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Capsaicin and nicotine-sensitive afferent neurones and nasal secretion in healthy human volunteers and in patients with vasomotor rhinitis

[1]*,†P. Stjärne, *,†L. Lundblad, †J.M. Lundberg, & *,†A. Änggård

[1]*Department of Oto-rhino-laryngology, Karolinska Hospital, 10401 Stockholm, Sweden and †Department of Pharmacology, Karolinska Institute, 10401 Stockholm, Sweden

1 Applications of capsaicin, nicotine and methacholine were made locally onto the nasal mucosa in human controls and patients suffering from hyperreactive nasal disorders. Perception of sensation was registered as a sympton score and secretion quantified. The sensory reaction (irritation – pain) to capsaicin was similar in the three groups studied, i.e. controls, a group of patients with the diagnosis of vasomotor rhinitis and a group of patients with increased nasal secretion as the main symptom of the hyperreactive disorder. Nicotine induced only a mild itching sensation in the three groups. However, capsaicin and nicotine challenge caused a significantly larger secretory response in the last group than in the unselected vasomotor rhinitis group and in the control group.

2 Pretreatment with muscarinic receptor antagonists almost completely abolished the secretory response to both capsaicin and nicotine, and blocked methacholine-induced secretion. Furthermore, pretreatment with a combination of local anaesthetic and vasoconstrictor agent abolished the capsaicin-induced irritation, as well as the capsaicin- and nicotine-induced secretion on both the ipsilateral and the contralateral side. Therefore, no clearcut contribution seems to be exerted by locally released peptides from sensory neurones as direct trigger substances for the secretory response to capsaicin.

3 In conclusion, the nasal secretory response, in man, to both capsaicin and nicotine, seems to be mediated via cholinergic parasympathetic reflexes. In patients with hyperreactive non-allergic disorders of the nasal mucosa with rhinorrhea as the main complaint, the enhanced secretion may be due to a hyperreactive efferent cholinergic mechanism rather than hypersensitive irritant receptors on capsaicin- and nicotine-sensitive sensory neurones. Challenge with irritant agents seems a useful test for the evaluation of both afferent and efferent reflexogenic responses in hyperreactive disorders of the nasal mucosa.

Introduction

The pathophysiology of non-allergic hyperreactive disorders of the nose, i.e. vasomotor rhinitis, is unclear. Increasing evidence from experimental studies suggests that unmyelinated, capsaicin-sensitive sensory C-fibres play an important role in a variety of protective reflexes, such as vascular and secretory reactions, in the nasal mucosa upon inhalation of irritants (Lundblad *et al.*, 1985). The capsaicin-sensitive nerves in the nose have been shown to contain tachykinins such as substance P (SP) (Hökfelt *et al.*, 1977; Lundblad *et al.*, 1983; Uddman *et al.*, 1983) and calcitonin gene-related peptide (CGRP) (Uddman *et al.*, 1985). These SP- and CGRP-containing nerves are distributed in a

[1] Author for correspondence: Department of Oto-rhino-laryngology, Karolinska Hospital, S-10401 Stockholm, Sweden.

dense network under and within the lining epithelium of the nasal mucosa as well as around blood vessels (Lundblad *et al.*, 1983, Uddman *et al.*, 1985.

Capsaicin, the pungent agent in hot pepper and paprica, which selectively activates chemosensitive C-fibre afferents, induces a local and central release of SP, CGRP and other neuropeptides from these neurones (Gamse *et al.*, 1979; Saria *et al.*, 1986; Hua *et al.*, 1986). In a preliminary study, we demonstrated that local application of capsaicin to the human nasal mucosa induces sneezing and secretion (Lundblad *et al.*, 1987a,b). The mechanism underlying this effect in man is unclear, even though SP is known to be a powerful secretagogue in some experimental animals (Pettersson *et al.*, 1985). Nicotine on the other hand, excites sensory nerves in the nasal mucosa which are probably different from those activated by capsaicin (Lundblad *et al.*, 1985).

