Rhinology, 22, 35-43, 1984

Effect of mechanical stimulation on mucociliary clearance of chicken sinus

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SUMMARY

The hypothesis that homeostatic control mechanisms control mucociliary function in ciliated mucous membranes was induced artificially by means of mechanical stimulation. The edge of right palatine cleft was stimulated mechanically by gentle touching with a dissecting needle, and sinus clearance time was recorded as soon as mechanical stimulation was initiated.

Mechanical stimulation caused acceleration of mucociliary flow of the sinus; sinus clearance time was accelerated on the side adjacent to the mechanically stimulated side of the palatine cleft, but not on the opposite side. Therefore, the reflex may be effective only on the stimulated side.

We investigated the effect of nerve blockers on mechanical stimulation. Mucociliary clearance in the chicken sinus was not affected by parasympatholytic agents, but was decelerated by the beta-adrenergic blocker. The effect of nerve blockers on the mechanical stimulation showed that parasympatholytic agents blocked mechanical stimulation, while sympatholytic agents did not completely block the response. These data suggest that mucociliary clearance may be regulated by the reflex of parasympathetic and partially sympathetic nerve fibers.

INTRODUCTION

Mucociliary clearance constitutes an effective protective mechanism against inhaled particles. Two different factors affect mucociliary clearance; the quality, or property, of the mucus, and the action, or beat, of cilia. Since both the direction and the rate of ciliary beat may be affected by changes in the quality and quantity of mucus due to infection or to exposure to toxic gases, one may conclude that the change of the mucus is of primary importance. Mucous quality and quantity may be altered not only by systemic dehydration, infectious agents, and exo- and endogenous irritants, but also by nerve reflexes by way of the central nervous system. Phipps and Richardson (1976) reported that mechanical stimulation of the nose and nasopharynx increased tracheal mucus output by reflexes which involved parasympathetic and probably also sympathetic motor pathways.

The acceleration of mucociliary clearance is known to result from injection with sympathomimetic (Camner et al., 1976) as well as parasympathomimetic (Saka-

kura and Proctor, 1972) drugs, while parasympatholytic (Pavia and Thomson, 1971) drugs appearently decelerate mucociliary clearance. We found mucociliary homeostasis following acute intermittent exposure to SO_2 and NDV infection (Wakabayashi et al., 1977; Ukai et al., 1983), and main factor in the mechanism of intranasal mucociliary homeostasis may be the reflex activity of the autonomic nervous system after stimulation of afferent nerve receptors in the nose by SO_2 or virus. The quality and quantity of mucus in the nose may be constantly regulated by autonomic nerve reflexes.

The present paper shows that mechanical stimulation on the chicken palatine cleft accelerates the rate of nasal mucociliary clearance and that this reflex is blocked by parasympatholytic and partially by sympatholytic agents.

MATERIALS AND METHODS

White Leghorn chickens were commercially obtained as 1-day old chicks, and they were maintained on Purina chow normal chick mash diet for 21 days. Mucociliary clearance time were measured under physiological conditions by using the method which have been previously described in detail (Wakabayashi et al., 1977); in briefly, the chicken's body was held in the plastic holder and its mouth was held open with an adjustable mouth opener. To measure mucociliary clearance, the entire apparatus was turned upside down and movement of mucus on the postnasal fossa through the palatine cleft was observed with a stereoscopic microscope, which has a bright cool spotlight, at ten-fold magnification. Three microliter of dye was injected through the thin membranous palate into the sinus, always at the site near the second row of dermal papillae.

The time required for arrival of the dye at choanal margins was measured. This is designated as sinus clearance time. The edge of right palatine cleft was stimulated mechanically by gentle touching with a dissecting needle, and sinus clearance time at the stimulated and unstimulated side were measured as soon as mechanical stimulation was initiated.

Nerve blockers were injected intramuscularly into the thigh. After preliminary tests to determine effective but nonlethal dosages, the following dosages of drugs were used; 2.5 mg/kg of atropine sulphate (Wyeth Lab.) 0.02 mg/kg of scopolamine (Pamine bromide, Upjohn), 5 mg/kg of propranolol hydrochloride (Inderal, ICI) and 0.5 mg/kg of reserpine (Serpasil, Ciba).

Statistical significance of results were evaluated by the paired t-test and Student's t-test.

