EFFECT ON CILIARY MOVEMENTS OF SOME AGENTS WHICH COME IN CONTACT WITH THE RESPIRATORY TRACT*

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Ciliary activity is one of the important protective mechanisms of the respiratory tract. Ciliary action keeps the viscous film of mucus moving towards the pharynx, thus helping to eliminate infectious agents and foreign particles. A defect in ciliary activity leads to stagnation of respiratory secretions which is a distinct aid to infection. Upper respiratory tract infections, bronchitis, pneumonia, asthma, pneumoconiosis, bronchiectasis, pulmonary atelectasis, etc. may result or be abetted by faulty ciliary function of the respiratory tract (1,4,5,9). No other tissue of the body with such a delicate system has to face, and now a days increasingly so, such a large number of abnormal gases and fumes like cigarette smoke, petrol gas, industrial fumes and gases, medicinal gases, vapours, aerosoles, etc. Body also excretes a number of drugs wholly or partly into the respiratory tract. A study of the effect of all those agents, which are either inhaled or excreted into the respiratory tract, on ciliary activity is of practical significance in relation to health and disease of the respiratory tract. The effects of a few volatile anaesthetics, volatile oils, alcohol and cigarette smoke on ciliary activity have been reported (6.7,8,10 to 16). The results of different workers are, however, variable probably because of the differences in the experimental models and solvents that have been used. It was, therefore, thought worthwhile to study the effect of a number of agents which are liable to affect the respiratory epithelium, on ciliary activity without using any special solvent.

MATERIALS AND METHODS

The ciliary motility of frog's oesophagus was studied by the method described earlier (2). Ringer solution of pH 7.5 containing NaCl, 111.11; KCl, 1.88; $CaCl_2$, 1.08; $NaHCO_3$, 2.38 and NaH_2PO_4, 0.083 *mM* was used. In all observations time taken by poppy seeds to travel a unit distance of 1 *cm* was calculated and the effect of the agents was expressed as per centage of the control effect (time to travel 1 *cm*) in each case. The effect of the agent was studied only when there were at least 3 nearly constant readings taken at intervals of 5 *min*. A number of control readings were always interpolated between the observations with the agents studied. Experiments were conducted with the following agents :—

Ethyl alcohol, paraldehyde, ether, chloroform, oil of peppermint, menthol, thymol, guaiacol, creosote, potassium iodide, oxygen, carbon dioxide, petrol gas and cigarette smoke. All

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agents were dissolved in Ringer solution. Effects of potassium iodide were compared with equimolar concentrations of potassium bromide and chloride.

Oxygen, carbon dioxide and petrol gas were bubbled through 50 *ml* of Ringer solution for 1 and/or 10 min and the solutions were then used for study. The smoke from one cigarette (Panama brand) was bubbled through 50 *ml* of Ringer solution by smoking it the *hookah* fashion. This was then used for study. The ash from the cigarette was carefully collected and put in another 50 *ml* of Ringer solution. The ash was shaken thoroughly and filtered. The filtrate was also used for study.

TABLE I

Effect of some agents on the ciliary movements

Agents and concentration	*Untreated*			*Pretreated with atropine* 10^{-6}		
	Number of experiments	*Travel time (% of control mean ± S.E.)*	*P value*	*Number of experiments*	*Travel time (% of control mean ± S.E.)*	*P value*
1. Alcohol 0.02%	6	64.1±4.1	<0.001			
„ 0.1%	5	150.7±6.3	<0.001			
„ 0.5%	5	183.2±16.2	<0.001			
„ 1.0%	5	208.6±15.1	<0.001			
2. Chloroform 0.5%	4	246.7±43.1	<0.01			
3. Ether 10.0%	10	61.8±2.3	<0.001	6	22.0±3.8	<0.001
4. Paraldehyde 1.0%	9	47.1±2.5	<0.001	4	25.3±3.1	<0.001
5. Oil of Peppermint 0.2%	6	58.2±3.8	<0.001	4	22.7±0.8	<0.001
6. Menthol**	10	71.9±3.0	<0.001	4	29.5±9.8	<0.001
7. Guaiacol 1.25%	4	57.3±3.2	<0.001	4	37.9±7.5	<0.001
8. Creosote 0.67%	4	50.8±5.8	<0.001	4	19.4±3.0	<0.001
9. Thymol 0.1%	8	172.6±7.3	<0.001			
10. Oxygen 10 min	4	57.0±5.7	<0.001			
11. CO_2(pH 6.3) 10 min	9	59.7±2.6	<0.001	4	54.3±3.0	<0.001
Ringer (pH 6.3)	7	110.2±3.1	<0.01	4	105.1±1.0	<0.05
12. Petrol gas (bubbled for 1 min in Ringer solution)	5	103.0±3.5	>0.05			
Petrol gas (bubbled for 10 min in Ringer solution)	10	160.8±5.3	<0.001			
13. Potassium iodide 0.1%	5	192.5±19.4	<0.005			
Potassium chloride*	4	107.4±7.9	>0.05			
Potassium bromide*	4	143.5±11.1	<0.01			
14. Acetylcholine 10^{-6}	8	60.8±5.2	<0.001	7	104.8±3.6	>0.05

*Concentration equimolar to potassium iodide 0.1%.