The parasympathetic nervous system innervates the glands of the nasal mucosa (Cauna *et al.*, 1972). Muscarinic receptor stimulation by methacholine induces a profuse watery secretion upon local challenge (Borum 1979). The preganglionic parasympathetic fibres to the nasal mucosa run together with the sympathetic fibres in the Vidian nerve to the sphenopalatine ganglion. It has been shown that Vidian neurectomy has an inhibitory effect on the rhinorrhea observed in patients with nasal allergy and intramuscular injection of atropine blocks the hypersecretion (Konno & Togawa, 1979a).

Histamine and methacholine challenge have been suggested as useful tests for evaluation of nasal hyperreactivity (Borum *et al.*, 1983). Histamine stimulates sensory receptors and produces reflex secretion from the nasal glands but in addition has a direct effect on the nasal glands, inducing secretion and transudation (Konno & Togawa, 1979b).

Methacholine on the other hand, acts directly on the glandular cells. Therefore, methacholine challenge evaluates only the final step of the efferent part of the reflex arc. To characterize nasal hyperreactivity, a substance which selectively activates the reflex arc by stimulating afferent sensory receptors in the nasal mucosa would be preferable.

The aim of the present study was to quantify and characterize the subjective sensation and secretory effect induced by two sensory irritants, capsaicin and nicotine. The secretory effects of the substances were compared to the effect of methacholine. The agents were applied locally to the human nasal mucosa in three different groups tested: normal subjects, patients with unspecific vasomotor rhinitis and patients with increased nasal discharge as the main complaint. Furthermore, anticholinoceptor drugs and local anaesthetics were given as pretreatment before capsaicin, nicotine and methacholine application to investigate the nature of the reflex mechanism.

Methods

Normal subjects were selected from medical students (mean age: 32 years, male/female: 50/50%) at the Karolinska Hospital. They had no history of nasal disease or nasal allergy, smoking or ongoing drug treatment. The patients with vasomotor rhinitis came from the outpatient department at the ENT-clinic (mean age: 39 years, male/female: 37/63%). Vasomotor rhinitis was diagnosed by the following clinical symptoms: sneezing and rhinorrhea and/or nasal congestion. There was no history of atopy or allergic disease. We chose to study an unselected group of patients with the diagnosis of vasomotor rhinitis (the group is referred to in the text as VMR) and compare it to another group of patients with vasomotor rhinitis with sneezing and rhinorrhea as dominating symptoms (VMR-d).

To evaluate the subjective sensation caused by the application of capsaicin to the nasal mucosa a symptom score was used (scale graded from 0–10, where 1 represented slight irritation, 5 burning sensation and 10 severe pain). The subject was asked to mark a subjective score on the scale after each application. The number of sneezing discharges within 2 min of application of the substances was also registered.

The exocrine secretion from the nasal mucosa was absorbed by a rectangular piece of absorbant paper, 5 × 50 mm (Knowles *et al.*, 1981; Pettersson *et al.*, 1985) preweighed in a test tube. The piece of paper was placed against the septal wall in the nasal cavity to be tested. In addition, in some experiments a piece of paper was also inserted into the contralateral nostril. After 2 min the paper was removed and again weighed. Drugs were applied in a volume of 50 μl to the anterior part of the inferior turbinate with a micropipette. The following substances, in stock solution, were used for application: capsaicin (initially dissolved in 70% ethanol and further diluted in saline; Sigma), nicotine bitartrate (diluted in saline) and methacholine bromide (diluted in saline). Atropine, 0.5 mg (ACO), was given i.m. in one group and in four of the VMR-d patients combined with ipratropium bromide 0.25 mg ml^{-1}, 200 μl (Atrovent, Boehringer-Ingelheim). The ipratropium was sprayed into the nasal cavity 30 min before the application of capsaicin.

