Rhinology, 30, 149-160, 1992

Influence of sympathetic and parasympathetic substances. in· clinical concentrations on human nasal ciliary beat

K.J.A.O. Ingels, F. Meeuwsen, K. Graamans and E.H. Huizing

Dept. of Otorhinolaryngology, University Hospital, Utrecht, The Netherlands

SUMMARY

The effect of autonomic substances on ciliary beat in mucosa! biopsies from the normal human nose was studied. The influence on ciliary beat frequency (CBF) as well as on ciliary beat harmony was measured photoelectrically. The sympathetic /3-agonist isoprenaline was found to have a stimulatory effect on CBF, whereas the a-agonist xylometazoline reduced CBF and ciliary beat harmony. The parasympathetic agonist carbachol had a positive effect on CBF. The sympathetic antagonists timolol (/3) and phentolamine (a) as well as the parasympathetic antagonist atropine had no effect. The effect of the agonists on ciliary beat was absent when they were administered in combination with their specific antagonists. These experiments indicate that, in clinical concentrations, the autonomic substances modify ci/ia,y beat by a direct effect on the ciliated cells.

INTRODUCTION

The effects of sympathetic and parasympathetic drugs (autonomic drugs or substances) on normal (Camner et al., 1976; Chopra, 1978; Camner et al., 1986; Konietzo et al., 1975; Kordik et al., 1952; Pavia et al., 1979; Verdugo et al., 1980; Pavia et al., 1983) and diseased (Mossberg et al., 1976; Ohashi et al., 1983; Santa Cruz et al., 1974) respiratory mucosa are well known.

Sympathetic /J-agonists were found to stimulate ciliary beat frequency **(CB:f)** both in vitro (Ohashi et al., 1983; Wolf et al., 1988) and in viva (Hybbinette and Mercke, 1982; Weiss et al., 1981). More specifically, they were shown to affect the ßrreceptors (Wolf et al., 1988; Wong et al., 1988). The effect of sympathetic a-agonists on ciliary beat has not yet been completely elucidated. They have been reported to decrease (Simon et al., 1977) as well as to increase (Sakethoo et al., 1978) mucociliary transport and, in a third study (Van de Dank et al., 1982), to

have no influence at all. Experiments with parasympathetic agonists resulted in even more contradictory results. Hybbinette and Mercke (1982) found that metacholine produced a dose-dependent acceleration of mucociliary activity. King and Viires (1979), on the other hand, reported a decrease in mucus transport in the canine trachea. A concentration of 10^{-3} M carbachol did not change CBF in the experiments of Wolf et al. (1988) with cultured human respiratory epithelium.

One of the main reasons for these contradictory results might be that the data are not comparable, because of the different measuring procedures for ciliary activity (mucociliary transport, ciliary beat frequency, mucociliary activity) that have been used. A second factor is the differences in mucosal biopsy technique and the origin of mucosa. It is important to take into account whether the drugs are administered directly to the mucosa (in vitro or in vivo) or systemically. The majority of the experiments was carried out in animals. Some experiments studied normal human mucosa, others studied diseased human mucosa. So far, little information is available on the effects of autonomic substances on nasal mucosa. We therefore investigated the effect of the /J-sympathomimetic isoprenaline sulphate, the a-sympathomimetic xylometazoline.HCl, and the parasympathomimetic carbachol on human nasal CBF as well as on ciliary beat harmony. The ciliary beat harmony was quantified in terms of photoelectrical-signal consistency (SC), according to a method described by our group elsewhere (Ingels et al., in press). Table 1 shows the agonists, with their antagonists, that were investigated in this study. The /J-sympathomimetic isoprenaline sulfate is frequently prescribed in bronchospasm as an aerosol with a concentration of 1 mg/ml, which is equal to 1.9×10^{-3} M. The a-sympathomimetic xylometazoline. HCl is a decongestant; its concentration in nose drops is usually 0.1 % equaling 3.6×10^{-3} M. The parasympathomimetic drug carbachol is used in eye drops in a concentration of 1.5% (= 8.2×10^{-2} M).

Table 1. Classification of autonomic substances used in the experiments.

	Agonist	Antagonist
Sympathetic:		
/J	isoprenaline	timolol
a	xylometazoline	phentolamine
Parasympathetic:	carbachol	atropine

MATERIALS AND METHODS

The effects of autonomic substances on ciliary beat were investigated in forceps biopsies taken from normal human nasal mucosa.

