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Effect of xylometazoline and antazoline on ciliary beat frequency

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Summary

The ciliotoxic effects of xylometazoline and antazoline were evaluated using cilia from the rat trachea. It is shown that both drugs exert dose-dependent ciliotoxic effects. A factorial experiment showed that the two drugs moderated each other's ciliotoxicity at concentrations of 0.05% for xylometazoline hydrochloride and 0.0007% for antazoline nitrate. No interaction was observed at higher concentrations up to 0.1% xylometazoline hydrochloride and 0.007% antazoline nitrate. The data suggest that the co-formulation of an H_1 antihistamine and an α_1 vasoconstrictor may indeed reduce their ciliotoxicity.

Introduction

Xylometazoline is a vasoconstrictor agent which is widely used for the relief of nasal congestion (Dudley and Cherry, 1978; Falck et al., 1990; Wight and Cochrane, 1990). It has the advantage, relative to many other topical vasoconstrictors, of being long-acting thereby requiring only twice daily applications (BNF, 1991). Reducing the frequency of applications is believed to lower the risk of rebound congestion, often observed with long-term use of nasal decongestants. Antazoline, on the other hand, is a topical H_1 antihistamine compound. It is co-formulated with vasoconstrictors into nose drops and sprays intended for symptomatic relief of allergic rhinitis (Li Wan Po, 1990). Such formulations have fallen into disrepute because of the known sensitization potential of antihistamine compounds following topical applications to the epidermis. There is no evidence that this risk is associated with application to mucous membranes and there is currently renewed interest in intranasal antihistamine applications. At least one antihistamine nasal spray has been licensed for clinical use during the past 2 years. For these reasons, we conducted a systematic evaluation of the ciliotoxicity of xylometazoline and antazoline. The objectives were to determine (i) whether the two compounds were ciliotoxic and (ii) whether the combination of the two compounds exerted additive, synergistic or antagonistic ciliotoxic effects.

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Materials and Methods

Materials

The Locke-Ringer (LR) solution used as the reference control solution in the antazoline study had the following composition: NaCl, 7.72 g; KCl, 0.42 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; dextrose, 1.00 g; $NaHCO_3$, 0.15 g and de-ionised water to 1 l. The pH of LR solution was 7.4 and the solution was sterilized before use in order to ensure absence of significant growth of micro-organisms during the studies. Medium 199 enriched with Hank's salts from Gibco Life Technologies Ltd, U.K., was used as reference control solution. Quantitatively identical results were obtained with both media. Medium 199 was preferred because of improved pH stability on storage. All the drug solutions were made by dissolving the required amount in the appropriate medium. The concentrations of antazoline nitrate used in the present study were 0.0001, 0.0007, 0.0005, 0.001, 0.005, 0.007, 0.01, 0.05 and 0.1% w/v. For xylometazoline hydrochloride, the concentrations were 0.05 and 0.1%.

The 2^2 factorial experiment included two factors: xylometazoline hydrochloride and antazoline nitrate subsequently referred to simply as xylometazoline and antazoline. The four combinations were 0.1% xylometazoline and 0.007% antazoline, 0.1% xylometazoline and 0.0007% antazoline, 0.05% xylometazoline and 0.007% antazoline and 0.05% xylometazoline and 0.0007% antazoline.

Preparation of ciliated trachea

Adult male Wirstar rats weighing between 350 and 450 g were used in the present study. Each rat was killed humanely by an experienced technician and the trachea immediately removed and incubated at 37°C in the appropriate medium.

