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# Computer-Assisted Video Measurement of Inhibition of Ciliary Beat Frequency of Human Nasal Epithelium In Vitro by Xylometazoline

Linda N. Curtis and Johnny L. Carson

*Department of Biology (L.N.C.), University of North Carolina at Greensboro, Greensboro; and Departments of Cell Biology and Anatomy and Pediatrics (J.L.C.), University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, U.S.A.*

The effect of xylometazoline, an alpha-adrenergic agonist, on ciliary beat frequency (CBF) was tested on samples of human nasal epithelium in vitro. Ciliated tissue was obtained from the inferior nasal turbinates of five normal individuals. CBF was measured from video recordings of ciliary activity using a computer-assisted photometric technique. The mean CBF of cells from the five subjects, followed for 40 min without xylometazoline, was 12.0 ± 1.1 Hz. All concentrations of xylometazoline significantly decreased ciliary beat frequency. After a 10-min exposure, the mean CBF dropped to 3.8 ± 0.4 with 0.1% xylometazoline, 4.9 ± 1.0 with 0.05%, and 8.1 ± 0.9 with 0.025%. Washing with control culture medium at least partially reversed the inhibition within 10 min. Phentolamine ($10^{-3}$ M), an alpha-adrenergic antagonist, did not alter CBF significantly when used alone, but partially blocked the strong cilioinhibitory effect of xylometazoline. This action of xylometazoline is similar to that of several commercially prepared decongestants that contain potentially ciliotoxic preservatives in addition to alpha-adrenergic agonists and supports the view that alpha-adrenergic agonists act directly on ciliated cells to inhibit ciliary activity.

**Keywords:** Cilia, Ciliary beat frequency, Human nasal epithelium, Alpha-adrenergic, Xylometazoline

## Introduction

Ciliary activity is an important component of the mucociliary transport mechanism responsible for clearance of airways. Slow, abnormal ciliary beating is associated with an increased incidence of respiratory tract infection including recurrent sinusitis, otitis media, and chronic bronchitis (Rossman et al., 1984; Pedersen et al., 1981). Because topical medications, such as decongestants, may alter the rate at which the cilia beat, it is important to understand the basis of their action.

Beta-adrenergic agonists, such as terbutaline and isoproterenol, stimulate both mucociliary clearance (Wood et al., 1975) and ciliary beat frequency (CBF) in a dose-dependent manner (Sanderson and Dirksen, 1989; Lopez-Vidriero et al., 1985; Verdugo et al., 1980; Melville and Iravani, 1975). Isoproterenol (Sanderson and Dirksen, 1989) and aminophylline (Melville and Irvanni, 1975) both increase intracellular cAMP and CBF. Similarly, a rise in intracellular, free calcium increases beating by vertebrate cilia (Sanderson and Dirksen, 1989).

There are several examples whereby alpha-adrenergic drugs inhibit cAMP production (Gilman, 1984) and might potentially inhibit ciliary beat frequency. Although known to reduce secretion and mucosal swelling by vasoconstriction, the effects of alpha-adrenergic drugs on mucociliary transport and CBF have resulted in conflicting evidence. Petruson and Hansson (1982) reported that mucociliary transport was unchanged by xylometazoline, an alpha-adrenergic agonist. In contrast, Dudley and Cherry (1977, 1978), Phillips et al. (1990), and Hybbinette and Mercke (1982) found that ciliary activity decreased for most concentrations of alpha-adrenergic agonists studied. Hybinnette and Mercke described the effects of alpha-adrenergic agents in vivo and mentioned the possibility that changes in blood

Address reprint requests to Dr. L.N. Curtis, 427 Eberhart Bldg., Department of Biology, UNCG, Greensboro, NC 27412, U.S.A.
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flow caused by alpha-adrenergic drugs might have caused a decrease in mucociliary wave frequency. The studies by Dudley and Cherry (1977, 1978) and by Phillips et al. (1990) used commercial preparations of alpha-adrenergic drugs containing preservatives. In a review of drugs that alter CBF, Hermens and Merkus (1987) noted that preservatives used in commercial nasal decongestants are ciliotoxic. They suggest that the cilioinhibitory effect of commercially prepared decongestants is due instead to additives such as preservatives.