Substances studied

The effect of isoprenaline sulphate on CBF and ciliary beat harmony was studied in six specimens, that of xylometazoline.HCl in five, and that of carbachol in six. All three drugs were dissolved in aqueous CMRL-1066, in a series of increasing concentrations of 10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2} M. They were tested in the pure form, without the addition of preservatives. The pH of all solutions remained 7.5 because of the buffer capacity of the medium. Osmolarity ranged from a minimum of 315 mosm/1 to a maximum of 335 mosm/1. In a previous study we found that within this range CBF is not affected (Ingels, 1991).

Biopsy method

The present study was performed on 23 volunteers, who were to be operated upon in our Department for reasons not related to respiratory mucosa! pathology. They all signed a volunteer declaration - as proposed in the 1964 "Declaration of Helsinki" of the World Medical Association, and reviewed in 1975 in Tokyo. The exclusion criteria were acute rhinitis in the two weeks preceding the study, chronic rhinitis, allergy, a history of nasal surgery, gross deformities of nasal anatomy, smoking, and all medications except contraceptives. The biopsies were obtained from the posterior part of the inferior turbinate of the most patent side of the nose. They were taken under general anaesthesia, prior to surgery, by means of a Gerritsma forceps (Entermed, Linschoten, The Netherlands), as described earlier by Fokkens et al. (1988) and Ingels (1991). Each specimen was collected into enriched CMRL-1066 medium (Yager et al., 1978). The medium was continually oxygenated with a mixture of 5% 0_2 and 95% CO_2.

Experimental design

The sample was fixed by two glasses with a silicone ring in between, leaving a compartment with a volume of 0.4 ml (Figure 1). In all experiments the biopsy

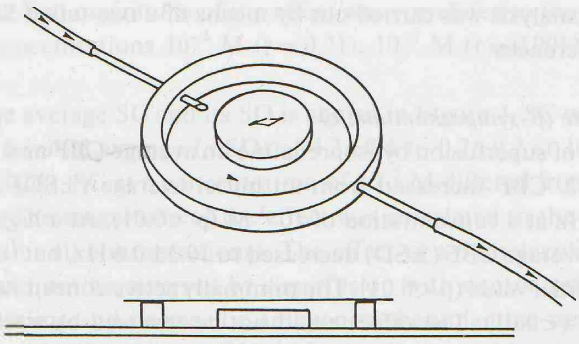

Figure 1. Schematic drawing of the superfusion chamber. Arrow heads indicate direction of fluid flow.

specimen was first superfused with CMRL-1066 medium before any initial pbotoelectrical recordings were made. The sample was then superfused with the lowest concentration of the drug, after which a second recording was made. Further superfusions were done with successively higher concentrations, and corresponding recordings were made. After each superfusion an attempt was made to reverse the effect by washing out the substance with neutral CMRL-1066 medium. All superfusions were done at a flow rate of 0.67 ml/min.

In order to establish whether a recorded effect was receptor mediated, a control experiment was carried out. The minimally effective concentration of the agonists (isoprenaline sulphate, xylometazoline.HCl and carbachol) was administered in combination with the specific antagonist (timolol maleate, phentolamine, atropine sulphate) in the same concentration. This control experiment was performed on a fresh sample. Prior to the control experiments, we excluded the possibility that the antagonist itself would affect ciliary beat.

Signal recording and analysis

Ciliary movements were recorded photoelectrically by the technique we described previously (Ingels et al., 1990). The combined signal, comprising different sinusoidal waves, was sorted out off-line by Fourier transform analysis. In the resulting power spectrum, the first harmonic represents the basal CBF. The reciprocal value of the average SD of the waveform over a 0.2s period is a measure for the photoelectrical-signal consistency (SC). This value is correlated to ciliary beat harmony, as we found in previous experiments (Ingels et al., in press). Care was taken to measure one single cell during the whole experiment, because of the intercellular differences in CBF (Ingels et al., 1990). All measurements were carried out 5 min after discontinuation of the superfusion, to exclude mechanical effects caused by the procedure (Ingels, 1991).

RESULTS

Statistical analysis was carried out by means of a two-tailed Student's t-test for paired differences.

Isoprenaline ((3-sympathomimetic)

The effect of superfusion by isoprenaline on average CBF and its SD is depicted in Figure 2. CBF increased from an initial average (±SD) of 8.2±0.6 Hz to 10.5±0.6 Hz at a concentration of 10^{-3} M ($p < 0.01$). At a higher concentration (10^{-2} M), average CBF (±SD) decreased to 10.2±0.6 Hz, but this was still higher than the initial value ($p < 0.01$). The minimally active concentration was found to be 10^{-5} M (<0.01). The effect could not be reversed by washing with neutral CMRL-1066 medium. There was no effect on the SC ($p > 0.75$, for all concentrations).