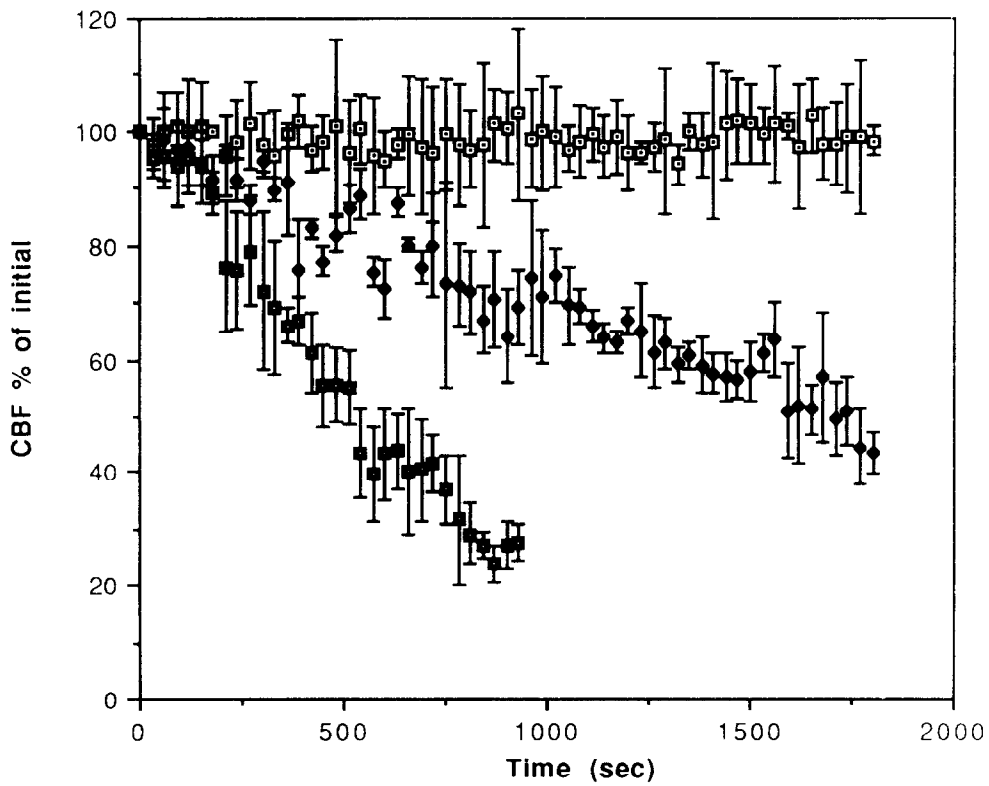

Fig. 1. Effects of xylometazoline hydrochloride on ciliary beat frequency (CBF) in rat trachea. (□) Control, (◆) 0.05% w/v xylometazoline hydrochloride, (■) 0.1% w/v xylometazoline hydrochloride.

Ring segments of about 1 mm were cut from the trachea and again stored in the enriched medium until required.

Recording of ciliary beat frequency

Before a measurement, a tracheal ring with healthy ciliated epithelium was placed in the microscope slide chamber. The correct position for viewing the ciliated epithelium was obtained using the microscope and a video monitor (Hitachi).

Measurements of the beat frequency were taken at three different sites on each tracheal explant. The initial beat frequency was initially measured at room temperature in drug-free solution. The reference solution was then removed and replaced with drug solution. The beat frequency was measured again and expressed as a percentage of the initial value. The tracheal rings were each monitored over 30 min.

New tracheal explants were used for each solution and the slide chamber was rinsed with control medium several times before introducing a new drug solution.

The equipment used for recording ciliary beat frequency consisted of an inverted binocular microscope (Olympus CK2-TRP) fitted with an X20 objective and an X10 ocular lens. A video camera (Hitachi KP-143) was attached to the phototube behind the eyepieces thereby enabling the image of the cilia to be displayed on a video monitor (Hitachi) and to be recorded on a video cassette recorder (VCR) (Akai VS-425 EK).

The movements of the cilia recorded on the video tape were detected by a data acquisition