**Saturated solution.

RESULTS

The results are summarised in Tables I and II. Ether, paraldehyde, oil of peppermint, menthol, guaiacol, creosote, oxygen, carbon dioxide and weak solution of alcohol (0.02%) significantly increased ciliary activity. In order to find out whether the excitant action of these agents was due to direct action or mediated through a local reflex arc (2), their effects were also studied on atropinised cilia. Atropine pretreatment did not block the excitant action of these agents while it completely blocked that of acetylcholine, indicating that they all acted as direct excitants of cilia. The hydrogen ion concentration of carbon dioxide Ringer being pH 6.3 the effect of normal Ringer with pH adjusted to 6.3 with the help of dilute hydrochloric acid was also studied. Acidic Ringer was found to be depressant indicating a specific stimulant action of carbon dioxide.

TABLE II

Effect of cigarette smoke and ash on ciliary movements

Agents	*Untreated*			*Pretreated with pentolinium* 2×10^{-5}		
	No. of experiments	*Travel time (% of control. mean ± S.E.)*	*P value*	*No. of experiments*	*Travel time (% of control. mean ± S.E.)*	*P value*
1. Smoke Ringer	9	59.7±5.7	<0.001	4	22.8±1.7	<0.001
Nicotine 10^{-6}	4	64.4±2.4	<0.001	4	102.1±2.5	>0.05
2. Ash Ringer (pH 9.0)	6	186.3±6.9	<0.001			
Ringer (pH 9.0)	4	253.1±8.9	<0.001			
Ash Ringer (pH 7.5)	4	85.3±2.0	<0.001	5	58.3±4.6	<0.001

Chloroform, thymol, petrol gas and higher concentrations of alcohol (0.1 to 1.0 %) caused significant depression of ciliary activity. Potassium iodide in the concentration of 0.1% (6.024 mM) produced a marked retardation of ciliary movements. To assess whether the effect was due to the anion or the cation, the effect was compared with those of equimolar concentrations of potassium chloride and potassium bromide. Potassium chloride had no significant action while potassium bromide was a moderate depressant.

The effect of cigarette smoke-Ringer and ash-Ringer are summarised in Table II. Since nicotine is one of the constituents of cigarette smoke, the effect of smoke-Ringer was compared with that of nicotine. Nicotine and cigarette smoke stimulated ciliary activity to nearly the same extent. Pretreatment of the epithelium with pentolinium tartarate completely blocked the nicotine response. Smoke-Ringer on the other hand markedly stimulated the ciliary activity which was significantly depressed by pentolinium treatment. Since the pH of ash-

Ringer was 9.0, normal Ringer solution with its pH adjusted to 9.0 with sodium bicarbonate was used for comparison. The hydrogen ion concentration of cigarette ash-Ringer was also adjusted to pH 7.5 using dilute hydrochloric acid and tested on ciliary movement. Normal Ringer of pH 9.0 produced a greater depression of ciliary movements than that of ash-Ringer of pH 9.0, while ash-Ringer of pH 7.5 produced slight stimulation of ciliary movements.

DISCUSSION

The ciliated epithelium of frog's oesophagus is a very simple, convenient as well as a very sensitive preparation for studying the effect of drugs on ciliary activity. It has been shown to respond to changes in temperature, osmotic pressure, hydrogen ion concentration, different anions and cations (3). In the present study oxygen and carbon dioxide have been found to stimulate ciliary activity. The action of carbon dioxide was direct and not due to change in hydrogen ion concentration. Carbon dioxide is known to stimulate the respiratory centre directly. It appears, therefore, that inhalation of fresh air in healthy individuals and of carbogen in diseased states is conducive for the health of respiratory tract.

With the extensive use of gas as fuel for domestic as well as industrial purposes and with the increasing use of gas driven vehicles, the inhalation of petrol gas has become common. Weak solution of petrol gas was found to have no significant effect on ciliary activity but in high concentration it was a depressant. This fact is of special significance to those who are engaged in such industries or vocations where the personnel are constantly exposed to petrol gas. The relationship of cigarette smoking with the incidence of respiratory infections and bronchogenic carcinoma is well known. Hilding (6,7) reported that cigarette smoke stopped ciliary beat after 11 min of exposure. He thought that stoppage might have been produced by a substance toxic to ciliated cells, probably nicotine. In the present study, however, cigarette smoke was found to stimulate ciliary activity. The difference in the results is probably because Hilding (6,7) studied the direct effect of smoke, while in the present study a dilute solution of smoke was used. There is no doubt that cigarette smoke affects ciliary activity, and it appears that weak inhalation may act as excitant while deep inhalation may act as depressant of ciliary activity. It is possible that prolonged heavy smoking for a number of years induces respiratory diseases because of its damaging effect on ciliated epithelium. The excitant principle in the smoke as well as the ash of cigarette does not appear to be nicotine as suggested by Hilding (6,7) because nicotine action could be blocked by a ganglion blocking agent while cigarette smoke as well as ash effect remained unaffected. Some other constituent of tobacco, viz. pyridine bases, organic acids, aldehydes, hydrocarbons, etc., may be responsible for the activity on cilia.