In the present study, we tested the hypothesis that xylometazoline, and not the preservatives, is responsible for the cilioinhibitory effect of xylometazoline-containing decongestants. To do this, we examined the effect of an alpha-adrenergic agonist, xylometazoline, applied directly to ciliated cells (in vitro). The potential interference of preservatives in commercial preparations was purposefully avoided by preparing the solutions just prior to use. Using a computer-assisted photometric technique and human nasal epithelium, we found that 0.1%, 0.05%, and 0.025% concentrations of xylometazoline significantly decreased ciliary beat frequency. Both the rapidity and extent of inhibition of CBF by xylometazoline were similar to that reported for commercially prepared alpha-adrenergic decongestants that contain preservatives that also inhibit ciliary activity (Melville and Iravani, 1975; Petruson and Hanson, 1982; Dudley and Cherry, 1977, 1978; Phillips et al., 1990). The specificity of action of xylometazoline was tested by blocking with phentolamine, a known competitor for alpha receptors. Although preservatives used in nasal decongestants may be toxic to ciliated cells, our study provides evidence that an alpha-adrenergic drug, acting alone on ciliated cells, can directly decrease CBF.

## Materials and Methods

### *Subjects and Sample Collection*

Five normal, nonsmoking subjects were the source of nasal epithelium used in this study. The experimental protocol was reviewed and approved by an institutional review committee on the protection of rights of human subjects. All volunteers provided informed consent prior to participation in the study. There were three females and two males ranging in age from 23 to 77 years. None of the subjects had chronic respiratory disease; and none had been symptomatic for acute upper respiratory tract infection for at least 1 month prior to being studied. Nasal ciliated epithelium was obtained from the inferior surface of an inferior nasal turbinate using a sterile, disposable curette (no. 2-0103 Rhino-probe, Synbiotics Corp., San Diego, CA). Next, the specimen was placed into Ham's F-12 Medium supplemented with 100 units/mL of penicillin and 100 mg of streptomycin/mL. (Gibco, Grand Island, NY). The epithelium was dispersed into small pieces, of approximately 20–200 cells. Several clusters of cells were placed in a coverslip "chamber" (Figure 1). Coverslip chambers were placed on a custom-built warming stage on a Zeiss WL Standard microscope. Water at 37°C was pumped to the warming stage to maintain constant temperature of the medium in the coverslip "chamber." Several clusters of cells were placed on a 24 × 50 mm coverslip to which no. 25-gauge syringe needles were attached. Polyethylene tubing connected the incurrent and excurrent needles to 1-cc syringes. A second coverslip, 18 × 18 mm, was placed over the tissue and sealed in place using a nontoxic melted mixture (1:1 wt/wt) of Vaseline and paraffin. The fluid volume of this chamber was 0.1 mL. The stage warmer was machined from aluminum and included a continuous $^{3}w4.5_{16}$-in water channel drilled in the periphery. A 12.5-mm aperture in the floor provided an opening for the light path. The stem of the thermometer was held in direct contact with a side of the chamber by florist clay. Although warming of tissues for 1 min or less was sufficient to support normal ciliary beat frequencies, tissues were allowed to equilibrate on the stage for 10 min prior to control measurements.