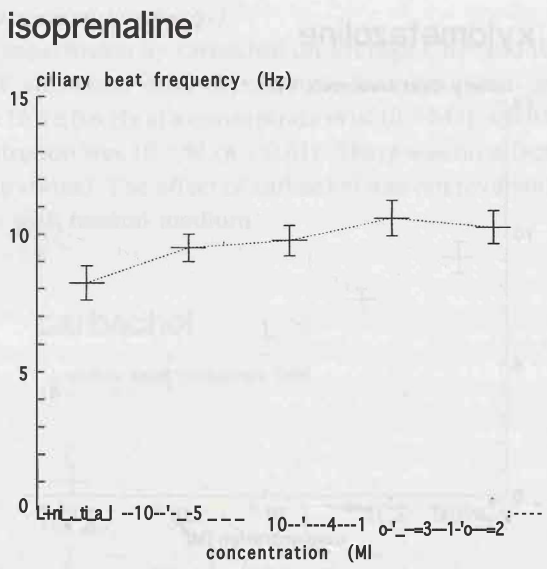

Figure 2. Average ciliary beat frequency (CBF) with SD in relation to increasing concentrations of isoprenaline (six subjects).

When 10^{-5} M isoprenaline was administered together with its specific antagonist timolol in the same concentration, no effect could be observed on either CBF or SC. Nor could an effect of 10^{-5} M timolol alone be recorded.

Xylometazoline (a-sympathomimetic)

The effect of xylometazoline on average CBF and its SD is shown in Figure 3. CBF decreased dose-dependently from an initial average (±SD) of 9.1±0.6 Hz to 0.6±0.6 Hz at a concentration of 10^{-2} M ($p < 0.01$). In four of the five cases, a complete ciliostasis was seen at 10^{-2} M. The minimally active concentration was 10^{-4} M ($p < 0.05$). The magnitude of the effect was correlated to the initial CBF values for the concentrations 10^{-4} M ($r = 0.71$), 10^{-3} M ($r = 0.90$) and 10^{-2} M ($r = 0.63$).

The effect on the average SC and its SD is shown in Figure 4. SC was found to decrease from an initial average (±SD) of 4.3±0.6 to 0.2±0.2 at 10^{-2} M xylometazoline ($p < 0.01$). SC at a concentration of 10^{-5} M differed from the initial value ($p < 0.05$). The magnitude of the effect was correlated to the initial CBF values ($r > 0.90$; for all concentrations). The effect of xylometazoline on both CBF and SC could not be reversed by superfusion with neutral medium.

When xylometazoline and its specific antagonist phentolamine were administered together, in a concentration of 10^{-4} M, no effect could be seen, either on CBF or on SC. Nor could an effect of phentolamine alone be recorded.

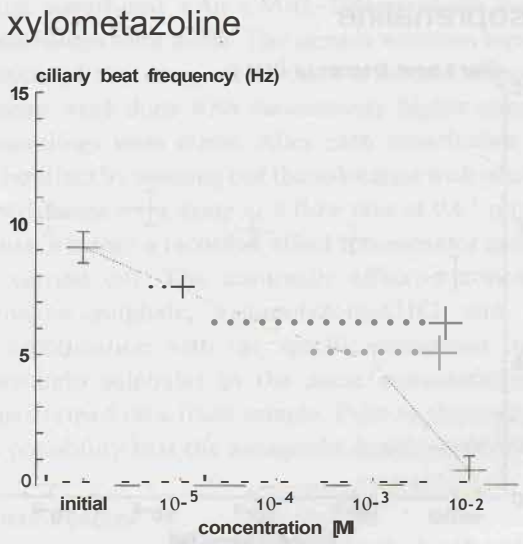

Figure 3. Average ciliary beat frequency (CBF) with SD in relation to increasing concentrations of xylometazoline (five subjects).