Cotton strips were soaked in lignocaine chloride 4% (Xylocaine, Astra); naphazoline chloride

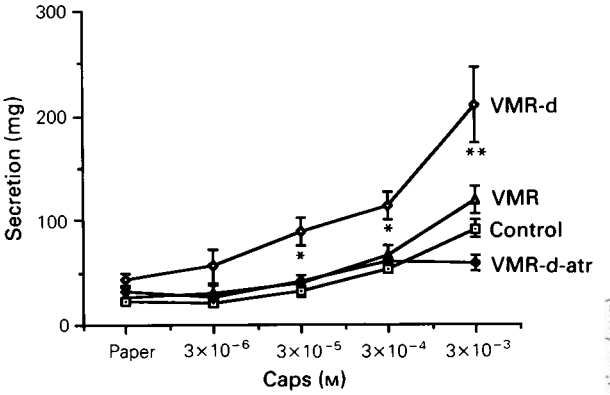

Figure 1 Secretory response upon the introduction of filter paper into the nasal cavity and subsequently upon local applications of capsaicin to the inferior turbinate of the human nasal mucosa in dilutions of 3.3×10^{-6} M – 3.3×10^{-3} M in a volume of 50 μl. Control group (□, $n = 17$), VMR-group (Δ, $n = 10$), VMR-d-group (◇, $n = 8$) and VMR-d pretreated with i.m. atropine and local ipratropium bromide (VMR-d-atr) (◆, $n = 4$). Responses are means with s.e.mean shown by vertical bars. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to control. Student's t test.

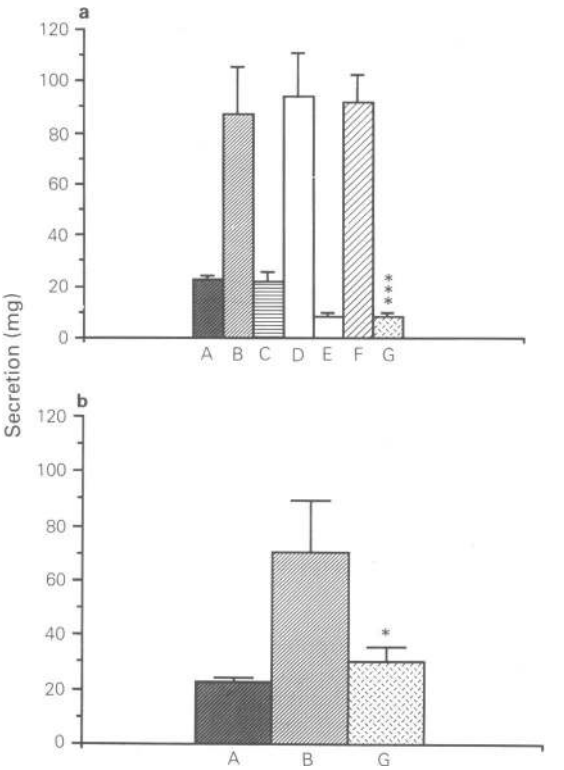

Figure 2 Secretory response upon local application of capsaicin, 3.3×10^{-3} M, in a volume of 50 μl to the inferior turbinate on the ipsilateral side (a) and on the contralateral side (b), before and after pretreatment on the stimulated side with lignocaine chloride 4% naphazoline chloride 0.02 mg ml^{-1} or lignocaine chloride 3.4% mixed with naphazoline chloride 0.02 mg ml^{-1}. (A) Control; (B) capsaicin 3.3×10^{-3} M; (C) lignocaine; (D) capsaicin and lignocaine; (E) naphazoline; (F) capsaicin and naphazoline; (G) capsaicin, naphazoline and lignocaine. The responses are means with s.e.mean shown by vertical bars. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Quade analysis. $n = 5$.

0.02 mg ml^{-1} (Naphazoline, Leo); naphazoline chloride 0.02 mg ml^{-1} mixed with lignocaine chloride 3.4% or ipratropium bromide 0.25 mg ml^{-1}. The strips were packed into the nasal cavity and left there for 15 min. Capsaicin 3.3×10^{-3} M, nicotine 6.5×10^{-3} M or methacholine 5×10^{-4} M, in a volume of 50 μl, was then applied locally to the inferior turbinate in the pretreated nostril.

The study was approved by the ethical committee of the Medical Faculty at the Karolinska Hospital. Informed consent was obtained from all the subjects included in the study.

Statistical and numerical analysis: the mean and standard error of the mean (s.e.mean) are used to describe central tendency and variation throughout the study. Student's t test and Quade analysis (Theodorsson-Norheim, 1987) were used to test the statistical significance. P values of <0.05 were taken as significant.

