EFFECTS OF \( \beta \)-ADRENOCEPTOR STIMULANTS ON THE CANINE TRACHEAL CILIATED CELLS

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Abstract—We investigated the effect of \( \beta \)-adrenoceptor stimulants on the canine tracheal ciliated cells. A mucosal specimen of the tracheal membranous wall was placed in a chamber containing Hanks’ solution. Membrane potential was recorded by an intracellular microelectrode, and ciliary beating was measured by a photoelectrical technique. Isoproterenol and salbutamol in \( 10^{-7} \) to \( 10^{-4} \) M caused a concentration-dependent increase in both frequencies of ciliary beating and small electrical oscillatories were superimposed on the membrane potential. Theophylline also increased frequencies of both activities in doses of \( 10^{-5} \) M and above. Onset of action of theophylline was later than that of isoproterenol or salbutamol. All the above drugs only slightly depolarized the membrane potential, in concentrations of \( 10^{-4} \) M. Propranolol (\( 10^{-7} \)–\( 10^{-4} \) M) alone had little effect on ciliary beating and electrical activity. However, the effects of isoproterenol and salbutamol were effectively antagonized by propranolol (\( 10^{-5} \) M). Dibutyryl cyclic AMP (\( 10^{-7} \)–\( 10^{-4} \) M) caused concentration-dependent increases in ciliary beating and electrical activity. These results suggest that potent bronchodilators such as \( \beta \)-stimulants and theophylline increase the activity of tracheal ciliated cells by stimulating the intracellular cyclic AMP system.

Mucociliary transport is an important defence mechanism in the airway. \( \beta \)-Adrenoceptor stimulants such as terbutaline and epinephrine which are used to treat chronic obstructive pulmonary disease, reportedly enhance mucociliary clearance in humans (1, 2) and in the isolated rat (3) and canine tracheae (4). Furthermore, \( \beta \)-adrenoceptor stimulants (NAB 365 and epinephrine) enhance secretory activity in the airway (3, 5, 6). Whether the increase in mucociliary clearance which is produced by isoproterenol can be ascribed to a direct action on the ciliated cells or to a change in rheological property of mucous secretions is unclear.

We investigated the direct effects of \( \beta \)-adrenoceptor stimulants, theophylline and cyclic AMP on tracheal ciliary activity, using microelectrode and photoelectrical methods.

MATERIALS AND METHODS

The method used in the present study was as described previously (7, 8). Briefly, the tracheae were excised from dogs (8–12 kg) anesthetized with sodium pentobarbital 30 (mg/kg. i.v.). A 5x5 mm piece of the tracheal mucosal membrane (smooth muscle layer-free) was placed in a 2 ml chamber containing Hanks’ solution (136.9 mM NaCl, 5.3 mM KCl, 1.1 mM CaCl2, 0.8 mM MgSO4, 0.3 mM Na2HPO4, 0.4 mM KH2PO4, 4.2 mM
NaHCO₃ and 11.1 mM glucose). The solution used in the experiment was adjusted to pH 7.2±0.1 and continuously oxygenated with a 95% O₂--5% CO₂ mixture. The temperature of the solution was kept at 36.0±0.1°C. The flow rate of the perfusing Hanks' solution in the experimental chamber was kept constant at 1 ml/min. Ciliary beating was measured using the photo-electrical technique described previously (8).

Membrane potential and oscillations were determined using the microelectrode method (7, 8). A microelectrode (less than 0.5 mm in external tip diameter and 30–60 MΩ in resistance) filled with 3 M KCl was used. As described previously (7), membrane potential of a tracheal ciliated cell is superimposed with small electrical oscillations. The oscillatory potentials were recorded using a high gain AC amplifier (Nihon Kohden, AVB-2), and frequency of the oscillations was determined to evaluate drug effects.

The ciliary beating and intracellular potential were recorded for 2 sec, every 30 sec during 5 min after initiation of the drug administration. The changes in frequencies of ciliary beating and oscillatory potentials were expressed as mean % change ±S.E. The percent changes were calculated by comparing the frequency during a 5-min period of drug treatment with that during a 5-min period before treatment. The Student's t-test was used for the statistical analysis of data.