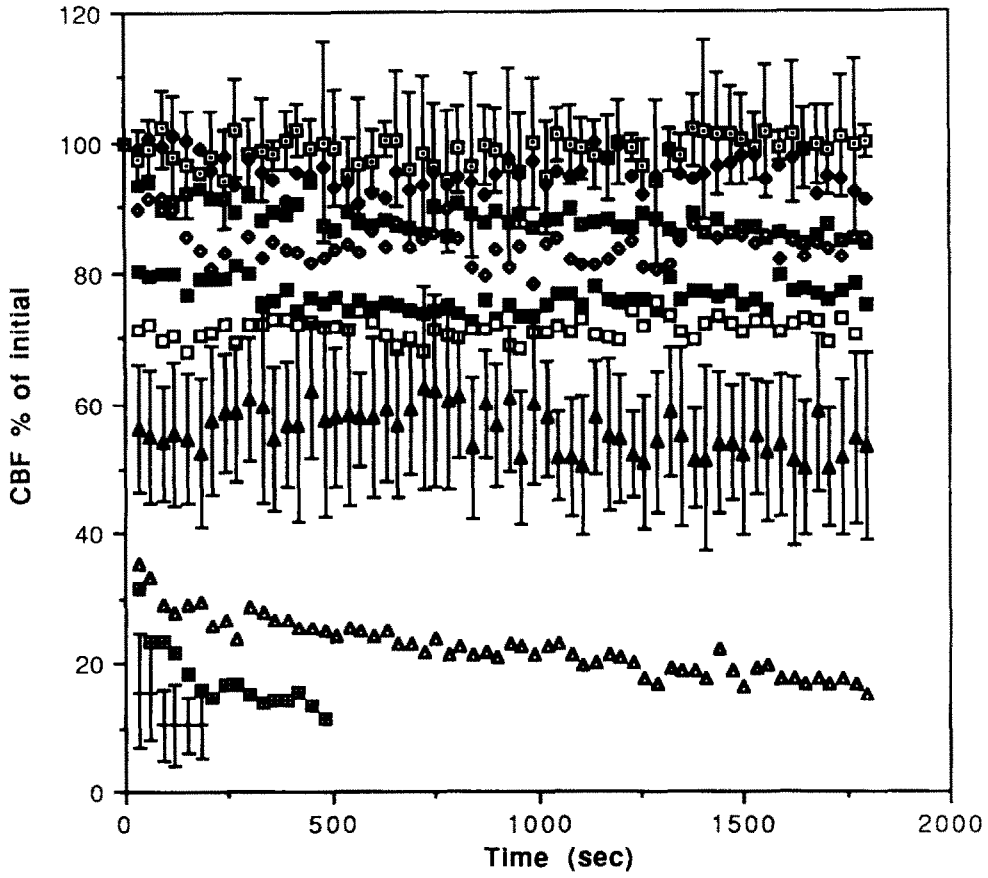

Fig. 2. Effect of antazoline concentration on ciliary beat frequency (CBF) in rat trachea. (⊡) Control, (◆) 0.0001%, (■) 0.0005%, (◆) 0.0007%, (■) 0.001%, (□) 0.005%, (▲) 0.007%, (▲) 0.01%, (⊞) 0.05%, (+) 0.1% antazoline.

unit based on equipment described by Teichtahl et al., (1986). The data acquisition unit consists of a probe, a low-pass filter and an Opus PC III computer with on-board A/D converter. The probe picks up an optical signal through its photo-cell and converts it into an electrical signal which is amplified to between 0 and +300 mV. The filter circuit takes the signal from the probe, filters out frequencies above 30 Hz, and adjusts the signal to 0–5 V.

A sampling period of 10 ms, corresponding to a sampling rate of 100 Hz was used. The highest frequency detectable was 50 Hz. The signal derived from the video monitor was converted by fast Fourier transform into a frequency spectrum using software written in C to run on an Opus PC III computer. Each sample consists of 256 data points corresponding to a time duration of 256 ms.

The average result is given for the group. A program called HARDCOPY is used to allow graphics dump from the screen to the printer/plotter.

Statistics

The area under the curve (AUC) values were calculated using the trapezoidal rule and a Lotus 1-2-3® Macro (Chan and Li Wan Po, 1992). Minitab® was used for carrying out the statistical analyses.