Results

Upon local nasal application of capsaicin all subjects responded with a concentration-dependent increase in nasal sensations, as revealed by the symptom score, and nasal secretion. Sneezing discharge was discarded as a parameter since it was quite clear that subjects could suppress the reaction. In a low dose the subjects mainly perceived irritation, while upon higher concentrations increasingly severe pain was reported. The subjective sensation plotted on a symptom score scale (not shown) showed no statistical differences between the groups. The secretory effect of capsaicin, on the other hand, was significantly greater in the VMR-d group than in the other groups in all but the lowest concentration (see Figure 1). There was also a tendency, although not statistically significant, for the VMR group to secrete more than the controls.

Atropine i.m. had no effect on nasal secretion induced by capsaicin application ($n = 10$, not

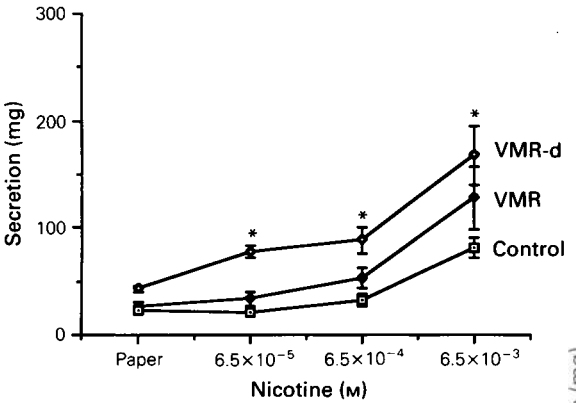

Figure 3 Secretory response upon local applicaion of nicotine to the inferior turbinate in dilutions of 6.5×10^{-5} M – 6.5×10^{-3} M in a volume of 50 μl. Control group ($n = 10$), VMR-group ($n = 10$) and VMR-d-group ($n = 4$). The responses are means with s.e.mean shown by vertical bars. $*P < 0.05$; $** < 0.01$. Student's t test, compared to controls.

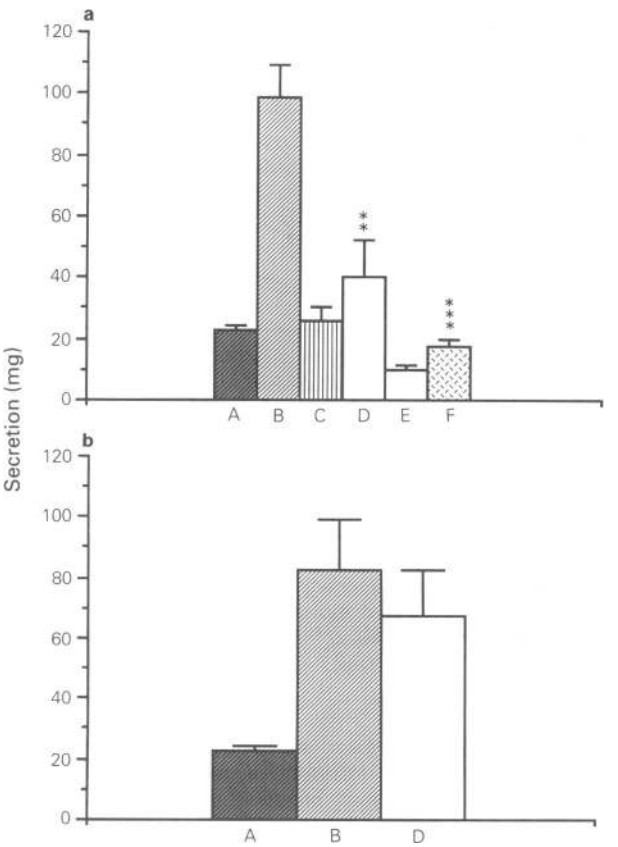

Figure 4 Secretory response upon local application of nicotine 6.5×10^{-3} M in a volume of 50 μl to the inferior turbinate on the ipsilateral side (a) and on the contralateral side (b), before and after pretreatment on the stimulated side with ipratropium bromide 0.25 mg ml^{-1} or lignocaine chloride 3.4% mixed with naphazoline chloride 0.02 mg ml.$^{-1}$ The responses are means with s.e.mean shown by vertical bars. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Quade analysis. $n = 5$. (A) Control; (B) nicotine 6.5×10^{-3} M; (C) ipratropium; (D) nicotine and ipratopium; (E) naphazoline; (F) nicotine, naphazoline and lignocaine.

shown). However, atropine i.m. in combination with ipratropium, sprayed locally into the nasal cavity, caused a marked reduction of the secretory response in the VMR-d group (Figure 1).