**Drugs:** d,l-Isoproterenol hydrochloride (Sigma) N⁶,O²'-dibutyryl-adenosine 3',5'-cyclic monophosphoric acid (DBcAMP, Sigma), salbutamol sulfate dihydrate (Sankyo), theophylline (Tokyo Chemical) and d,l-propranolol hydrochloride (ICI) were dissolved in Hanks' solution. Drug solutions were applied to the ciliated epithelial preparation by perfusing the solutions in Hanks' medium into an experimental chamber at a rate of 1 ml/min.

**RESULTS**

**Basal membrane potential and ciliary beating:** The average membrane potential was -29.2±1.5 (S.E.) mV (N=48) in untreated ciliated cells. The frequency and amplitude of oscillatory potentials were 13.2±1.1 Hz and 0.21±0.02 mV, respectively. The frequency of ciliary beating was 13.3±1.8 beats/sec. These values were coincident with

![Graph](https://via.placeholder.com/150)

Fig. 1. Effects of isoproterenol on frequencies of ciliary beating (left) and oscillatory potentials (right) of canine tracheal ciliated cells. Each point represents the mean percent change with S.E. from control for 5 to 7 experiments.
those obtained previously (7, 8). The oscillations were stable at least for one hour.

Effect of isoproterenol: Application of d,l-isoproterenol (10^{-7}–10^{-4} M) into the chamber increased the frequency of ciliary beating, concentration-dependently, within 30 sec after a start of drug perfusion (Fig. 1). The enhanced ciliary activity decreased to the control level after isoproterenol solution was displaced by control Hanks’ solution. The frequency of oscillatory potentials was also increased. In 10^{-4} M, the mean frequencies of ciliary beating and oscillatory potentials during a 5-min period increased by 31.5±5.2% (p<0.001) and 26.7±6.8% (p<0.01), respectively (Fig. 2). On the other hand, only a slight depolarization (approximately 3 mV) occurred following treatment with isoproterenol (10^{-4} M).

Effect of salbutamol: Salbutamol (10^{-7}–10^{-4} M) caused a concentration-dependent rise in the frequencies of ciliary beating and oscillatory potentials (Fig. 3). Salbutamol was less potent than isoproterenol. Salbutamol only slightly depolarized the membrane in 10^{-5}–10^{-4} M. In 10^{-4} M, the frequencies of ciliary beating and oscillatory potentials increased by 27.2±5.1% and 17.2±2.5% (p<0.001), respectively.

Effect of theophylline: Theophylline increased the frequency of ciliary beating in concentrations above 10^{-5} M. The change in ciliary beating appeared 60–120 sec after the administration of theophylline (Fig. 4). The frequency of oscillatory potentials was also increased. In 10^{-4} M, the frequencies of ciliary beating and oscillatory potentials increased by 17.6±4.8% (p<0.01) and 13.5±2.8% (p<0.01), respectively.

Effects of β-stimulants and theophylline in the presence of propranolol: Propranolol in 10^{-8} to 10^{-5} M alone did not affect ciliary activity. In 10^{-4} M, the β-blocking agent produced a slight decrease in the frequency

![Fig. 2. Effects of isoproterenol on frequencies of ciliary beating (left) and oscillatory potentials (right) of canine tracheal ciliated cells. Each column represents the mean with S.E. for 5 to 7 experiments. The percent changes were calculated by comparing the frequency during a 5-min period of drug treatment with that during a 5-min period before treatment. Significantly different at *p<0.05, **p<0.01 and ***p<0.001](image)

![Fig. 3. Effects of salbutamol on ciliary beating (left) and oscillatory potentials (right) of canine tracheal ciliated cells. Other explanations as in Fig. 2.](image)

![Fig. 4. Effects of theophylline on ciliary beating (left) and oscillatory potentials (right) of canine tracheal ciliated cells. Other explanations as in Fig. 2.](image)
of ciliary beating. After treatment with $10^{-5}$ M propranolol for 5 min, various concentrations of isoproterenol ($10^{-7}$–$10^{-4}$ M) had no effect on ciliary beating and oscillatory potentials (Figs. 5 and 6). The slight depolarization produced by high concentrations of isoproterenol was also blocked by propranolol. The blocking effect of propranolol was reversible; that is, isoproterenol again enhanced the ciliary activities after the tissue preparation had been washed with control Hanks’ solution for 15 min. After treatment with $10^{-5}$ M propranolol for 5 min, the stimulating effect of salbutamol ($10^{-5}$ M) on ciliary beating was also blocked (Fig. 6). On the other hand, propranolol did not significantly inhibit the action of theophylline (Fig. 6), although the stimulating effect of theophylline appeared after a more prolonged time lag of 2 to 3 min in the presence of propranolol (Fig. 7).