Results and Discussion

There is little doubt that both xylometazoline (Fig. 1) and antazoline (Fig. 2) exert an adverse effect on ciliary beat frequency in a dose-dependent manner. However, it is interesting to note

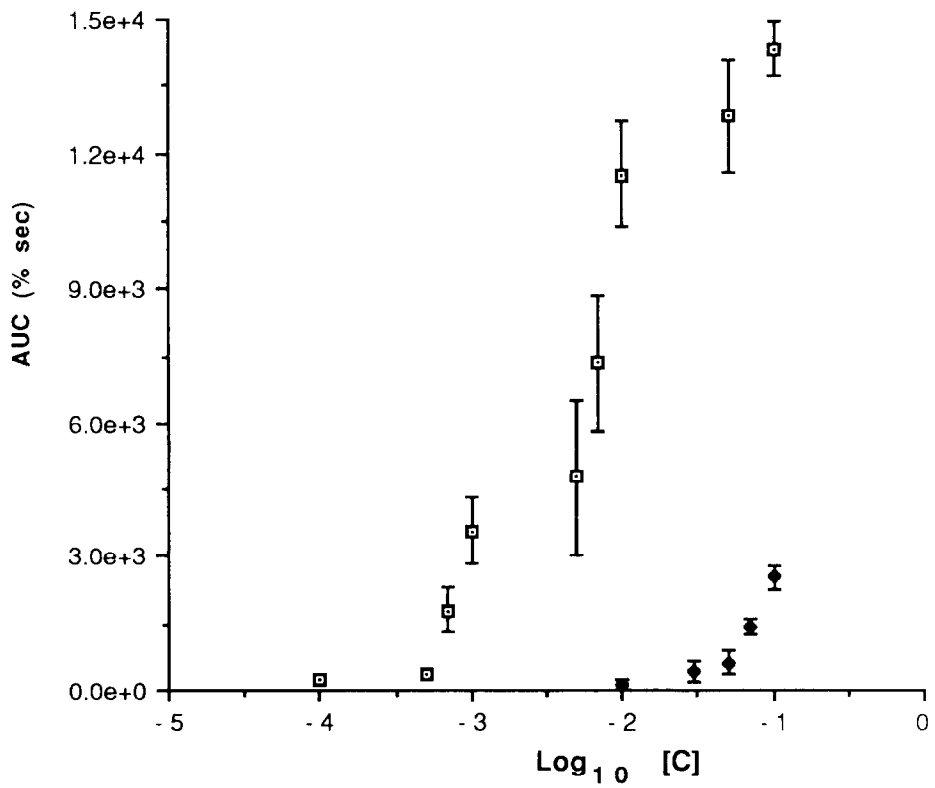

Fig. 3. Comparison of AUC values between antazoline (⊡) and xylometazoline (◆). See Materials and Methods for definition of AUC.

TABLE 1

Analysis of variance table for comparing the effects of xylometazoline hydrochloride 0.05% and antazoline nitrate 0.0007%, alone and in combination with each other in a 2^2 factorial experiment

Source	Sum of squares	Degrees of freedom	Mean squares	*F* values	*P*
Xylometazoline	747.2	1	747.2	13.89	< 0.001
Antazoline	959.8	1	959.8	17.84	< 0.001
Interaction	5 582.1	1	5 582.1	103.76	< 0.001
Error	1 721.2	32	53.8		
Total	9 010.3	35			

that the decay in ciliary beat frequency induced by xylometazoline is essentially zero order but that, in the case of antazoline, there is an apparent biphasic decay. An initial rapid drop in ciliary beat frequency is followed by a more gradual decay which was particularly noticeable at the higher concentrations of antazoline (Fig. 2). The reasons for such different effects are unclear. A more detailed examination of the dose-response relationship for antazoline showed that the profile was sigmoidal (Fig. 3).