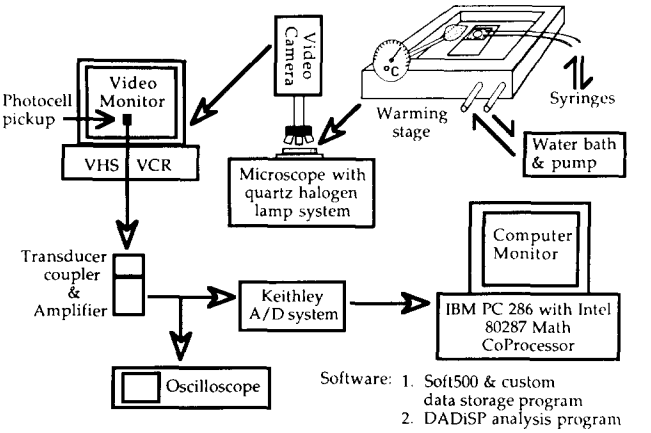


**Figure 1.** A schematic diagram illustrating the system for measuring ciliary beat frequency from previously recorded video images using a cadmium selenite photocell.

Video tape recordings of ciliary beating were made on favorably oriented, undamaged cells located at the edge of the tissue clusters. After equilibration in control culture media, cells with actively beating cilia were recorded for 1 min. The rate of ciliary beating was followed for 40 min in control culture medium. Cells were exposed to three concentrations of xylometazoline hydrochloride (Sigma, St. Louis, MO): 0.1% wt/vol, 0.05% wt/vol, and 0.025% wt/vol in culture medium.

The same field and, in most cases, the same individual cells that were recorded at 0 min were followed throughout the experiment. Following equilibration in control culture medium and a control recording (0 min), 1 mL of the chosen concentration of drug was perfused through the coverslip chamber. After 5 and again 10 min, ciliary beating was recorded. At the end of 10 min, the drug was washed out by perfusing with 1 mL of control culture media. Recordings at 15 and 20 min represent the recovery of ciliary beating after 5 and 10 min, respectively.

Phentolamine hydrochloride (Sigma), an alpha-adrenergic blocking agent, was used to determine whether the effect of xylometazoline was due to specific binding of alpha receptors. Solutions containing phentolamine were made fresh immediately before use. Ciliary beating was measured before and after 5 and 10 min of exposure to phentolamine and measured again after 5 and 10 min of exposure to 0.05% xylometazoline plus phentolamine.

### *System for Quantification of Ciliary Beating*

The design of our system (Curtis et al., 1991) for photoelectric measurement of ciliary beat frequencies (Figure 1) incorporates important features of that described by Sanderson and Dirksen (1985) and by Teichtahl et al. (1986). Ciliary beating was viewed using a Zeiss WL Standard light microscope equipped with a 40× (0.75 NA) phase-contrast objective. The microscopic image was recorded by a Dage MTI model 65 vidicon camera using its 2× enlargement feature; and data was stored by a Zenith Model 255 VHS videorecorder on 0.5-in cassettes. For measurement of ciliary beating, tapes were displayed on a 22-cm diagonal screen Sanyo MDC-56 Monitor at a final magnification of 1130×. The photoelectric transducer (model no. 323, Narco Bio-Systems, Inc., Austin, Texas) containing a 4-mm diameter photocell was positioned over the video image of the cilia. Movement of the cilia interrupting the light path caused changes in light intensity recorded by the photocell transducer. The photocell directly covers and acquires a strong signal from an area within the image that is 3.5 μm in diameter. These dimensions are well suited to recording from individual ciliated cells. The signal passed through a no. 7173 transducer coupler and a no. 7070 amplifier equipped with a 30-Hz electronic filter (Narco Bio-Systems, Inc.). The amplified signal was sent to both an oscilloscope and to a Keithley Instruments, Inc. (Cleveland, OH) series 5000 data acquisition system equipped with an AIM1 module. The analog signal was converted to digital and stored by a model 286 IBM computer equipped with an 80287 Intel math co-processor chip. A short basic program using Keithley Soft500 V.2 recorded the photocell data.

