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Correlation between the sensitivity of the ciliary beat frequency of human adenoid tissue and chicken embryo tracheas for some drugs

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SUMMARY

The effects of benzalkonium chloride, chlorbutol,xylometazoline and naphazoline on the ciliary beat frequency of human adenoids and chicken embryo tracheas have been determined and compared.

Chlorbutol 0.5% appeared to arrest ciliary motion in both tissues within 5 minutes. Rinsing with Locke Ringer solution (LR) restored the ciliary motion almost completely in both cases.

Benzalkonium chloride 0.006% + EDTA 0.1% decreased the ciliary beat frequency 35% for the human tissues and 50% for the chicken tissues after a contact of 20 minutes. In both cases the frequency hardly changed after rinsing with LR.

Naphazoline nitrate 0.1% and xylometazoline HCl 0.05% have reversible effects on the ciliary beat frequency of both human adenoids and chicken embryo tracheas. Cilia of human adenoids appeared to be more sensitive for xylometazoline than for naphazoline; whereas cilia of chicken embryo tracheas were more affected by naphazoline than by xylometazoline. The results with human adenoids and chicken embryo tracheas show a strong correlation (correlation coeff. = 0.82, $p < 0.005$). In the initial response the differences in sensitivity to preservatives and drugs were in many cases statistically significant, but the final effects were similar.

INTRODUCTION

In a former study Van de Donk et al. (1980a) described a photo-electric registration device with which the ciliary beat frequency can be determined "in vitro". With this instrument the influence of preservatives (Van de Donk et al., 1980b) and nasal drops (Van de Donk et al., 1981) on the ciliary beat frequency of chicken embryo tracheas have been determined.

The question arises whether a correlation exists between the sensitivity of the

ciliary activity of chicken embryo tracheas and that of the human nasal epithelium. An indication has been given by the results of investigations with transmission electron microscopy: the anatomy of cilia of humans and all animals are quite similar (Satir, 1980).
However, anatomical identity does not guarantee physiological identity. Therefore we investigated the influence of two preservatives (chlorbutol and benzalkonium chloride + EDTA) and two drugs (xylometazoline HCl and naphazoline nitrate) on the ciliary beat frequency of human adenoids and compared these results with the effects on the ciliary beat frequency of chicken embryo tracheas.

MATERIALS AND METHODS

The experiments were performed in Locke Ringer solution (LR), pH = 7.4 and a tempeature of 25 °C. The ciliary beat frequency was assessed by a photo-electric registration device (Van de Donk et al., 1980a). The effect of each compound was assessed on six different chicken embryo tracheas and on six different human adenoids. The preparation of the chicken embryo tracheas is described by Van de Donk et al. (1980a). The human adenoids were obtained from children (younger than 13 years) with infected adenoids. Anaesthesia was performed with a nitrous oxide/oxygen mixture; local anaesthetics were not used. The adenoids were removed by adenoidectomy.
The adenoids were collected in LR and kept at room temperature. The time between the adenoidectomy and the start of the experiments was about two hours. Slices were cut off of approximately 1 mm thick. The slices were turned on their sides and inspected for the presence of motile cilia. When the frequency of the ciliary beat exceeded 8 Hz, which was sufficient for our experiments, the piece of tissue was judged suitable for the experiments. The effects of chlorbutol 0.5%, benzalkonium chloride 0.006% + EDTA 0.1%, naphazoline nitrate 0.1% and xylometazoline HCl 0.05% in LR on both human adenoids and chicken embryo tracheas were determined.
The differences in sensitivity were submitted to a student test. The correlation was assessed with a spearman-rank correlation test.

RESULTS

On about 50% of the adenoids we were able to find motile cilia in the not inflamed areas of the adenoids. The mean ciliary beat frequency of the adenoids, used for the experiments, was 11.1 Hz $\pm$ 1.7 and of the chicken tracheas 16.9 Hz $\pm$ 1.8 (mean $\pm$ s.d.).
The effects of chlorbutol 0.5% and of benzalkonium chloride 0.006% + EDTA 0.1%, xylometazoline HCl 0.05% and naphazoline nitrate 0.1% are demonstated by Table 1.
The first column shows the ciliary beat frequency just before the experiment

Table 1. The effects of chlorbutol, benzalkonium chloride + EDTA, xylometazoline HCl and naphazoline nitrate on ciliary movement.