Antazoline and xylometazoline combinations

The effects of combinations of antazoline and xylometazoline were first investigated using two 2^2 factorial experiment. In such experiments, two

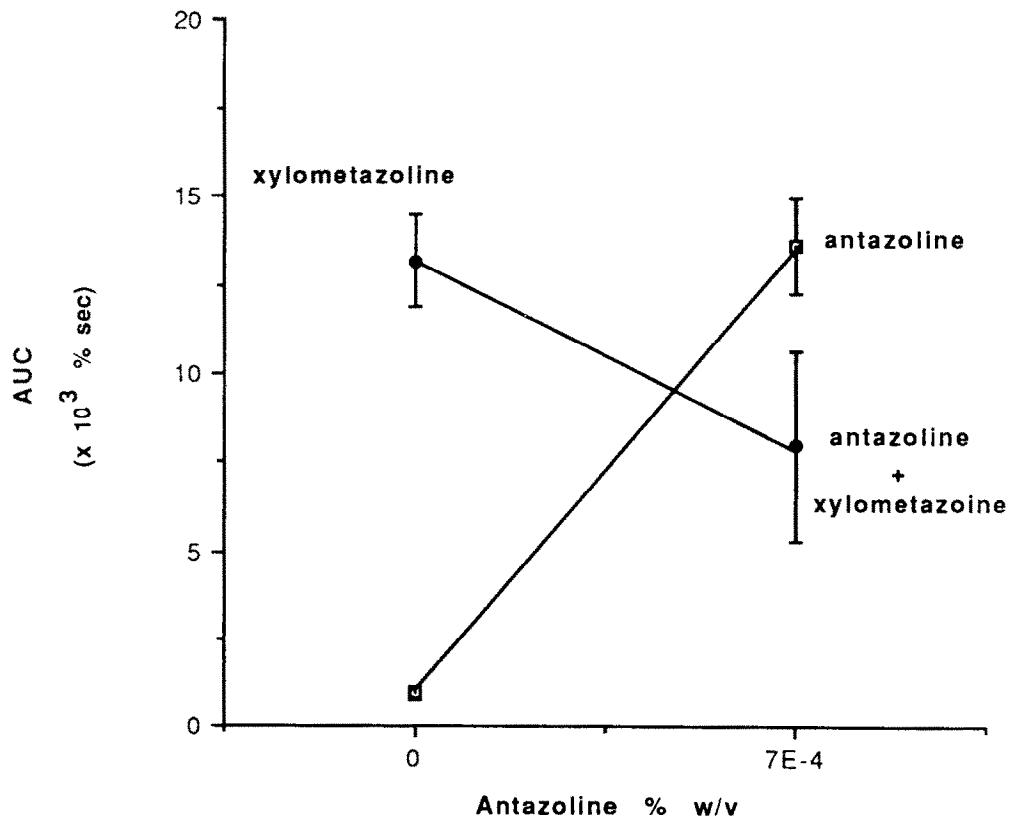

Fig. 4. Effect of antazoline and xylometazoline combination on ciliary activity. See Materials and Methods for definition of AUC.

TABLE 2

Analysis of variance table for comparing the effects of xylometazoline hydrochloride (0.05 and 0.1%) and antazoline sulphate (0.0007 and 0.007%) in a 2^2 factorial experiment

Source	Sum of squares	Degrees of freedoms	Mean of squares	*F* values	*P*
Xylometazoline	135136	1	135136	49.51	< 0.001
Antazoline	17905	1	17905	6.56	0.015
Interaction	153	1	153	0.06	0.814
Error	87340	32	2729		
Total	240534	35			

factors (xylometazoine and antazoline) are set at two levels each. The levels are referred to as low and high. In the first 2^2 factorial experiment, the low level is chosen as the absence of the drugs concerned in the solutions and the high level is selected to be 0.05% for xylometazoline and 0.0007% for antazoline. The four test solutions therefore contained 0.05% xylometazoline, 0.0007% antazoline, 0.05% xylometazoline and 0.0007% antazoline, and control medium only.

The AUC values were calculated from each ciliary beat frequency time curve. The beat frequencies were normalised with respect to the initial value (100%) and the AUC was the area between the line $y = 100\%$ and the decay curve. Analysis of variance of the AUC for the various solutions showed that the two drugs moderated each other's activity (Table 1). The combination of 0.05% xylometazoline and 0.0007% antazoline was less ciliotoxic than the individual drugs on their own, at the same concentration (Figs. 4 and 5).