### *Analysis of Data*

The DaDISP Worksheet Program V1.05B by DSP Development Corporation (Cambridge, MA) was used to display data sets and ascertain beat frequency. A resolution of 0.1 Hz was obtained using a fast Fourier transform (FFT) analysis of 8192 data points, recorded at an interrupt rate of 1 per millisecond. As shown in Figure 2, the beat frequency was identified as the fastest major peak other than the 60-Hz scanline peak. *Major peaks* were defined as 10 times greater than background. The use of a 30-Hz electronic filter decreased the amplitude of the scanline peak by about 75% without altering the amplitude or frequency of the CBF signal. The size of the scanline signal varied according to the brightness of the video image and remained even when no evidence of cell movement was present.

A potential error in using the FFT with a photometric technique to determine CBF is aliasing. This can occur when the signal is sampled too slowly resulting in false, low-frequency components of the FFT (Weaver, 1989). Sampling a signal at the Nyquist frequency (twice the highest frequency of interest in the signal) prevents aliasing for perfect signals. Sampling the signal at a rate five times the Nyquist frequency is often recommended to ensure adequate sampling. For a CBF of 15 Hz, the Nyquist frequency would be 30 Hz, and the recommended sampling rate would be 150 Hz. The sampling rate of 1000/sec used by our data acquisition system easily exceeds this. However, the video-field sampling rate of 60 Hz is only twice the Nyquist frequency. Thus, the video sampling rate is the limiting factor of our system (Curtis et al., 1991). Standard video systems, such as ours, are effective for recording CBF from both normal cells and patients who have ciliary defects that reduce CBF but might produce low CBF measurements in studies of drugs or other factors that increase CBF above normal.

### *Statistics*

The results were expressed as mean ± SEM. Data were analyzed using the StatView SE+ statistical program by Abacus concepts (Berkeley, CA). Data for each treatment were examined by a one-factor, repeated-measures analysis of variance (ANOVA) to evaluate change with time, for example, the difference between 0 and 5 min with 0.1% xylometazoline. Next, all data were examined together by a one-factor ANOVA to compare treatments (xylometazoline concentrations) The Fisher Protected Least Significant

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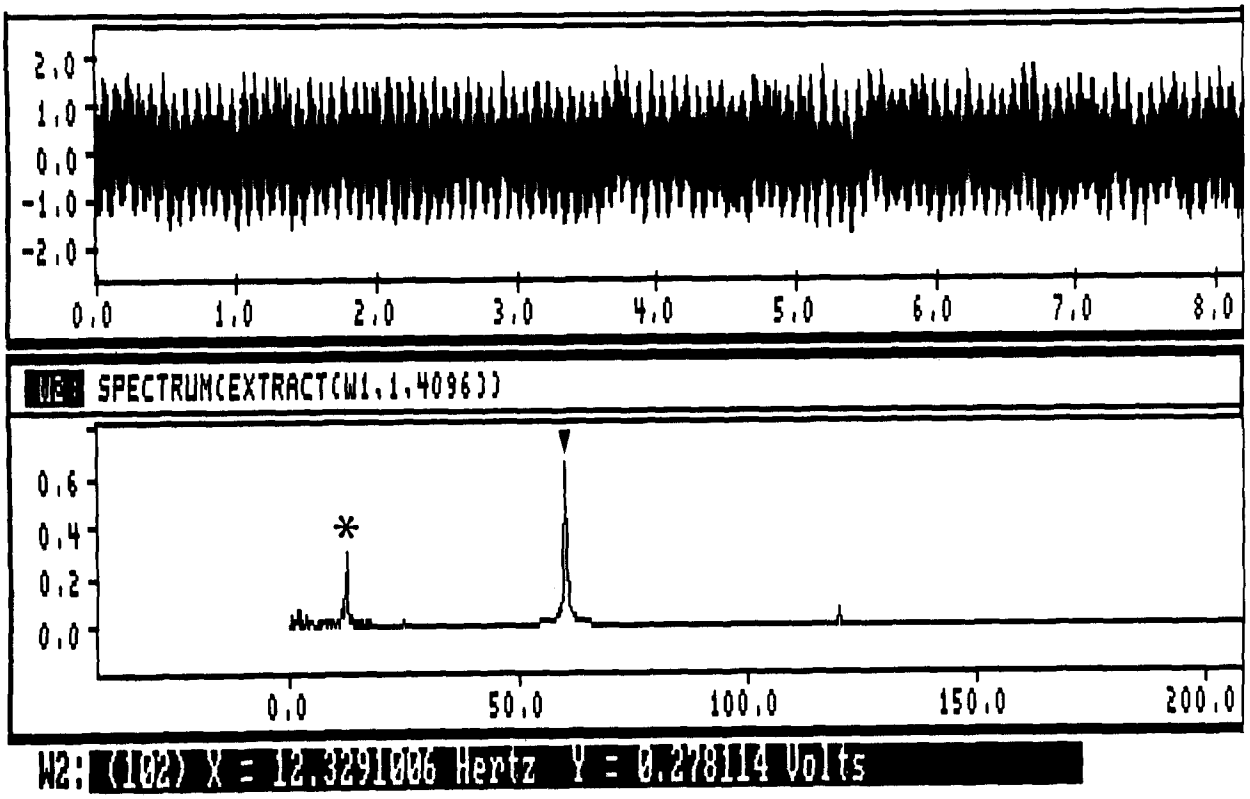