Effects of a few anaesthetic gases and vapours on ciliary activity have been reported (8,15). Nitrous oxide even in high concentrations was found to be without any action on ciliary activity. But chloroform has been reported to be a marked depressant; this has been confirmed in the present study. Ether vapour, however, has been reported to have no specific effect on ciliary movement. Any depressant action was thought to be due to the cooling effect of ether.

Direct application of ether was found to stop ciliary activity temporarily, and withdrawl of ether was followed by a short period of exaggerated activity. In the present study. however, ether solution in Ringer was found to excite ciliary activity. This may be due to the known irritant action of the anaesthetic. It is probable that long continued irritation of respiratory epithelium for several hr during ether anaesthesia may result in an exhaustion of ciliated cells, which may be responsible for increased incidence of respiratory tract infections reported after ether anaesthesia.

Alcoholism being a social problem in any society, a number of workers have studied the effect of alcohol on ciliary activity (8,10,11,12,13,16). Alcohol in high concentrations (5 to 20%) has been reported to retard or arrest ciliary motility of the trachea of horse, rabbit and fowl and the oesophagus of frog. There was, however, wide variations in the actual concentration of alcohol which depressed ciliary activity probably because of the different biological preparations used. In the present study very dilute solutions of alcohol were used. In a very low concentration (0.02%) it was found to stimulate ciliary activity while in concentrations of 0.1 to 1.0% it caused significant depression. The present study thus shows that alcohol has an effect on ciliary activity and it is possible that the deleterious action of alcohol on ciliary function may be one of the factors responsible for making the alcoholics prone to respiratory infections. One per cent solution of paraldehyde was found to be a stimulant of cilia. However, the concentrations in which it is expected to be excreted in the lungs in clinical practice are very much lower, and in these concentrations it might not have any significant action on the cilia.

Several non-specific substances including volatile solids, oils, etc. find their place in various formulations used for respiratory tract inflammations. Oils of clove, peppermint, spearmint, cinnamon and thymol have been reported to depress ciliary activity (11,16), while menthol and camphor have not been found to have any significant effect (14,18). In the present study, oil of peppermint, guaiacol and creosote markedly stimulated ciliary activity while menthol had a mild stimulant action. Thymol definitely retarded ciliary beat confirming previous reports. Thymol, a phenol derivative, having a weak local anaesthetic and anthelmintic actions may be directly depressant to the cilia. The ciliary stimulant action of oil of peppermint, creosote and guaiacol may be responsible for their expectorant actions.

Potassium iodide often forms a constituent of various formulations used for respiratory diseases. Potassium iodide in concentrations of 0.1% was found to markedly depress ciliary motility. Potassium chloride was not found to have any significant action while the bromide salt was moderately depressant . The results indicate that the depressant action was not due to the cation but because of the anion . It was reported earlier that the chloride ion in the Ringer solution cannot be replaced by any other ion (3), and the present study further shows that the bromide and iodide ions are actually depressant, the latter being more so, possibly because of its irritant action. The depressant action of potassium iodide is not of any practical significance a far as systemic use of the drug is concerned because its therapeutic dose is very small. However, potassium iodide should not be used in nasal drops.

It is evident that ciliary activity is affected by a variety of drugs, and as normal function of the ciliated epithelium is necessary for the health of respiratory tract, it is necessary that all drugs which are expected to come in contact with the epithelium of the respiratory tract should be investigated for their toxicity on ciliary activity.

SUMMARY

The effect of a number of agents which are either inhaled or excreted through the respiratory tract or are used for the treatment of inflammatory conditions of respiratory tract was studied on ciliated epithelium of frog's oesophagus. All the agents studied were dissolved in normal Ringer solution. The ciliary motility was found to be stimulated by very weak solution of ethanol (0.02%), ether, paraldehyde, oil of peppermint, menthol, guaiacol, creosote, oxygen, carbon dioxide and cigarette smoke. Relatively higher concentration of ethanol (0.1 to 1.0%), chloroform, thymol, petrol gas, potassium iodide and potassium bromide were found to depress ciliary motility. The stimulant actions did not appear to be mediated by neuro-humoral mechanisms. The action of carbon dioxide was not due to any change in hydrogen ion concentration, and the effect of cigarette smoke was possibly not because of nicotine. Of the three anions chloride, bromide and iodide, the iodide ion was the most depressant. The results have been discussed.

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