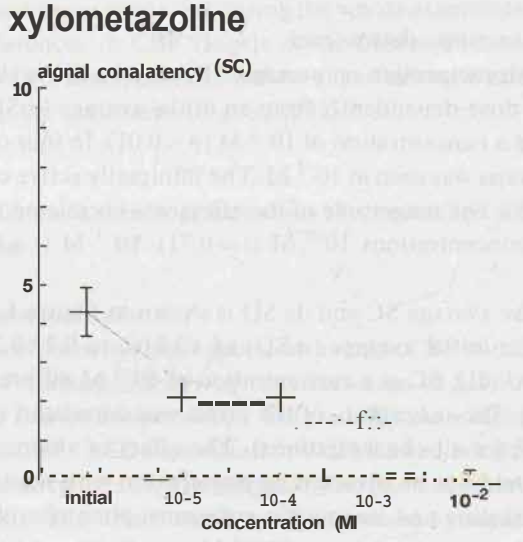

Figure 4. Average photoelectrical-signal consistency (SC) with SD in relation to increasing concentrations of xylometazoline (five subjects).

Carbachol (parasympathomimetic)

The effect of superfusion by carbachol on average CBF and its SD is shown in Figure 5. CBF increased dose-dependently from an initial average (±SD) of 8.7 ± 0.7 Hz to 10.3 ± 0.6 Hz at a concentration of 10^{-2} M ($p < 0.01$). The minimally active concentration was 10^{-3} M ($p < 0.01$). There was no effect on SC ($p > 0.33$; for all concentrations). The effect of carbachol was not reversible by washing out the substance with neutral medium.

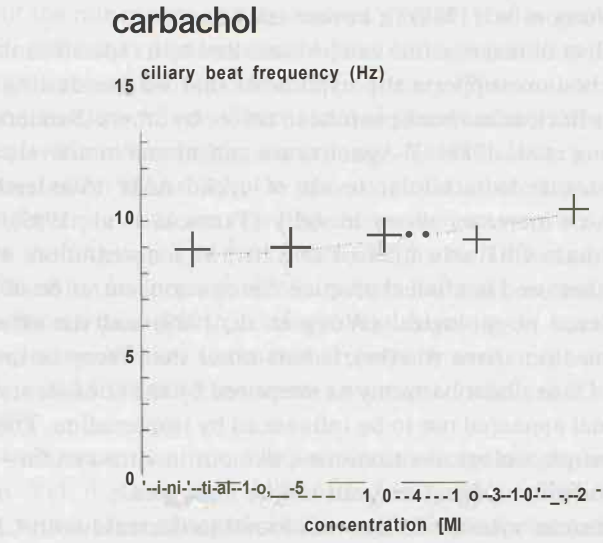

Figure 5. Average ciliary beat frequency (CBF) with SD in relation to increasing concentrations of carbachol (six subjects).

When carbachol was administered together with its specific antagonist atropine, in a concentration of 10^{-3} M, no effect could be demonstrated. Nor could an effect of atropine alone be recorded.

DISCUSSION

Experiments on mucosal samples in vitro have the advantage that the direct effects on ciliary activity are measured. A second advantage is that human material can be used. A disadvantage of this method is that ciliary activity in vitro may decline with time. This problem can largely be overcome by careful regulation of the measuring factors, as we discussed previously (Ingels et al., 1990; Ingels, 1991). We prefer the in vitro model using human nasal mucosa obtained by means of a forceps biopsy.

Orthosympathetic system

The non-selective (J-sympathomimetic isoprenaline was found to have a stimulatory effect on CBF. The maximum increase of CBF occurred at a concentration of 10^{-3} M and amounted to 28%. This is in agreement with previous results obtained in cultured rabbit tracheal ciliated cells by Sanderson and Dirksen (1989), who found a 27% increase, and with results obtained by Hybbinette and Mercke (1982), who measured a 24.7% increase in rabbits. It is lower, however, than the 44% increase found by Ohashi et al. (1983) in sinus mucosa samples, the 100% increase measured by Verdugo et al. (1980) in rabbits, and the 100% increase found by Wong et al. (1988) in bovine trachea.

The stimulatory effect of isoprenaline can be cancelled by its specific antagonist timolol. This observation supports the hypothesis that we are dealing with a receptor-mediated effect, as has been postulated before by others (Sanderson and Dirksen, 1989; Wong et al., 1988). (J-Agonists are considered to activate adenylate cyclase and elevate intracellular levels of cyclic AMP (Sanderson and Dirksen, 1989), which increases ciliary motility (Tamaoki et al., 1989). In our experiments, maximum CBF was attained at a 10^{-3} M concentration, which is about the same as that used in clinical practice. Since a concentration of 10^{-3} M can not be considered physiological (Wong et al., 1988) and the effects are irreversible, the question arises whether factors other than receptor-mediated effects play a role. Ciliary beat harmony as measured by the consistency of the photoelectrical signal appeared not to be influenced by isoprenaline. This is not surprising as in near-physiological situations - like our in vitro system - ciliary beat harmony is probably optimal and can not be improved.