**Figure 2.** Representative output of ciliary beat frequency using a computer-assisted photometric approach. The upper window displays a photocell signal. The lower window shows the amplitude spectrum of the above signal. The spectrum is the magnitude of the first half of the fast Fourier transform divided by the number of sample points. The *beat frequency* was defined as the fastest major peak (other than the 60-Hz peak caused by scanlines on the monitor). When the cursor (not shown is placed at the top of the peak indicated by the asterisk, the frequency of the peak is displayed below the graph. The CBF in this example was 12.3 Hz. An arrowhead marks the 60-Hz peak.

Difference (PLSD) (Milliken and Johnson, 1984) test of means was used to compare means between groups.

## Results

The ciliary beat frequency of five normal individuals was measured over a 40-min period as shown in Figure 3. The initial measurement was 12.2 ± 0.4 Hz, and the value after 40 min was 12.0 ± 1.1 Hz. There was no significant decline in CBF when the means for each time were compared using one-factor, repeated-measures ANOVA with a confidence value of $p = 0.01$, and the Fisher Protected Least Significant Difference test of means.

The effect of three concentrations of xylometazoline hydrochloride was measured on subsequent samples from the five subjects. Figure 3 displays the time course of the experiment with measurement of CBF after 5 and 10 min of exposure to xylometazoline followed by washing with 1 ml of control culture medium (15 and 20 min). The strongest concentration, 0.1% xylometazoline, caused a decrease in the CBF of 69.1%

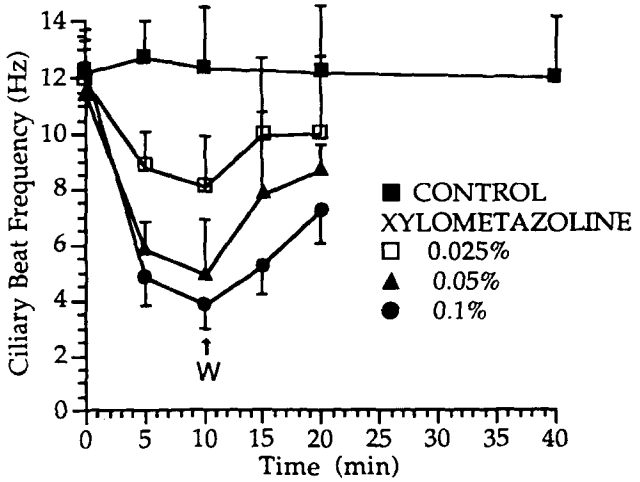


**Figure 3.** The effect of xylometazoline hydrochloride on ciliary beating. $n = 5$; means ± SEM. Data for 5 and 10 min represent effects of the drug. Beginning at 10 min as indicted by the letter "W," cells were washed with control culture medium.

after 10 min. Washing with control medium restored the CBF to 58.5% of initial values after 10 min. The same pattern was seen for 0.05% xylometazoline except that the decrease in CBF at 10 min was not as large. After 10 min washing, the CBF for 0.05% was somewhat higher than that for 0.1%. The weakest concentration tested, 0.025%, caused the smallest decrease. After 10 min washing, the CBF approached normal.