When capsaicin (3.3×10^{-3} M) was applied to the inferior turbinate, a matched secretion was also evoked from the contralateral side which was not significantly lower than that on the stimulated side (see Figures 2a and b). Pretreatment with 4% lidocaine did not have any effect on basal secretion (see Figure 2a). Capsaicin (3.3×10^{-3} M), when applied in the lidocaine pretreated nostril, induced a secretory effect which was not significantly lower than the control response (see Figure 2a). Furthermore, the individuals reported a symptom score of the sensation which was similar to the control situation (not shown). Naphazoline, a vasoconstrictor agent, caused a significant reduction *per se* (about 60%) in the basal secretion level (see Figure 2a). However, when capsaicin (3.3×10^{-3} M) was applied to the inferior turbinate of the naphazoline pretreated side, the secretory effect was not inhibited (see Figure 2a). To improve the action of local anaesthetics, lignocaine was combined with naphazoline as pretreatment. Upon subsequent capsaicin (3.3×10^{-3} M) application, the subjects perceived no sensation of irritation and, furthermore, the secretory effect was abolished (the ipsilateral secretion level measured was lower than the basal level obtained when inserting the filter paper in the unstimulated nostril) (see Figures 2a and b).

Nasal application of nicotine produced a concentration-dependent secretion in all the groups studied (see Figure 3). There was a significantly greater secretory response in the VMR-d group compared to the normal subjects (controls). Nicotine induced only a mild itching sensation, which did not seem to be dose-dependent (not shown). Nicotine (6.5×10^{-3} M) caused a secretory response on the contralateral side that was similar to that on the stimulated side (see Figures 4a and b). Unilateral pretreatment with ipratropium caused a significant reduction of the secretion on the nicotine stimulated

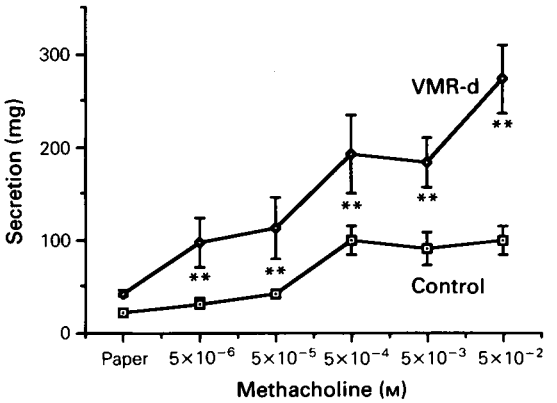

Figure 5 Secretory response upon local application of methacholine to the inferior turbinate in dilutions of 5×10^{-6} M – 5×10^{-2} M in a volume of 50 µl. Control group ($n = 10$), VMR-d-group ($n = 8$). The responses are expressed as means with s.e.mean shown by vertical bars. $*P < 0.05$, $**P < 0.01$, $***P$0.001. Student's t text.

side, but not on the contralateral side (see Figures 4a and b). After pretreatment with a combination of lignocaine and naphazoline the secretory effect of nicotine was abolished (see Figure 4a).

Nasal application of methacholine induced a concentration-dependent secretion (see Figure 5). The methacholine response was significantly higher

