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ATP induces respiratory ciliostimulation in rat and guinea pig in vitro and in vivo

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SUMMARY

Adenosine triphosphate (ATP) has been shown to revitalize the disturbed nasal mucociliary function in man. We investigated the effects of ATP on the ciliary beat frequency (CBF) in animals by immersing tracheal exp/ants from rats in various concentrations of ATP, and by infusing ATP intravenously to guinea pigs. CBF was measured with a photodetector technique from the swface of the exp/ants or from the incised trachea. ATP (from 0.01 to 1 mg/ml) in vitro increased CBF in rat tracheal exp/ants up to 10.5% ($p < 0.05$). In viva ATP (1 mg/kg) increased the CBF by 29% ($p < 0.01$) in the guinea pig trachea. As the CBF was increased by ATP, both in vitro and in viva, it can be suggested that the improvement in mucociliary transport by exogenous ATP as shown in previous studies is caused by the ciliostimulatory effect of ATP.

INTRODUCTION

Mucociliary function is one of the most important defense mechanisms of the respiratory airways against microbes and other foreign particles. Lack of cilia, changes in the ultrastructure of the cilia or in the visco-elastic properties of the respiratory mucus, or dysfunction of the ciliary movements impair the mucociliary transport.

Dysfunction of airway mucosal cilia has been shown to be frequently associated with the diseases of the respiratory tract (Rossman et al., 1980). Changes in the physico-chemical properties (e.g. pH, osmolality) of the respiratory mucus and many exogenous factors can decrease the ciliary beat frequency (CBF), for example ethanol (Maurer and Liebman, 1988) and various drugs (Pariente, 1988). Viruses (Sakakura et al., 1985) as well as toxins produced by bacteria, e.g. in chronic bronchitis, inhibit mucociliary clearance by disturbing the ciliary beating movements (Dirksen et al., 1987; Ohashi et al., 1987; Weich et al., 1988).

In patients with immotile cilia syndrome the incidence of respiratory infections is

high. The reason for impaired ciliary activity is the absence of the dynein arms normally found in the nine peripheral, microtubular doublets. These arms contain the adenosine-triphosphatase-containing protein dynein. Vorhaus and Deyrup (1953) showed, using an aluminium-foil particle technique in vitro, that local administration of adenosine triphosphate (ATP) improved mucociliary transport of the pharyngeal mucosa of the frog. **ATP** induces active movement also in the cilia of patients with immotile cilia syndrome in vitro (Forrest et al., 1979).

ATP has been also tried on patients with impaired mucociliary function for reasons other than classical immotile cilia syndrome. In patients with chronic rhinitis, for instance, promising results have been achieved (Nuutinen et al., 1988). The exact site and the mechanism ofaction ofATP is obscure. In this study we investigated the nature of the effect of ATP on ciliary activity in rats and guinea pigs. Mucosa! explants from rats were immersed in a solution ofATP, and ATP was injected intravenously to anaesthetized guinea pigs, and changes in ciliary function were measured photo-electrically.

MATERIAL AND METHODS

Animals

Male Wistar rats (weight 270-350 g, N = 5 in each of the eight groups) and female Dunkin Hartley guinea pigs (average weight 1000 g, N = 6 in each of the two groups) were used. Until sacrifice, they were kept in laboratory conditions (temperature 20± 1 °C, relative humidity 55-75%, lights on from 7 a.m. to 9 p.m.) and fed ad libiturn.

In vitro study

Rats were decapitated, the neck was dissected, and a piece of trachea from the second to the sixth cartilage caudally from the larynx was excised and opened along the midline. The explant was attached, inner surface outwards, to a slightly convex piece of silicone rubber, which was placed into a measurement chamber, which was kept warm (37±0.1 °C) from beneath by circulating water, and moist from above with a flow (2 l/min) of heated (37±0.1 °C), humid air (relative humidity 95%).