To explore this interaction further, a second factorial experiment was carried out with the two xylometazoline levels being 0.05 and 0.1% and the two antazoline levels being 0.0007 and 0.007%. In this instance, the four solutions therefore contained xylometazoline and antazoline at the fol-

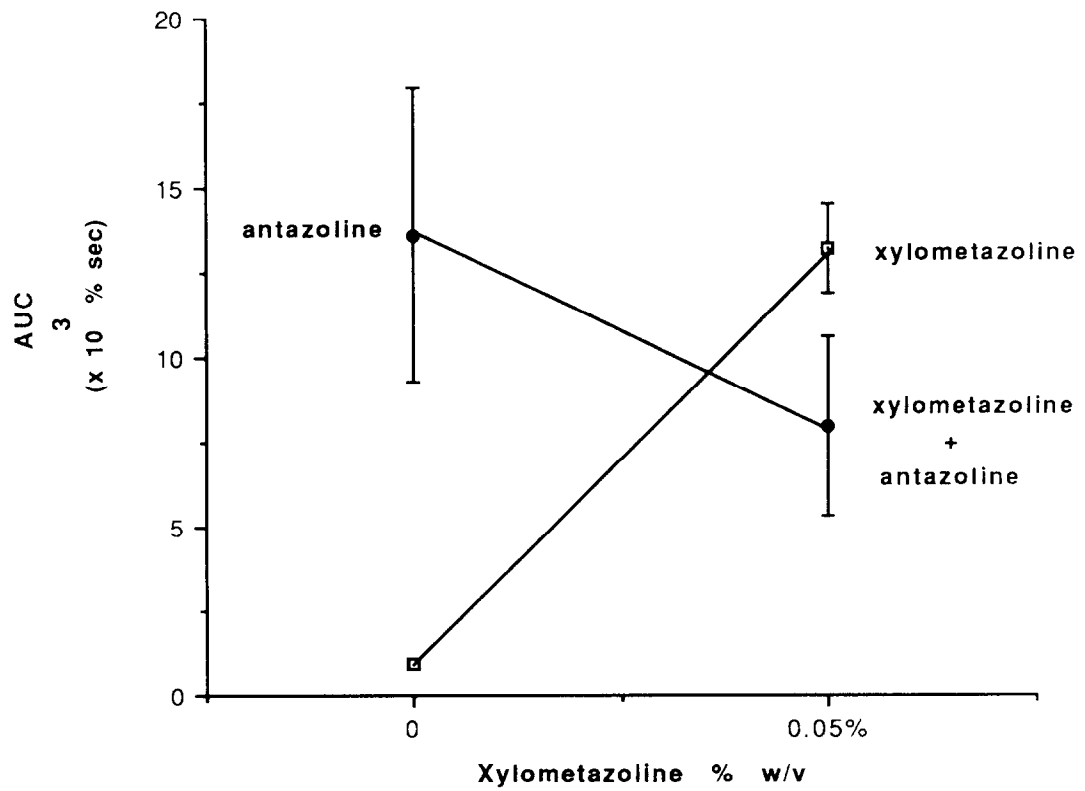

Fig. 5. Effect of xylometazoline and antazoline combination on ciliary activity. See Materials and Methods for definition of AUC.

lowing respective concentrations: (i) 0.05% + 0.0007%, (ii) 0.1% + 0.0007%, (iii) 0.05% + 0.007% and (iv) 0.1% + 0.007%.

Analysis of variance of the AUC values as previously described showed that within this higher concentration range, no interaction was evident (Table 2). The increase in ciliotoxicity, as reflected by the AUC, when the xylometazoline concentration was increased from 0.05 to 0.1%, was not significantly different in the presence of 0.0007 and 0.007% antazoline or when the concentration was raised from 0.0007 to 0.007% when xylometazoline concentration was raised from 0.05 to 0.1% (Figs 6 and 7).