compound	species	initial[1]) frequency	frequency[2]) $t = 5$ min.		t (min)[3]) LR	frequency[2]) $t = 0.33$ h		P[4])
chlorbutol 0.5%	human	10.6 ± 1.6	0%		5	65%		0.95
	chicken	17.1 ± 2.0	0%		5	81%		
			frequency[2]) $t = 0.33$ h	P[4])	t(h)[3]) LR	frequency[2]) $t = 0.67$ h	P[4])	$t = 1$ h	P[4])
benzalkonium chloride 0.006% + EDTA 0.1%	human	11.4 ± 1.7	65%	0.995	0.33	65%	0.9	66%	0.995
	chicken	17.4 ± 1.9	50%		0.33	57%		49%	
xylometazoline HCl 0.05%	human	11.4 ± 1.2	57%	0.95	0.33	88%	0.75	102%	0.75
	chicken	16.6 ± 2.0	75%		0.33	101%		100%	
naphazoline nitrate 0.1%	human	11.0 ± 1.8	67%	0.9	0.33	95%	0.9	100%	0.75
	chicken	16.3 ± 1.4	52%		0.33	79%		92%	

[1]) Initial frequency and standard deviation in beats/sec.
[2]) Percentage of the initial frequency after the indicated time.
[3]) Time after which the rings were rinsed with LR.
[4]) Probability of the difference between the beat frequency of human and chicken cilia.

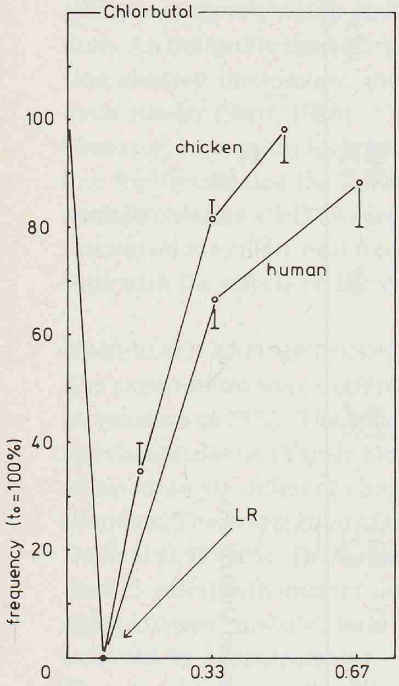

Figure 1.
Time versus frequency plot: chlorbutol 0.5% washed after 5 min. with LR.

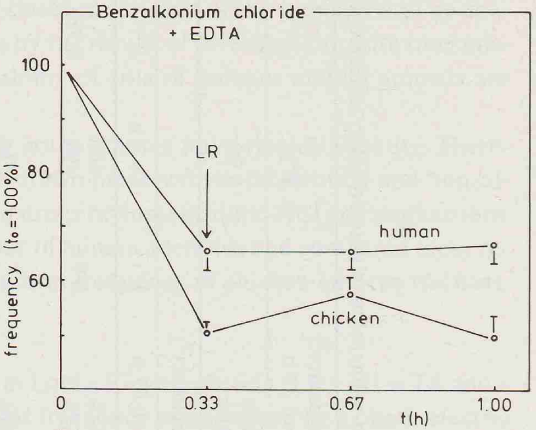

Figure 2.
Time versus frequency plot: benzalkonium chloride 0.006% + EDTA 0.1% washed after 20 min. with LR.

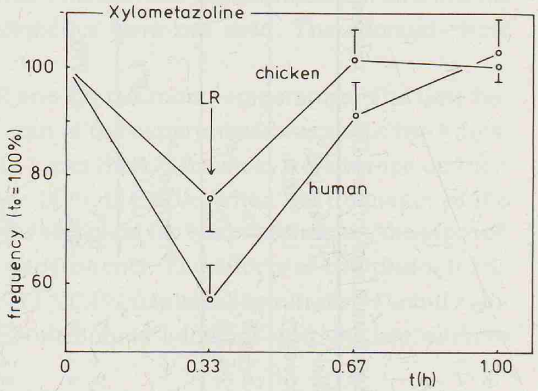

Figure 3.
Time versus frequency plot: xylometazoline HCl 0.05% washed after 20 min. with LR.

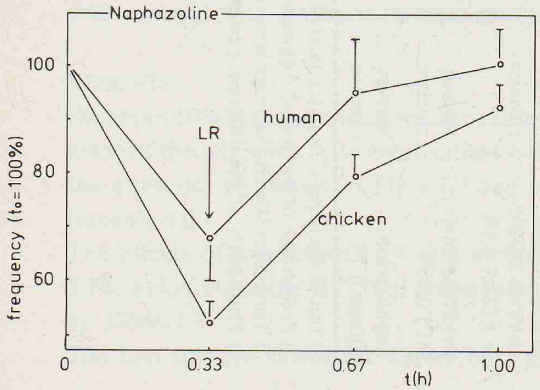

Figure 4.
Time versus frequency plot: naphazoline nitrate 0.1% washed after 20 min. with LR.

started. The second column gives the frequencies at the first measurement, as a percentage of the initial frequency. The effect of chlorbutol was first measured after a 5 minutes' contact, the other after a 20 minutes' contact.
After the first measurement the tissues were rinsed with Locke Ringer solutions. The last columns show the frequency as a percentage of the initial frequency after rinsing with LR.
The probabilities of the differences between the ciliary beat frequencies of human adenoids and chicken embryo tracheas at the same drug concentration and contact time are indicated next to the frequencies.
The Figures 1–4 demonstrate the effects of the preservatives and the two decongestants in more details. The S.E.M. values are indicated by vertical bars.