In viva study

Guinea pigs were anaesthetized with a combination of 7.5 mg/kg midazolam, 0.473 mg/kg fentanyl and 15 mg/kg fluanisone intraperitoneally. The vena jugularis was cannulated, and the trachea was carefully freed from the surrounding tissues. Then, the trachea was incised from the second to the sixth cartilage caudally from the larynx, and gently elevated from below by a slightly convex steel support to prevent the respiratory movements from shaking the

measurement area. The animal was placed on an electrically heated blanket, and the dissected area was sheltered with a plastic cover, into which warm (3 7± 1 °C), humid air (relative humidity 95%) was blown continuously (2 1/min).

Drug treatment

In the in vitro studies, the explants were immersed in Locke-Ringer solution containing either 0.005, 0.01, 0.05, 0.1, 1.0, 5 or 10 mg/ml of ATP. Control explants were immersed in drug-free Locke/Ringer solution. The solution was carefully pipetted on the bottom of the measurement chamber (not directly on the explant), until the surface of the liquid covered the explant totally. Before the measurement of the CBF, the liquid was removed by suction. After measurement, which took approximately one minute, the chamber was again filled with solution. Each explant was treated only with one ATP concentration. In the in vivo experiments, drug solution was injected 15 min after finishing the incision of the trachea into the venajugularis interna; injection took one minute.

Measurement of ciliary activity

CBF was measured using a modification (Karttunen et al., 1990) of the method published by Mercke et al. (1974). Measurements were commenced 10 min after the completion of the installations and preparations.

Analysis

CBF (Hz; beats/sec) was calculated from the undistorted sections of the recording. In the in vitro study, the significance of the differences (ATP-treated vs. controls) in CBF values was analyzed using analysis of variance (Student-Newman-Keuls method). Differences in CBF values within each group (before vs. after immersion) were analyzed using two-way ANOVA and Scheffe's test. In the in vivo study, the differences between $t=0$ and $t=20$ min CBF values were analyzed using a paired t-test, and the differences between control and ATP-treated guinea pigs were analyzed with Student's two-tailed t-test. Differences producing values of $p<0.05$ were considered as statistically significant.

RESULTS

CBF in vitro

In control explants, as well as in those immersed in 0.005 mg/ml concentration of ATP, CBF decreased time-dependently ($p<0.05$); Table 1. ATP increased the rat tracheal CBF. The effect appeared to be time-dependent, but dose-dependency was not so apparent: ATP concentrations of 0.005, 5 and 10 mg/ml were ineffective, but concentrations of 0.01, 0.05, 0.1 and 1 mg/ml increased the CBF by 10.5, 8.1, 5.8 resp. 8.7% (Figure 1).

Table 1. Ciliary beat frequency in rat tracheal explants immersed in ATP-containing Locke-Ringer solution. Values are beats/min (Hz; mean ± SEM, N = 5 with each concentration).

ATP (mg/ml)	duration of immersion (min)					
	0	10	20	30	45	60
0	18.0±0.1	17.9±0.1	17.4±0.1^{+}	17.4±0.1^{+}	17.5±0.1^{+}	17.2±0.1^{+}
0.005	18.0±0.0	17.7±0.1	17.4±0.1^{+}	17.3±0.1^{+}	17.2±0.1^{+}	17.1±0.1^{+}
0.01	18.1±0.4	18.2±0.2	18.4±0.3	18.4±0.3	18.5±0.3	19.0±0.3*$^{+}$
0.05	18.2±0.3	18.5±0.6	18.3±0.2	18.4±0.4	18.6±0.4*	18.6±0.4*
0.1	18.0±0.1	18.0±0.1	18.1±0.1	18.2±0.1	18.2±0.1	18.2±0.1
1.0	18.2±0.3	18.5±0.4	18.4±0.4	18.4±0.4	18.2±0.2	18.7±0.4*
5.0	17.8±0.4	17.8±0.3	17.7±0.6	17.8±0.1	17.5±0.3	17.5±0.2
10.0	17.7±0.4	17.6±0.5	17.4±0.4	17.5±0.4	17.7±0.3	17.7±0.3

* = significantly ($p < 0.05$) different from respective value in control group.
$^{+}$ = significantly ($p < 0.05$) different from value before immersion.