A one-factor, repeated-measures ANOVA indicated that differences within each xylometazoline concentration series was significant at the $p = 0.01$ level. Using the Fisher PLSD test, means for each of the three concentrations of xylometazoline were significantly different from the 0-min control ($p < 0.05$) after 5 and 10 min exposure. Differences between 0 and 20 min (10-min washout) were significant ($p < 0.05$) for all concentrations of xylometazoline. The same was true between 10 min when the xylometazoline effect was greatest and 20 min (or 10 min after washout). When all data sets were examined together by a one-factor repeated-measures ANOVA to compare treatments (xylometazoline concentrations), differences between treatments were again significant at the $p = 0.01$ level. The Fisher PLSD test was used to compare means between groups. They were identical to those for the first comparison.

The effect of an alpha-adrenergic antagonist on CBF is shown in Table 1. Exposure to $10^{-3}$ *M* phentolamine alone caused a 9.4% decrease in CBF after 10 min. However, this decrease was not statistically significant when examined by ANOVA and the Fisher PLSD test of means. In contrast, 0.05% xylometazoline caused a 52.8% decrease by 5 min and a 58.5% decrease by 10 min that were statistically significantly ($p < 0.05$). When cells were pretreated with phentolamine for 10 min and then exposed to a combination of 0.05% xylometazoline together with phentolamine, there was a decline of 8.5% by 5 min and 28.3% by 10 min. The change at 5 min was not significantly different from 0 min, but, by 10 min, the change was significant. The difference between means for xylometazoline alone and combined with phentolamine was 4.7 Hz or 44.3% at 5 min and 3.2 Hz or 30.2% at 10 min; both differences were statistically significant ($p < 0.05$).

## Discussion

Ciliary beat frequency of human nasal epithelium was stable for 40 min when maintained in control culture medium. The mean for five individuals at 0 min was 12.2 ± 0.4 Hz and was essentially unchanged at 12.0 ± 1.1 Hz after 40 min. This is important because it indicates that any changes in ciliary beat frequency occurring during the shorter 20-min test period used for experiments with xylometazoline were due to the drug and not to a general decline in performance of the cells caused by uncharacterized factors of the in vitro environment. The values we obtained for control human tissue are comparable to those reported by others (Yager et al., 1978).

Xylometazoline decreased CBF in a concentration-dependent manner. The highest concentration, 0.1%, produced the strongest inhibition of ciliary beating. As expected, the effect by 0.05% was greater than 0.025%. CBF at both times and all concentrations was significantly different from the control at the same time. Also, the decrease in activity was more pronounced after exposure for 10 min.

Washing with control media, after 10 min exposure to the drug, partially restored CBF to control level. At the lowest concentration tested, 0.025%, the mean was not significantly different from control 10 min after a control rinse. This suggests that xylometazoline temporarily, but reversibly, inhibits ciliary beating.

The alpha-adrenergic blocking drug phentolamine ($10^{-3}$ *M*) caused a slight (9.4%) decrease in CBF as compared to the control medium. This decrease was not statistically significant. Similarly, Melville and Iravani observed a decline of 13% with phentolamine in intact rat trachea. Phentolamine at $10^{-3}$ *M* prevented a significant decrease in beat frequency by 0.05% xylometazoline for the shorter, 5-min exposure. This suggests that the cilioinhibitory action of xylometazoline is due to binding of alpha-adrenergic receptors. The fact that there is a decline in CBF of 28.3% by 10 min