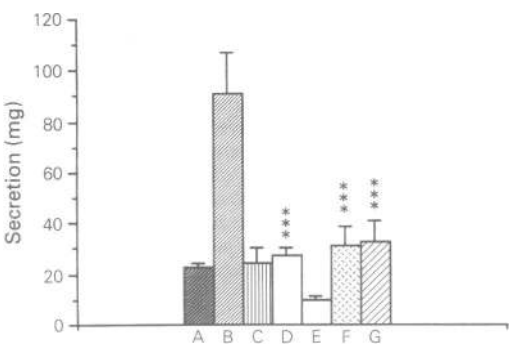

Figure 6 Secretory response upon local application of methacholine, 5×10^{-4} M, to the inferior turbinate before and after pretreatment with ipratropium bromide 0.25 mg ml^{-1}, naphazoline chloride 0.02 mg/ml^{-1} or lignocaine chloride 3.4% mixed with naphazoline chloride 0.02 mg ml^{-1}. (A) Control, (B) methacholine; (C) ipratropium; (D) methacholine and ipratropium; (E) naphazoline; (F) methacholine, naphazoline and lignocaine; (G) methacholine and naphazoline. The responses are means with s.e.mean shown by vertical bars. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Quade analysis. $n = 5$.

in the VMR-d group than in the controls (see Figure 5). Methacholine did not produce any subjective sensation. After pretreatment with ipratropium, the secretory response to methacholine 5×10^{-5} M was abolished (see Figure 6). Pretreatment with lignocaine combined with naphazoline caused a significant reduction of the secretory response to methacholine 5×10^{-4} M (see Figure 6). However, pretreatment with naphazoline alone also caused a marked reduction in the secretory response to methacholine (Figure 6).

Discussion

In the present study, capsaicin locally applied to the human nasal mucosa elicited a concentration-dependent sensation of irritation and pain as well as a secretory response. The capsaicin-induced secretion was considerably larger in the vasomotor rhinitis patients with enhanced secretion as the main complaint. When using methacholine challenge to reveal secretory hyperreactivity (Borum, 1979), there has been some question whether nasal absorption of the applied substance was more prominent in the hyperreactive group so explaining the observed increased response. The present study indicates that this is not the case with capsaicin. Thus, there was no significant difference in the subjective symptom scores in the groups upon capsaicin application.

Various methods have been described for measuring nasal secretion to a challenge. Konno & Togawa (1979a) used suction, Secher *et al.* (1982) preferred weighing handkerchiefs after nose blowing and Johansson & Deuschl (1976), Linder *et al.* (1983) and Naclerio *et al.* (1983) all describe different techniques for nasal lavage with or without a tracer substance. The technique used in our study for the collection of nasal secretion has been described earlier by Knowles and co-workers (1981) and by Pettersson *et al.* (1985). This technique obviously has its limitations, since the method will only permit the collection of secretion from a selected area of the nasal mucosa. However, an equal proportion was likely to have been obtained in each experiment since in all subjects the filter paper and substances were applied to the same anatomical site. However, it seems that the method underestimates a high secretory response.

The mechanism underlying the secretory effect of capsaicin and nicotine may be complex. It has been shown that SP- and CGRP-IR nerves are present in the human nasal mucosa around some exocrine elements, mainly ducts (Stjärne *et al.*, unpublished observations). SP is known to be a potent secretagogue, at least in the nasal mucosa of the rat and

dog (Rackham *et al.*, 1981; Pettersson *et al.*, 1985). Hypothetically, the SP-induced secretion may be secondary to mast cell degranulation (Hägermark *et al.*, 1978) and subsequent histamine release (Konno & Togawa, 1979b). From experimental studies (Lundblad & Lundberg, 1984), it can be deduced that the mechanism underlying the secretory response to capsaicin may be: (1) a local axon reflex with the subsequent release of mediators from sensory nerves acting on the exocrine elements in the nasal mucosa, (2) a parasympathetic reflex relayed via axon collaterals of sensory nerves in the sphenopalatine ganglion, or (3) a centrally-mediated parasympathetic reflex (see Figure 7).