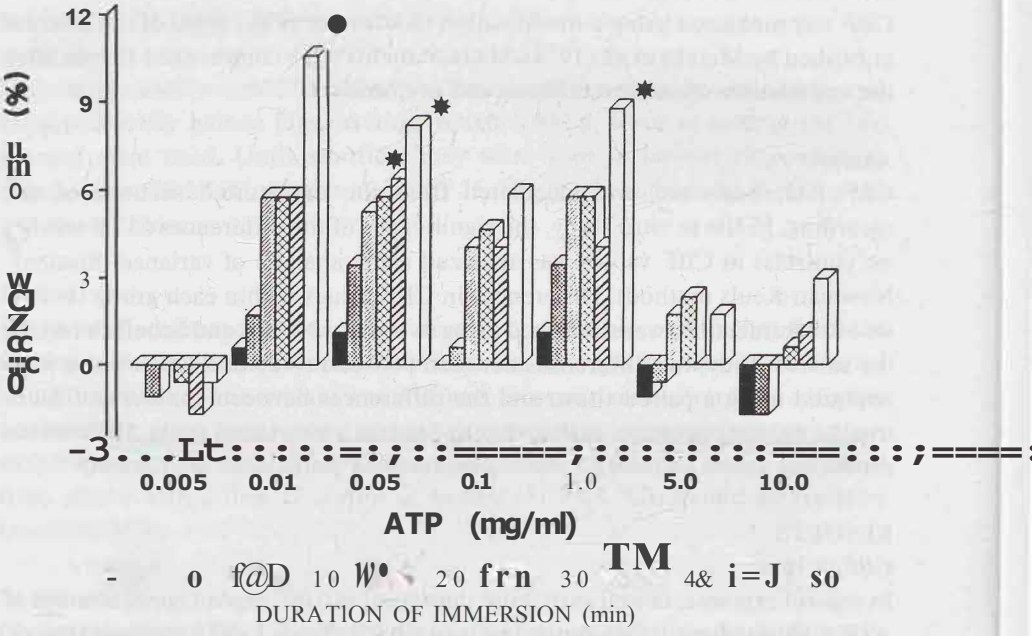

Figure 1. Effect of ATP on the ciliary beat frequency in rat tracheal explants. Values are changes in percentages (means from 5 explants/concentration) observed after 10, 20, 30, 45 and 60 min immersion as compared with control values measured from explants immersed in ATP-free Locke-Ringer solution. Black columns indicate the difference in ciliary beating frequency before immersion as compared to controls.
* = $p < 0.05$, calculated from the differences between values in Hz (beats/sec).

CBF in viva

CBF was increased by 29% ($p < 0.01$) in guinea pigs that were given 1 mg/kg of ATP. CBF was not altered in control animals during the 20-min observation period (Table 2).

Table 2. Effect of ATP (1 mg/kg i.v.) on ciliary beat frequency (Hz, mean ± SEM, N = 6 in both groups) in the trachea of anaesthetized guinea pigs. NS = not significant ($p > 0.05$).

	at time 0 min	at time 20 min	change
controls	16.6±0.5	16.5 ± 0.5	- 0.6%, NS
ATP-treated	15.1±0.8	19.5±1.4	+29.0%, $p<0.01$
difference between groups	NS	NS	

DISCUSSION

Ciliary activity of the respiratory airways may be impaired for a variety of reasons (Rossman et al., 1980), and only in part of the cases is a curative treatment (e.g. eradication of bacteria) possible. In many cases a treatment that would support the insufficient ciliary clearance would be useful, but there is a lack of possibilities for improving mucociliary function, although various compounds and factors are known to increase the mucociliary clearance of the respiratory airways. Mechanical stimulation of the cell surface increases the CBF (Sanderson and Dirksen, 1989), which is why in this study the tracheal tissue was not touched or mechanically manipulated after the setting up procedure of the preparation. Pharmacologically, ciliary function may be enhanced by i.a. histamine (Garrard et al., 1989) and bradykinin (Tamaoki et al., 1989), but these compounds are not suitable for clinical therapy. Terbutaline, fenoterol and mabuterol, which have been shown to increase mucociliary clearance (Miyata et al., 1987; Pavia et al., 1987; Weich et al., 1988), represent a therapeutically and potentially useful group. /J-Mimetics probably activate cilia by increasing the concentration of cyclic AMP in the tissue (Sanderson and Dirksen, 1989).