**Table 1.** Effect of $10^{-3}$ *M* Phentolamine Alone and with 0.05% Xylometazoline

| Drug | 0 Min | 5 Min | % of Initial Value | 10 Min | % of Initial Value |
|---|---|---|---|---|---|
| Control | 12.2 ± 0.4[a] | 12.4 ± 0.2 | 101.6 | 11.7 ± 0.5 | 95.9 |
| $10^{-3}$ *M* Phentolamine | 11.7 ± 1.0 | 11.3 ± 1.3 | 96.6 | 10.6 ± 0.9 | 90.6 |
| 0.05% Xylometazoline | 10.6 ± 1.4 | 5.0 ± 0.5[b] | 47.2 | 4.4 ± 1.2[b] | 41.5 |
| 0.05% Xylometazoline + $10^{-3}$ *M* Phentolamine | 10.6 ± 0.9 | 9.7 ± 0.3 | 91.5 | 7.6 ± 0.8[b] | 71.7 |

[a] Ciliary beat frequency in hertz is listed as mean ± SEM; $n = 3$. The same cells from three subjects were followed for phentolamine alone and combined with xylometazoline.

[b] A Fisher PLSD test of means was used to compare 0–5 and 10 min of exposure; $p < 0.05$.

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may be explained by the exchange between phentolamine and xylometazoline at receptor binding sites. This seems especially likely since 0.05% xylometazoline is $1.8 \times 10^{-3}$ *M* or 1.8 times greater that the $10^{-3}$ *M* concentration of phentolamine used here.

On the surface, our finding of a decrease in CBF is contrary to the finding by Petruson and Hansson (1982) of unaltered, long-term, mucociliary transport times. Their subjects used xylometazoline three times per day, but the time elapsed between drug exposure and mucociliary transport test is not stated. A lengthy interval between exposure to drug and measurement of saccharin transport could miss a short-term, but reversible decrease. However, our finding of a rapid decrease (within 5 min) in CBF agrees well with Simon et al. (1977) that xylometazoline prolongs transport times using $^{51}Cr$ as a tracer. It is notable that they measured transport soon (12–15 min) after exposure to xylometazoline.

A number of drugs used to treat respiratory diseases have been reported to impair CBF when applied locally. Karttunen et al. (1990) found that two antitussives, vadocaine and dextromethorphan, decrease ciliary beat frequency by as much as 20%, and that the antihistamines, diphenhydramine and hydroxyzine, caused ciliary beating to decrease and stop totally within 20 min. Dudley and Cherry (1977) found that mucolytic agents, including 10% acetylcysteine, 7.5% sodium bicarbonate, and 5.6% L-arginine reduced the number of cells showing ciliary activity. In examining the effects of several commercially prepared nasal decongestants including Otrivin, which contains xylometazoline hydrochloride, Dudley and Cherry (1978) found evidence of "cilotoxicity" for cultured chicken trachea. Whether this effect is due to the action of xylometazoline is not entirely clear since commercially prepared nasal decongestants also contain preservatives (Dudley and Cherry, 1978; Hermens et al., 1987; van de Donk et al., 1982) such as chlorbutal, benzalkonium chloride, and the mercury compound thiomersal that may be responsible for toxicity to cells when applied locally.

Our finding that the alpha-adrenergic agonist xylometazoline inhibited ciliary beating supports and extends earlier work by Dudley and Cherry (1977, 1978) on commercially prepared alpha-adrenergic decongestants. The fact that cilioinhibition was at least partially blocked by phentolamine suggests that the effect of xylometazoline is specifically due to binding of alpha-adrenergic receptors of human nasal epithelium. It is also consistent with a pharmocokinetic pattern of alpha-adrenergic agonists in the inhibition of ciliary activity that is independent of the effect of preservatives used in nasal sprays and nasal drops. Photometric measurement of CBF in vitro is advantageous for the study of ciliary regulatory mechanisms because it isolates cell clusters from potentially uncontrolled variables such as nervous and endocrine regulation. This approach should facilitate future clinical studies of ciliary dysfunction at the cell level.

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