The a-sympathomimetic xylometazoline was found to decrease both CBF and SC in a dose-dependent way. This finding is in agreement with that of the in vivo experiment by Hybbinette and Mercke (1982). Phillips et al. (1990) found a CBF increase when phenylephrine was administered at a 0.01% concentration, a decrease at 0.1 %, and ciliostasis at 0.5%. The different results may be explained by the fact that phenylephrine is predominantly an a_1-agonist while xylometazoline is an a_{2A}-agonist. The latter executes its effect by lowering cyclic AMP (Watson and Abbott, 1990).

The deteriorating effect of xylometazoline on ciliary function was cancelled by simultaneous administration of the antagonist phentolamine. This result confirms that its action is receptor mediated, although we have to stress that we were not able to reverse its effect by washing out. Whether the strong effect of the higher concentrations is still receptor mediated seems questionable. In clinical practice, xylometazoline is administered at a 0.1 % concentration; in our experiment, this resulted in a decrease of CBF by more than 48% and of SC by more than 61 %. This means that the ciliary beat harmony is disturbed.

Parasympathetic system

The parasympathomimetic carbachol was found to increase CBF only at high (non-physiological) concentrations of at least 10^{-3} M. This is in agreement with the findings of Hybbinette and Mercke (1982) from their in vivo model after intra-arterial administration of metacholine in rabbits. However, they found a much greater increase (57.7%) than measured in our in vitro model (17.8%). Wolf et al. (1988), on the other hand, did not measure a change in CBF in cultured human ciliary cells of the upper respiratory tract with 10^{-3} M carbachol. These differences may partly be explained by the fact that carbachol has no specificity for one of the muscarinic cholinoceptors. The M_2 cholinoceptor is probably not involved, since that pathway would have brought about a reduction of cyclic AMP with a subsequent decrease of frequency (Watson and Abbott, 1990).

We found that the effect of carbachol is absent when the parasympatholytic atropine is administered simultaneously. Nevertheless, we assume that the stimulatory effect of carbachol is not receptor-mediated, as the concentrations required for stimulation are very high. Moreover, we were not able to reverse the effect by washing out. The increase of CBF by high doses of carbachol was not accompanied by an increase of ciliary beat harmony as measured by SC. This again supports the idea that ciliary beat harmony is near its maximum in physiological conditions. Carbachol is clinically used in eye drops in a 1.5% concentration, and from our experiments we may conclude that it enhances human nasal CBF.

We are well aware that, in our experiments, we are not dealing with an in vivo situation. Yet, it seems that isoprenaline and carbachol can be prescribed for patients, without fear of negative effects on ciliary activity. As far as xylometazoline is concerned, a possible inhibition of ciliary function has to be taken into account.

ACKNOWLEDGEMENTS

We are grateful to J.F.L. Klis for his statistical advice. We also want to thank F. Engels for solving the pharmaceutical problems.