RESULTS

Normal sinus clearance time

A total of 2485 measurements of sinus clearance time were attempted on 327 normal chickens on two occasions per day. Measurements were not successfully

completed in 7.8% of its attempts in sinus. The mean sinus clearance time for normal chickens measured on two occasions per day in each animal for 13 consecutive days from the 16th to the 28th day of age was roughly 2–4 minutes. The standard deviation was less than one and a half minutes. There was no statistical difference related to age and deviation of sinus time per day.

Effect of mechanical stimulation on time of sinus clearance

Clearance time from the right sinus was measured on nine chickens once daily for 16 to 28 consecutive days, and was measured after four episodes of mechanical stimulation on the right edge of cleft palate on the 22nd to the 25th days (Figure 1). The mean ± standard deviation of the sinus clearance time was 131.0 ± 16.7 seconds (n = 8) on the nonmechanical stimulation and 96.8 ± 9.5 seconds (n = 4) after mechanical stimulation. Mechanical stimulation caused a significant acceleration in sinus clearance time, and this response was reversible because of showing normal mean values on the 26th to the 28th days. There was a significant difference between the sinus clearance time after and without mechanical stimulation when a comparison of mean value was made ($p < 0.01$). We have analysed the effect of mechanical stimulation on the individual chicken. Clearance time from the right sinus was measured twice daily for 15 to 29 consecutive days, and was measured after seven episodes of mechanical stimulation on the 23rd to the 29th days. The conveyance time and its mean ± SD after and without stimulation

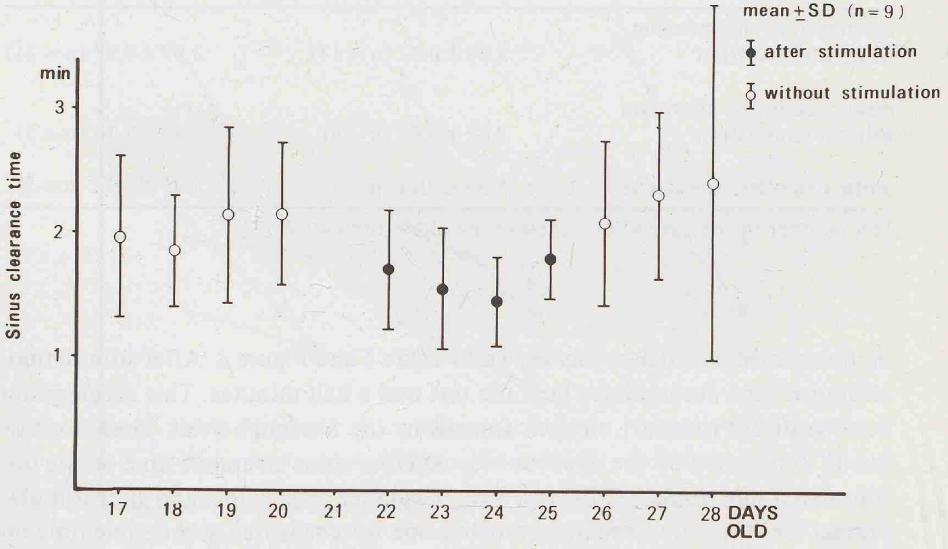

Figure 1. Effect of mechanical stimulation on the sinus clearance time. Numbers on the ordinate show sinus clearance time in minutes, those on the abscissa the ages of chickens in days.

Table 1. Actual conveyance times (minutes) in the individual animal. Numbers in the parentheses show the conveyance times after stimulation.

age	time	#82	#87
15	pm	3.13	ND
16	am	2.57	2.08
	pm	4.15	2.53
17	am	1.39	2.23
	pm	2.39	3.14
18	am	4.22	2.47
	pm	2.44	3.31
19	am	3.14	2.52
	pm	2.18	5.45
21	pm	2.30	3.11
23	am	2.47	3.06
	pm	(1.29)	(1.15)
24	am	2.23	3.13
	pm	(1.46)	(1.16)
25	am	2.49	4.30
	pm	(1.10)	(1.57)
26	am	3.19	4.25
	pm	(1.30)	(1.32)
27	am	2.14	3.52
	pm	(1.50)	(1.27)
28	am	2.33	1.19
	pm	(1,45)	(1.34)
29	am	2.45	3.42
	pm	(1.32)	(1.49)
mean ± sd in the morning without stimulation		2.49 ± 0.42 (n = 11)	3.09 ± 0.57 (n = 11)
mean ± sd in the afternoon without stimulation		2.57 ± 0.43 (n = 6)	3.43 ± 1.10 (n = 5)
mean ± sd after stimulation		1.35 ± 0.13 (n = 7)	1.33 ± 0.16 (n = 7)