The secretory effect of capsaicin could be totally blocked, both on the side of application and on the contralateral side, using a combined pretreatment with local anaesthetic and a vasoconstrictor agent. This indicates that the major portion of the secretory effect of capsaicin is reflex mediated. The failure of lignocaine alone to block the secretion is probably due to the fact that the nasal mucosa is highly vascularized, which prevents the relatively more hydrophilic lignocaine molecule penetrating as deeply into the tissue as capsaicin, which is known to be very lipophilic. This is supported by the fact that subjects upon capsaicin application after lignocaine pretreatment indicated a symptom sensation score that was not significantly lower than in the control situation. In subjects pretreated with lignocaine/naphazoline, where the secretion was almost abolished, no sensation was perceived upon the capsaicin application. To verify that the blocking effect of lignocaine/naphazoline on the secretory response, was due to a nerve blockade and not a secondary effect due to the vasoconstriction induced by naphazoline, pretreatment with naphazoline alone was performed. The subsequent capsaicin application induced a nasal secretion which was not significantly lower than in the control situation with stimulation by capsaicin in the untreated nostril. This suggests that the blood flow decrease induced by naphazoline, which reduced the secretory response seen on methacholine application, was counteracted upon capsaicin application by an associated vasodilatation induced by local release of neuropeptides, most likely of both sensory (SP, CGRP) and parasympathetic (vasoactive intestinal polypeptide, VIP) origin (Lundblad *et al.*, 1983).

The reflex seems to be centrally-mediated since the ipsilateral application of capsaicin induces a contralateral secretory response. The finding that locally applied ipratropium together with systemic pretreatment with atropine almost abolished the secretory response to capsaicin strongly suggests a parasympathetic cholinergic involvement in the reflex arc, acting on muscarinic receptors as the final step

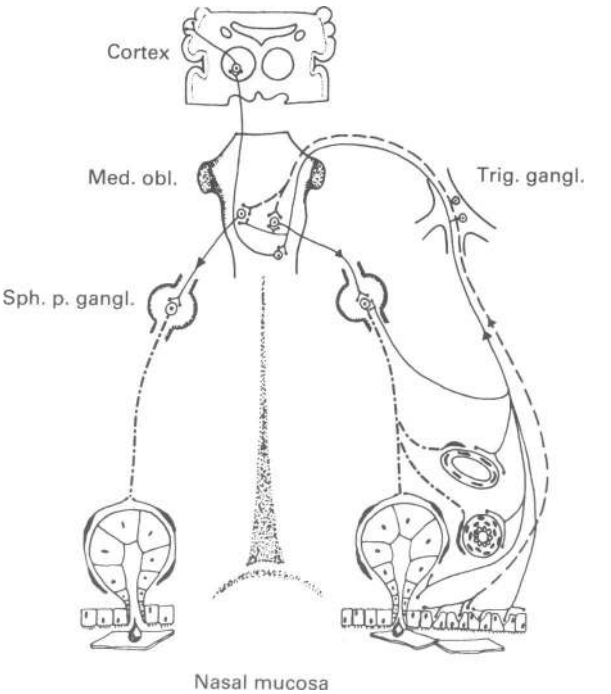

Figure 7 Schematic drawing of the vascular and secretory innervation of the human nasal mucosa. At least two different populations of chemo-sensitive sensory neurones seem to be present. One is the capsaicin-sensitive C-fibre afferents (———), containing substance P and calcitonin gene-related peptide, with axonal branches to the arterioles and venous sinosoids, to the ductal portion of the exocrine glands and to the epithelium which they penetrate. The other population is the nicotine-sensitive afferents (- - - - -). Both types of sensory nerves terminate in the medulla oblongata, (Med. obl.,) where they are relayed to the cortex and to the preganglionic parasympathetic neurones that project to the sphenopalatine ganglion cells (Sph. p. gangl) in the pterygo-palatine fossa; here they synapse with postganglionic cholinergic neurones (–.–.–), also containing vasoactive intestinal peptide, in the final step of the reflex arc to exocrine secretion.

(Davies *et al.*, 1982). Similar results have been obtained with capsaicin-induced salivation in man (Duner-Engström *et al.*, 1986). However, a slight nasal secretory response was still present at high doses in the present study, indicating either incomplete blockade of muscarinic receptors or an effect on the exocrine glands mediated by locally released neuropeptides.

Nicotine is likely to activate a population of sensory nerves in the nasal mucosa other than the

capsaicin-sensitive C-fibre afferents, at least as regards sneezing discharges in the guinea-pig (Lundblad *et al.*, 1985). A similar situation seems to be present in man, since completely different sensations were perceived upon nicotine and capsaicin applications. Nicotine, however, also induced nasal secretion, which indicates that at least two populations of sensory nerves in the human nasal mucosa are linked to parasympathetic reflexes. This was also verified by the finding that pretreatment with lignocaine/naphazoline blocked the nasal secretory response upon nicotine application and that local pretreatment with ipratropium significantly reduced the nasal secretion upon nicotine application on the stimulated side, whereas it had no effect on the secretory response on the unpretreated contralateral side. Concerning the irritant effect of cigarette smoke on the nasal mucosa, however, vapour phase components other than nicotine seem to be the main irritants, at least in the guinea-pig (Lundblad & Lundberg, 1984).