Effect of DBcAMP: In concentrations of...
10^{-7} to 10^{-4} M, DBcAMP enhanced ciliary activities within 30–60 sec after application of the agent (Fig. 8). DBcAMP only slightly depolarized the membrane in 10^{-5}–10^{-4} M.

**DISCUSSION**

β-Adrenergic stimulants such as isoproterenol and epinephrine reportedly stimulate mucociliary transport in humans (1) and canine trachea (4). Both ciliary activity and overlying mucus are involved in mucociliary transport. Iravani et al. (3, 6) observed that NAB 365, a selective β2-agonist, caused an increase in mucus production in the rat and cat trachea. It has, thus, been unclear whether the stimulating effects of β-adrenergic stimulants on mucociliary transport are due to a direct effect on ciliated cells or to effects on mucus rheology.

The present study showed that isoproterenol and salbutamol caused a concentration-dependent enhancement of electrical activity (oscillatory potentials) as well as an increase in ciliary beating of tracheal ciliated cells, indicating that β-stimulants have a direct stimulating effect on the ciliary activity. Salbutamol, a relatively selective β2-agonist, had a potent cilio-exciting action similar to that seen with isoproterenol and, in addition, the enhanced ciliary activities induced by the two drugs were markedly inhibited by butoxamine, a β2-antagonist (unpublished data). Therefore, the effects of β-adrenoceptor stimulants on tracheal ciliated cells are probably mediated by β2-adrenoceptors on the cells.

Theophylline, another type of a bronchodilator which is widely used for treatment of bronchial asthma, also enhanced ciliary beating and oscillatory potentials. This finding indicates that theophylline has a direct stimulating effect on ciliated cells. As well as its cilio-exciting action, a stimulating action of theophylline on mucus production may also be partly responsible for the enhanced mucociliary transport (9–11). The cilio-exciting effect of theophylline appeared after a time lag of 1–2 min. Theophylline probably produces an inhibition of phosphodiesterase which leads to an accumulation of cyclic AMP in the cells. The time lag would be the period for accumulation of the cyclic AMP. In the presence of propranolol, the time lag in onset of action of theophylline was lengthened and such may be due to the local anesthetic effect of propranolol (12, 13).

Effects of cyclic AMP on tracheal ciliated cells have not been reported. We found that application of DBcAMP increased ciliary beating and electrical activity. The enhancement of the activities may be related to an increased energy supply through an activation of intracellular glycolysis or to an increased phospholylation of tubulin and dynein (cilia components) (14). DBcAMP caused an enhanced ciliary activity in relatively low concentrations, possibly because cyclic AMP-dependent protein kinase is abundant in the axoneme and membrane of the cilia (15, 16).

It was shown in the present study that isoproterenol has a potent cilio-exciting activity. The increased mortality in isoproterenol-treated patients with bronchial
asthma (17, 18), may therefore not be ascribable to a cilio-inhibition, but to a cardiac side effect or enhanced viscosity of intrabronchial mucus (19, 20).

The cilio-excitating as well as bronchodilating effects of isoproterenol, salbutamol and theophylline should be beneficial in the therapy for chronic obstructive pulmonary diseases such as bronchial asthma and chronic bronchitis. In addition, the present study suggests that the adenylate cyclase-cyclic AMP system plays an important role in $\beta$-adrenergic action that leads to a cilio-excitating in the trachea.

REFERENCES


19) Sturgess, J. and Reid, L.: The effect of isoprenaline and pilocarpine on (a) bronchial mucus-secreting tissue and (b) pancreas, salivary gland, heart, thymus, liver and spleen. Brit. J. exp. Pathol. 54, 388-403 (1973)