Various authors have reported on the ciliotoxicity of topical nasal medications. One of the first reports was by Dudley and Cherry (1978) who described their results with commercially available nasal formulations. Using direct microscopy to grade chicken tracheal cilia with respect to extent and vigour of activity, they reported that xylometazoline depressed ciliary activity in a dose-dependent manner. However, they admitted that it was difficult to dissociate the effects of the drug from that of the preservatives present in the formulation. Indeed, Van de Donk et al. (1981) and Hermens and Merkus (1987) suggest that xylometazoline exerts only moderate ciliotoxicity in the absence of mercurial preservatives. The same authors also suggest that combinations of decongestants may show more pronounced ciliotoxicity. A more recent study (Wight and

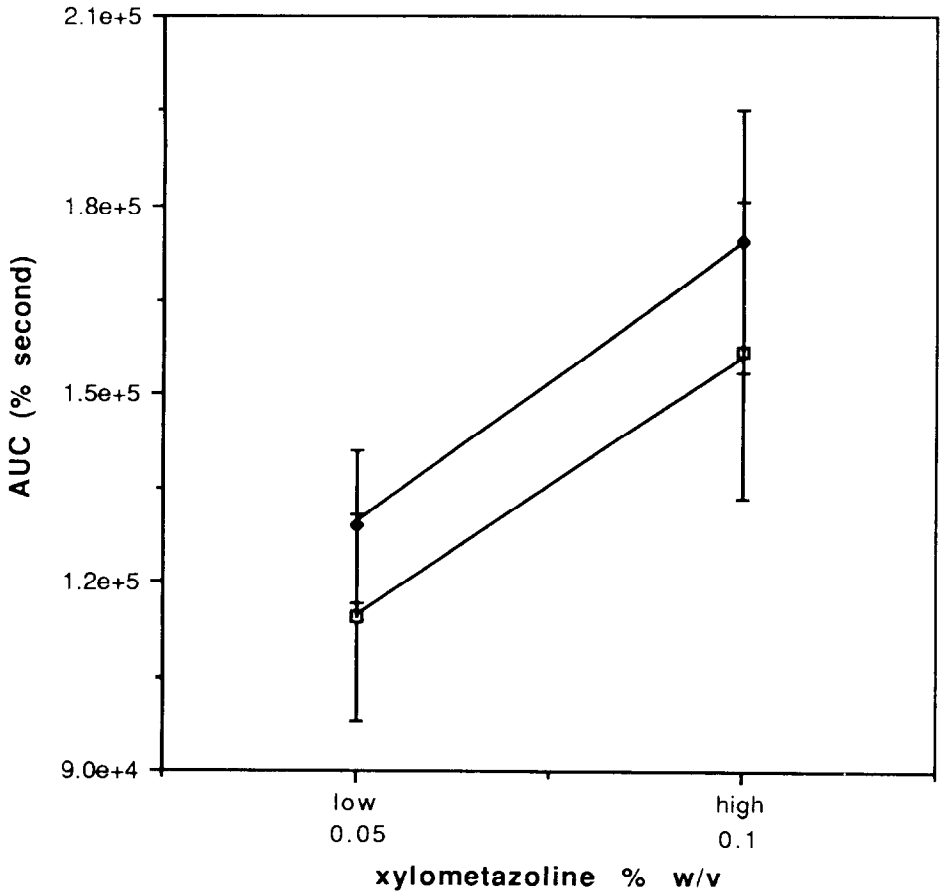

Fig. 6. Effect of xylometazoline in combination on ciliary activity in rat trachea. (□) 0.0007% antazoline and (♦) 0.007% antazoline. See Materials and Methods for definition of AUC.

Cochrane, 1990) on the effect of xylometazoline on blood flux showed that the drug decreased blood flux and nasal airway resistance but again interpretation of the results is made somewhat difficult due to the presence of preservatives in the formulations. However, the results are in agreement with those reported by Falck et al. (1990).