According to Mygind (1978), the vasomotor rhinitis patients may be divided into two 'main symptom' groups suffering from nasal congestion ('blockers') or hyperrhinorrhea ('runners'). It is possible that these two groups represent conditions with different pathophysiological mechanisms. Assuming that the same population of sensory nerve fibres is involved in sensation and in the reflex arc evoked secretion, our finding that there was no significant differences between the groups regarding the symptom score for irritation and pain upon capsaicin application, together with the fact that the VMR-d group produced a significantly higher secretory response than the controls on capsaicin, nicotine and methacholine application, would indicate that the hyperreactivity is most likely not directly related to the sensory nerve endings. Thus, the hypersecretory response may be related to a change at the cholinoceptor site on the glandular cell and/or glandular hypertrophy since the capsaicin-induced secretion, but not sensation, was almost abolished by ipratropium pretreatment. In patients with nasal allergy (Ishibe *et al.*, 1983), it has been demonstrated that there is an increase in the number of muscarinic receptors and a decrease in the number of α_1- and β-adrenoceptors. Whether this occurs in non-allergic disorders of the nasal mucosa is not known.

It is known from earlier studies that tachykinins induce a slow e.p.s.p. from ganglion cells and this could result in a facilitation of ganglionic transmission (Otsuka & Konishi, 1983). However, the symptom score for the subjective sensations in the VMR-d group was not significantly higher than in the other groups, which indicates that sensory transmission to the CNS was not altered in the diseased group. On the other hand, a constant exposure to irritants might, via increased sensory activity and parasympathetic tone, lead to a hypertrophy of the glandular tissue. However, it is important to point out that activation of receptors on the sensory neurones in the nasal mucosa is of primary importance in the protective responses induced by irritants in the environment. In this respect, the parasympathetic secretory reflex is one in an arsenal of protective responses that can be activated via the sensory irritant receptors (Lundblad & Lundberg, 1984). However, it remains to be established whether capsaicin desensitization and damage of the afferent part of the reflex arc can reduce the nasal secretory hyperreactivity to environmental stimuli in man, considering that the nicotine- sensitive sensory nerves are likely to be left intact.

During the completion of our study, Gepetti and co-workers (1988) have published results which in some aspects are contradictory to our findings. These authors proposed that capsaicin induces secretion in the human nasal mucosa primarily via local release of transmitter(s) from peripheral terminals of primary sensory neurones, but reported that local application of SP and CGRP did not reveal any secretory response. Our data with local anaesthetics and muscarinic receptor antagonists suggest that local release of tachykinins and CGRP may only play a minor direct role in the secretory response to local capsaicin application upon the human nasal mucosa *in vivo*. Furthermore, the finding of Gepetti *et al.* (1988) that ipratropium had no effect on capsaicin-induced secretion is also in apparent contradiction to the present data, but may be due to an incomplete blockade of muscarinic receptors.

In conclusion, our findings suggest that the secretory effect of capsaicin and nicotine in the human nasal mucosa is mediated via a central parasympathetic reflex arc with a final muscarinic receptor mechanism. Capsaicin and nicotine seem to activate different populations of sensory neurones. According to our data, no clearcut contribution is exerted by locally released tachykinins and CGRP as direct trigger substances for the secretory response to capsaicin. We propose that challenge with irritant agents is the appropriate test when evaluating both the afferent and the efferent pathways of reflexes in hyperreactive disorders of the nasal mucosa. The cause of enhanced secretion in patients with hyperreactive non-allergic disorders in the nasal mucosa is less likely to depend on hypersensitive irritant receptors on capsaicin-sensitive sensory neurones, providing that the same population of afferents mediate both the irritation sensation and the reflex cholinergic secretory response.

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