The number in the parenthesis shows time after stimulation.

on the individual chicken was shown to Table 1 and Figure 2. After stimulation, clearance time accumulates near the one and a half minutes. This acceleration was significant ($p < 0.01$ on each animal) by the Student's t-test. These experiments were tested on the opposite side whether sinus clearance time on the unstimulated side would be affected. Sinus clearance time before and after stimulation on the stimulated and unstimulated side were masured at the same time on five chickens on the 28th to the 30th days (Figures 3 and 4). Sinus clearance time on the stimulated side was accelerated significantly but not on the unstimulated side.

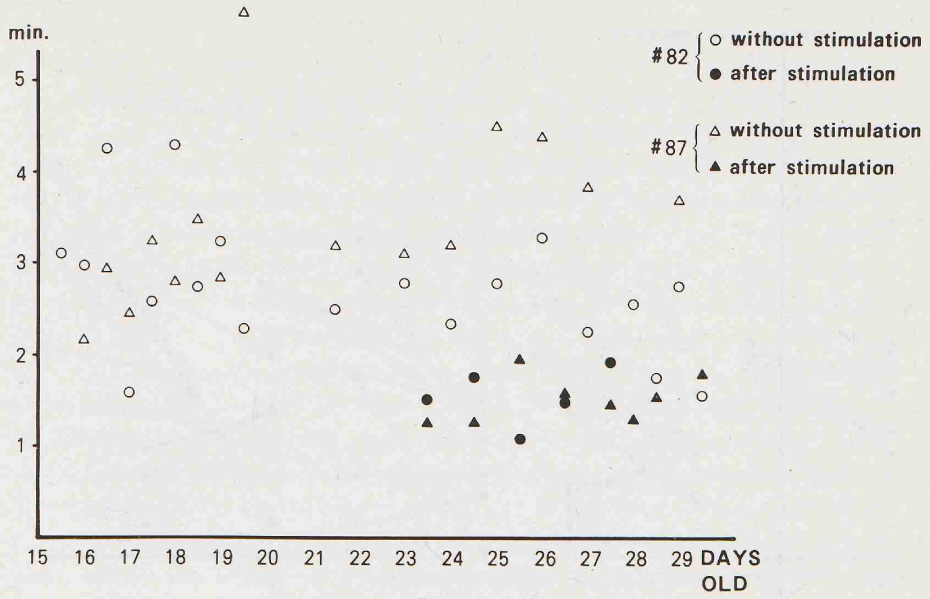

Figure 2. Effect of mechanical stimulation on the individual sinus clearance time. Numbers as in Figure 1.

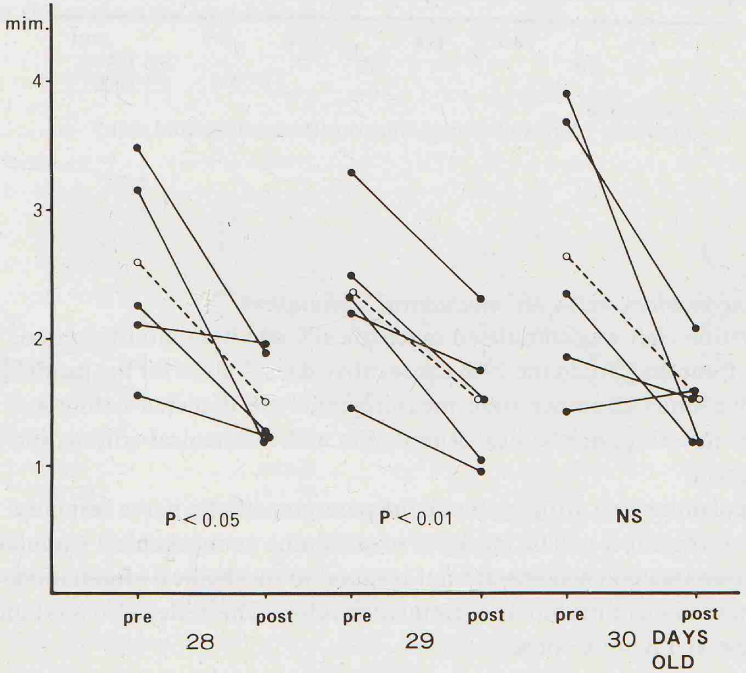

Figure 3. Sinus clearance time on the stimulated side. Open circles show the total averages.