DISCUSSION

The purpose of this study was to compare ciliary epithelium of humans and chicken embryos.
The pieces of tissues must have a surface of at least a few square millimeters to perform the experiments. As we had human adenoids at our disposal we did not ask volunteers to provide us with pieces of ciliary epithelium. We investigated chlorbutol as a representative of the lipophilic preservatives with fast but reversible effects on ciliary motion.
Benzalkonium chloride, mostly combined with EDTA, belongs to the polar preservatives with slow but irreversible effects on ciliary motion. Naphazoline nitrate and xylometazoline HCl were tested because they are extensively used as drugs in nasal medications.
With the human adenoids, we performed experiments without pieces of tissue that could serve as a reference, because the intact area was mostly too small. Therefore we focused our experiments on the reversibility of the effects. In case of complete reversibility, the tissues served as their own references.
This procedure was effective in the cases of chlorbutol, xylometazoline and naphazoline; the ciliary beat frequency returned respectively to 87%, 102% and 100% of the initial frequencies.
The effects of chlorbutol 0.5% on the ciliary beat frequency of human adenoids and chicken embryo tracheas (Table 1, Figure 1) are quite comparable. In both cases the cilia are immotile after a 5 min. contact. This effect is a bit more reversible for the chicken tracheas, which can be explained by the condition of these tissues. The difference after 20 min. is significant ($p < 0.05$). The chicken trachea tissues turned out to be significantly more sensitive than the adenoid tissues for the effects of benzalkonium chloride 0.006% + EDTA 0.1% (Table 1, Figure 2). The decrease of ciliary beat frequency during contact with this preserving combination and subsequently no more change in frequency after rinsing with LR, is however the same for both tissues.

Human adenoids are more sensitive for xylometazoline HCl 0.05% than chicken tracheas are (Table 1, Figure 3). For naphazoline nitrate 0.1% this situation is just the reverse (Table 1, Figure 4). In both cases the differences are significant after 0.33 h and 0.67 h, but not after 1 h.

Again the tendency: decrease of ciliary beat frequency during contact with the drugs and increase of the frequency up to about 100% of the initial frequency after rinsing with LR, is for both drugs and both kinds of tissue the same.

The overall correlation coefficient for all the experiments with regard to the sensitivity of human adenoids and chicken embryo tracheas is 0.82 ($p < 0.005$).

The differences in the sensitivity of human adenoids and chicken embryo tracheas to the drugs and the preservatives are not likely explained by variations in the pathological status of the tissues, as the S.E.M. values of the ciliary beat frequency of the human adenoids (Figures 1–4) are hardly larger than those of the chicken embryo tracheas.

We conclude that there exist some differences in sensitivity between the two species. The differences are mainly of a kinetic nature: differences in the time necessary to achieve an effect rather than differences in the extent of the eventual effect.

RÉSUMÉ

On a déterminé et comparé les effets du chlorure de benzalkonium, du chlorobutanol, du xylométazoline et du naphtazoline sur la fréquence du battement ciliaire des amygdales pharyngées humaines et des tracheas d'embryons de poulet.

Le chlorobutanol 0,5% produisait un arrêt du mouvement ciliaire en moins de 5 minutes, dans les deux tissues. Dans les deux cas, le mouvement ciliaire reprenait presque complètement après rinçage avec une solution de Locke Ringer (LR). Après avoir été exposé pendant 20 minutes au chlorure de benzalkonium 0,006% plus l'édétate de sodium 0,1%, la fréquence du mouvement ciliaire diminuait de 35% pour les tissues humains et de 50% pour les tissues de poulet. Dans les deux cas, la fréquence ne changeait guère après rinçage avec LR. Le nitrate de naphtazoline 0,1% et le chlorhydrate de xylométazoline 0,05% ont des effets réversibles sur la fréquence du battement ciliaire des amygdales pharyngées humaines aussi bien que sur celle des tracheas de poulet. Il apparaissait que les cilia des amygdales pharyngées humaines étaient plus sensibles au xylométazoline qu'au naphtazoline; les cilia des tracheas d'embryons de poulet, par contre, étaient plus affectés par le naphtazoline que par le xylométazoline. Les résultats des expériences faites avec les amygdales pharyngées humaines et les tracheas d'embryons de poulet montrent une corrélation très étroite (coefficient de corrélation = 0,82, $p < 0,005$). Quant à la réaction initiale les différences dans la sensibilité aux conservateurs et aux médicaments étaient statistiquement significatives en beaucoup des cas, mais finalement les effets étaient à peu près semblables.

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