In our study, all the solutions used were preservative-free so that confounding was therefore not a problem. Our results clearly indicate that in the absence of blood flow, both antazoline and xylometazoline are ciliotoxic, although the latter is significantly less so. In one of the earliest studies of intranasal application of antihistamines, Gundrum (1951) reported that a combination of antistine hydrochloride (0.5%) and naphazoline hydrochloride (0.025%) caused necrosis of the nasal area in rabbits. More recently, Stillwagon et al. (1987) showed that the antihistamine (chlorpheniramine maleate)-decongestant (phenylpropanolamine) combination, given orally, protected against the decrease in nasal airway resistance induced by pollen challenge in patients with allergic rhinitis. On the other hand, no improvement in eustachian tube function could be observed following administration of an oral combination of an antihistamine and a decongestant (Virtannen, 1982). The interaction between antihistamine and decongestants on nasal function is

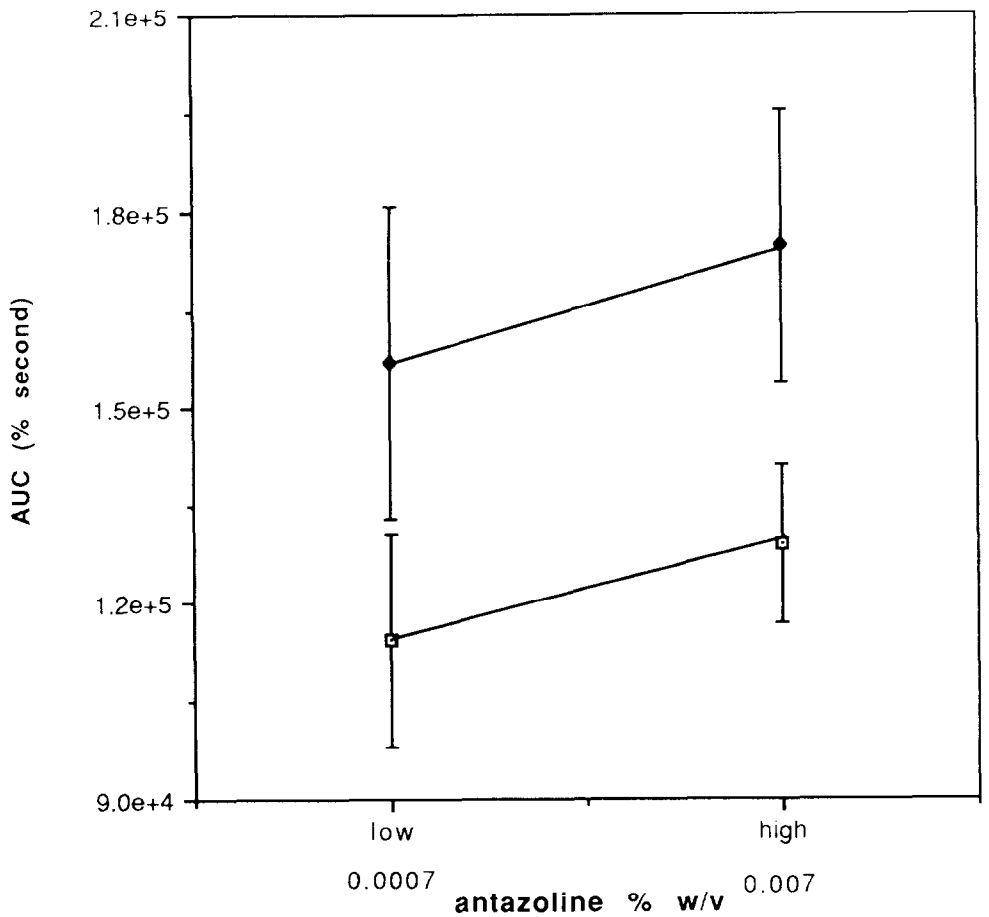

Fig. 7. Effect of antazoline in combination on ciliary activity in rat trachea. (⊡) 0.05% xylometazoline and (◆) 0.1% xylometazoline. See Materials and Methods for definition of AUC.

therefore complex with benefits observed only when there is a clear histamine involvement.

Conclusion

Our study shows that both xylometazoline and antazoline exert a cilio-inhibitory effect on in vitro rat tracheal cilia. The present factorial experiments indicate that xylometazoline and antazoline may moderate each other's effect. The pharmacological basis for this effect remains as yet unknown to us but is well worth further investigation. While ex vivo models such as that proposed by Levrier et al. (1989) are claimed to reflect the in vivo situation better, in our view, the system described herein is probably a useful first screen which will obviously need validation with in vivo experiments in humans.

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