Saavedra and Renaud (1975) managed to reactivate the ciliary movements of the ciliated protozoan *Tetrahymena pyriformis* with ATP. The mucociliary transport in human nose is accelerated by local administration of ATP (Nuutinen, 1985a and b) which implies that ATP either has an effect on the secretion and properties of the respiratory mucus, or on the activity of the cilia. Our present results suggest that the effect of ATP on ciliary function is ciliostimulation. In vitro, the explants were without the cover of respiratory mucus, and in spite of this the ciliary beating activity was increased after immersion in ATP solution.

The present results from rats can be interpreted to support our previous

suggestion (Saano et al., 1990) that, when the cilia are functioning normally, the effect of exogenous ATP on rat tracheal ciliary b at frequency in vivo is not very extensive. In experimental conditions, due to lack of blood circulation (in explants) or due to surgical incision (in vivo study), there is a leakage of ATP from the tissue. In that case, the administration of ATP normalizes the energy supply to cilia. The longer the experiment lasts, the more severe is the deterioration in the ciliary activity, and the ciliostimulatory effect of ATP is more clearly observable. The lack of a straightforward dose-dependency also supports this explanation.

The dose-response curve has a humped form, the high concentrations (5 and 10 mg/ml) showing no ciliostimulatory effect. This may be due to lack of effect at low concentrations, and due to high concentrations of ATP being ciliotoxic (Saavedra and Renaud, 1975).

In our study on anaesthetized rats, intravenous ATP (10 mg/kg) did not accelerate the CBF, but helped to maintain ciliary activity, which deteriorated in control animals (Saano et al., 1990). In the present study, intravenous ATP increased guinea pig tracheal CBF by 29%. Also in our in vitro studies on the effect of ATP on human maxillary sinus samples up to 20% increase in the CBF after immersion in ATP solution was obtained, whereas lower concentrations caused a smaller effect (Saano et al., 1991). Rats are not as sensitive as guinea pigs for the demonstration of drug effects on the respiratory system (Friebel, 1969), and this seems to be the case also with the ciliostimulatory action of ATP. With isoproterenol, however, increases of 50% or even more have been produced in rat tracheal explants (Lopez-Vidriero et al., 1985). Thus, also in rat, it is possible to pharmacologically increase the CBF markedly above the normal level.

The details of the mechanisms, how ATP acts, and how it reaches the ciliary structures, are still unclear. It has been suggested that ATP may enter the cilia by diffusion from the cytoplasmic matrix (Saavedra and Renaud, 1975). On the other hand, the vasodilatory effect of ATP on many vessels may have some importance on the ciliary function. Hybbinette (1981) suggested the energy supply of the mucosal vascular bed to be an important factor for the ciliary activity. However, further studies on the mechanism of ATP-induced ciliostimulation are needed. Among other questions, the possible role of adenosine-and purine receptors must be investigated. ATP is rapidly metabolized into adenosine, which has been suggested (Newberg et al., 1985) to be responsible of the cardiovascular effects seen after intravenous infusion of ATP (Saano et al., 1990).

In conclusion, ATP can stimulate the ciliary function in vitro and in vivo, and the ATP-induced improvement in mucociliary clearance (Nuutinen 1985a and b; Nuutinen et al., 1988) is most probably caused by increased ciliary beat frequency. Many compounds presently used as drugs or as preservatives in drugs decrease ciliary beat frequency (Van de Donk et al., 1981; Stanley et al., 1985;

Hermens and Merkus, 1987). From the clinical point of view, concurrent administration of ATP with these drugs may help in preventing these untoward ciliostatic effects.

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