REFERENCES

1. Camner P, Strandberg K, Philipson K. Increased mucociliary transport by cholinergic stimulation. Arch Environ Health 1976; 29: 220-224.
2. Camner P, Strandberg K, Philipson K. Increased mucociliary transport by adrenergic stimulation. Arch Environ Health 1976; 39: 79-82.
3. Chopra SK. Effect of atropine on mucociliary transport velocity in anestbetized dogs. Am Rev Respir Dis 1978; 118: 367-371.
4. Donk van de HJM, Jadoenath B, Zuidema J, Merkus FWHM. The effects of drugs on ciliary motility. I: Decongestants. Int J Pharm 1982; 12: 57-65.
5. Fokkens WJ, Vroom ThM, Gerritsma V, Rijntjes E. A biopsy method to obtain high quality specimens of nasal mucosa. Rhinology 1988; 26: 293-295.
6. Hybbinette JC, Mercke U. Effects of the parasympatbomimetic drug methacholine and its antagonist atropine on mucociliary activity. Acta Otolaryngol (Stockh) 1982; 93: 465-473.
7. Hybbinette JC, Mercke U. Effects of sympathomimetic agonists and antagonists on mucociliary activity. Acta Otolaryngol (Stockh) 1982; 94: 121-130.
8. Ingels KJAO, Meeuwsen F, Van Strien HLCJ, Graamans K, Huizing EH. Ciliary beat frequency and the nasal cycle. Eur Arch Otorhinolaryngol 1990; 248: 123-126.
9. Ingels KJAO. Factors influencing ciliary beat measurements. Rhinology 1991; 29: 17-26.
10. Ingels KJAO, Van Strien HLCJ, Graamans K, Smoorenburg GF, Huizing EH. A study of the photoelectrical signal from human nasal cilia under several conditions. Acta Otolaryngol (Stockh), in press.
11. King M, Viires N. Effect of methacholine chloride on rheology and transport of canine tracheal mucus. J Appl Physiol 1979; 47: 26-31.
12. Konietzko N, Klopfer M, Adam WE, Matthys H. Die mukociliare Klarfunktion der Lunge unter beta-adrenerger Stimulation. Pneumologie 1975; 152: 203-208.
13. Kordik P, Bulbring E, Burn JH. Ciliary movement and acetylcholine. Br J Pharmacol 1952; 7: 67-79.
14. Mossberg B, Strandberg K, Philipson K, Camner P. Tracheobronchial clearance and beta-adrenoreceptor stimulation in patients with chronic bronchitis. Scand J Respir Dis 1976; 57: 281-289.
15. Ohashi Y, Nakai Y, Zushi K, Muraoka M, Minowa Y, Harada H, Masutani H. Enhancement of ciliary action by a.,8-adrenergic stimulant. Acta Otolaryngol (Stockh) 1983; Suppl 397: 49-59.
16. Pavia D, Bateman JRM, Sheahan NF, Clarke SW. Effect of ipratropium bromide on mucociliary clearance and pulmonary function in reversible airways obstruction. Thorax 1979; 34: 501-507.
17. Pavia D, Sutton PP, Lopez-Vidreiro MT, Agnew JE, Clarke SW. Drug effects on mucociliary function. Eur J Respir Dis 1983; Suppl 128: 304-317.
18. Phillips PP, McCaffrey TV, Kern EB. The in vivo and in vitro effect of phenylephrine (Neo Synephrine) on nasal ciliary beat frequency and mucociliary transport. Otolaryngol Head Neck Surg 1990; 103: 558-565.
19. Saketkhoo K, Yergin BM, Januszkiewicz A, Kovis K, Sackner MA. The effect of nasal decongestants on nasal mucous velocity. Am Rev Respir Dis 1978; 118: 251-254.
20. Sanderson JS, Dirksen ER. Mechanosensitive and beta-adrenergic control of the ciliary beat frequency of mammalian respiratory tract cells in culture. Am Rev Respir Dis 1989; 139: 432-440.
21. Santa Cruz R, Landa J, Hirsch J, Sackner MA. Tracheal mucous velocity in normal man and patients with obstructive lung disease: Effects of terbutaline. Am Rev Respir Dis 1974; 109: 458-463.

22. Simon H, Drettner B, Jung B. Messung des Schleimhaut-transportes in menslichen Nase mit 5lcr markierten Harzkiigelchen. Acta Otolaryngol (Stockh) 1977; 83: 378.
23. Tamaoki J, Kondo M, Takizawa T. Effect on cAMP on ciliary function in rabbit tracheal epithelial cells. J Appl Physiol 1989; 66: 1035-1039.
24. Verdugo P, Johnson NT, Tam PY. fi-Adrenergic stimulation of respiratory ciliary activity. J. Appl. Physiol 1980; 48: 868-871.
25. Watson S, Abbott A. TiPS receptor nomenclature supplement. Trends Pharmacol Sciences 1990; 11: 1-30.
26. Weiss T, Dorrow P, Felix R. Effects of a beta adrenergic drug and a secretolytic agent on regional mucociliary clearance in patients with COLD. Chest 1981; 80: 881-885.
27. Wolf G, Saria A, Koidl B. Pharmakologische Untersuchungen an kultivierten humanen Flimmerzellen des oberen Respirationstraktes. Laryng Rhino! Otol 1988; 67: 518-522.
28. Wong LB, Miller IF and Yeates DB. Regulation of ciliary beat frequency by autonomic mechanisms: In vitro. J Appl Physiol 1988; 65: 1895-1901.
29. Yager J, Chen TM, Dulfano MJ. Measurement of frequency of ciliary beats of human respiratory epithelium. Chest 1978; 73: 627-633.

Dr. K.J.A.O. Ingels
Dept. of Otorhinolaryngology
University Hospital Ghent
De Pintelaan 185
B-9000 Ghent
Belgium