson PS. – The action of dust in the airways on secretion into the trachea of the cat. *J Physiol*, 1983; 342: 327–334.
6. Borson DB, Charlin M, Gold WD, Nadel JA. – Neural regulation of $^{35}SO_4$ -macromolecule secretion from tracheal glands of ferrets. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1984; 57: 457–466.
7. Baker B, Peatfield AC, Richardson PS. – Nervous control of mucin secretion into human bronchi. *J Physiol*, 1985; 365: 297–305.
8. Barnes PJ, Dewar A, Rogers DF. – Human bronchial secretion: effects of substance P, muscarinic and adrenergic stimulation *in vitro*. *Br J Pharmacol*, 1986; 89: 767P.
9. Rhodin JAG. – Ultrastructure and function of the human tracheal mucosa. *Am Rev Respir Dis*, 1966; 93: 1–15.

10. Dalsgaard CJ, Lundberg JM. – Evidence for a spinal afferent innervation of the guinea-pig lower respiratory tract as studied by the horseradish peroxidase technique. *Neurosci Lett*, 1984; 45: 117–122.
11. King M. – Magnetic microrheometer. *In*: Braga PC, Allegra L, eds. Methods in Bronchial Mucology. Raven Press, 1988; pp: 73–88.
12. King M, Vires N. – Effect of methacholine chloride on rheology and transport of canine tracheal mucus. *J Appl Physiol*, 1979; 41: 26–31.
13. Rubin BK, Ramirez O, King. M. – Mucus depleted frog palate as a model for the study of mucociliary clearance. *J Appl Physiol*, 1990; 169: 424–429.
14. King M. – Role of mucus viscoelasticity in cough clearance. *Biorheology*, 1987; 24: 589–597.
15. Lundberg JM, Brodin E, Saria A. – Effects and distribution of vagal capsaicin-sensitive substance P neurons with special reference to the trachea and lungs. *Acta Physiol Scand*, 1983; 119: 243–252.
16. Lundberg JM, Hökfelt T, Martling CR, *et al.* – Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. *Cell Tissue Res*, 1984; 235: 251–261.
17. Rangachari PK, McWade D, Donoff B. – Luminal tachykinin receptors on canine tracheal epithelium: functional subtyping. *Peptides*, 1987; 18: 101–108.
18. Luts A, Sundler F. – Peptide-containing nerve fibers in the respiratory tract of the ferret. *Cell Tissue Res*, 1990; 258: 259–268.
19. Baraniuk JN, Lundgren JD, Okayama M, *et al.* – Substance P and neurokinin A in human nasal mucosa. *Am J Respir Cell Mol Biol*, 1991; 4: 228–236.
20. Terenghi G, McGregor GP, Bhuttarcharji J, *et al.* – Vagal origin of substance P-containing nerves in the guinea-pig lung. *Neurosci Lett*, 1983; 36: 229–236.
21. Borson DB, Corrales R, Varsano S, *et al.* – Enkephalinase inhibitors potentiate substance P-induced secretion of $^{35}SO_4$-macromolecules from ferret trachea. *Exp Lung Res*, 1987; 12: 21–36.
22. Gashi AA, Borson DB, Finkheimer WE, *et al.* – Neuropeptides degranulate serous cells of ferret tracheal glands. *Am J Physiol*, 1986; 251: C223–C229.
23. Mizoguchi H, Hicks CR. – Effects of neurokinins on ion transport and sulfated macromolecule release in the isolated ferret trachea. *Exp Lung Res*, 1989; 15: 837–848.
24. Webber SE. – Receptors mediating the effects of substance P and neurokinin A on mucus secretion and smooth muscle tone of the ferret trachea: potentiation by an enkephalinase inhibitor. *Br J Pharmacol*, 1989; 98: 1197–1206.
25. Shimura S, Saskai T, Ikeda K, *et al.* – Neuropeptides and airway submucosal gland secretion. *Am Rev Respir Dis*, 1991; 143: S25–S27.
26. Coles SJ, Bhaskar KR, O'Sullivan DD, *et al.* – Airway mucus: composition and regulation of its secretion by neuropeptides *in vitro*. *Ciba Found symp*, 1984; 109: 40–60.
27. King M, Brock G, Lundell C. – Clearance of mucus by simulated cough. *J Appl Physiol*, 1985; 58: 1776–1782.
28. Girod S, Galabert C, Pierrot D, *et al.* – Role of phospholipid lining on respiratory mucus clearance by cough. *J Appl Physiol*, 1991; 71: 2262–2266.
29. De Sanctis GT, Kelly SM, Saetta MP, *et al.* – Hyporesponsiveness to aerosolized but not to infused methacholine in cigarette-smoking dogs. *Am Rev Respir Dis*, 1987; 135: 338–344.
30. Reasor MJ, Cohen D, Proctor DF, Rubin RJ. – Tracheobronchial secretions collected from intact dogs. II. Effects of cholinomimetic stimulation. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1978; 45: 190–194.
31. Leikauf GD, Ueki IF, Nadel JA. – Autonomic regulation of viscoelasticity of cat tracheal gland secretion. *J Appl Physiol: Respirat Environ Exercise Physiol*, 1984; 56: 426–430.
32. Webber SE, Widdicombe JG. – The actions of methacholine, phenylephrine, salbutamol and histamine on mucus secretion from the ferret *in vitro* trachea. *Agents Action*, 1987; 22: 82–85.
33. Litt M, Khan MA, Chakrin LW, *et al.* – The viscoelasticity of fractionated canine tracheal mucus. *Biorheology*, 1974; 11: 111–117.
34. Webber SE, Lim JCS, Widdicombe JG. – CGRP and methacholine-induced mucus secretion and epithelial albumin transport. *Eur Respir J*, 1990; 3: 280S.
35. Fewtrell CMS, Foreman JC, Jordan CC. *et al.* – The effects of substance P on histamine and 5 - hydroxytryptamine release in rat. *J Physiol*, 1982; 330: 393–411.
36. Lundberg JM, Saria A, Lundblad L, *et al.* – Bioactive peptides in capsaicin-sensitive C-fiber afferents of the airways: functional and pathophysiological implications. *In*: Barnes PJ, ed. The Airways: Neural Control in Health and Disease. Dekker, 1987; pp. 417–445.
37. Jeanneret-Grosjean A, King M, Michoud MC, *et al.* – Sampling technique and rheology of human bronchial mucus. *Am Rev Respir Dis*, 1988; 137: 707–710.
38. King M. – Interrelation between mechanical properties of mucus and mucociliary transport: effect of pharmacologic interventions. *Biorheology*, 1979; 16: 57–68.
39. King M, Rubin BK, Ramirez O, Zayas JG. – Mucus velocity, water content and viscoelasticity in beagle trachea show seasonal variations. *Chest* 1990; 98: 67s.