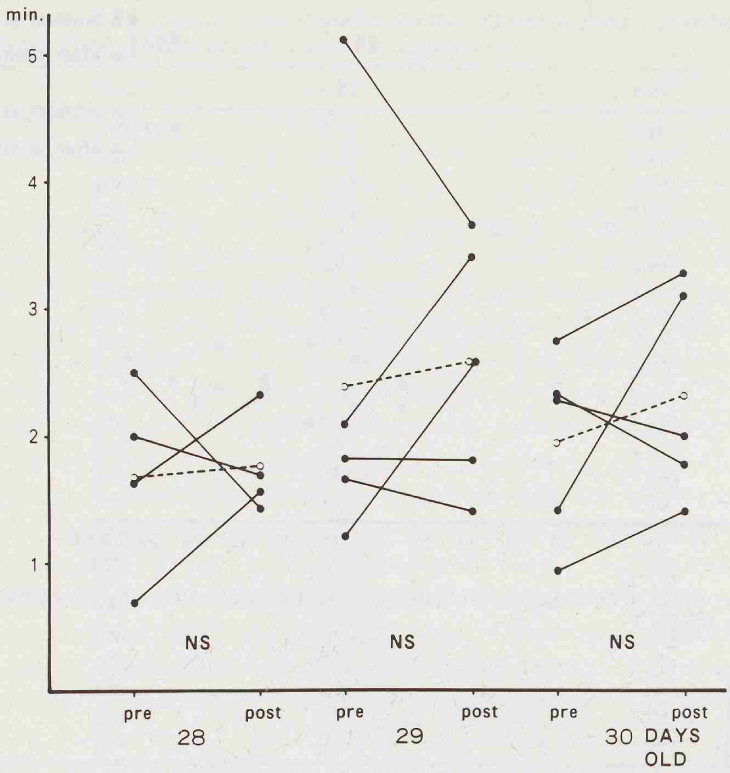

Figure 4. Sinus clearance time on the unstimulated side.

Effect of nerve blockers on the mechanical stimulation

Right palatine cleft was stimulated mechanically one hour after injection. This was done from the 23rd to the 29th consecutive days. Values for the mean of two consecutive sinus clearance time measurements are depicted before and one hour after injection, mechanical stimulation and mechanical stimulation plus nerve blocker.

Both scopolamine and atropine block the parasympathetic nerve response. Figure 5 shows the effect of 0.02 mg/kg of scopolamine on mechanical stimulation. Scopolamine treated chickens did not respond to mechanical stimulation, suggesting that scopolamine blocked stimulation reflex. This reflex also was blocked by atropine as Figure 6 shows.

Propanolol and reserpine block sympathetic response. One hour after injection of propranolol, 9 of 10 animals showed deceleration in sinus clearance time. While

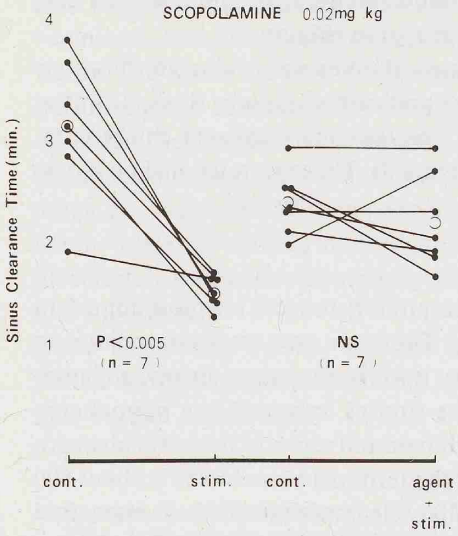

Figure 5. Effect of 0.02 mg/kg of scopolamine on mechanical stimulation. Open circles show the total averages.

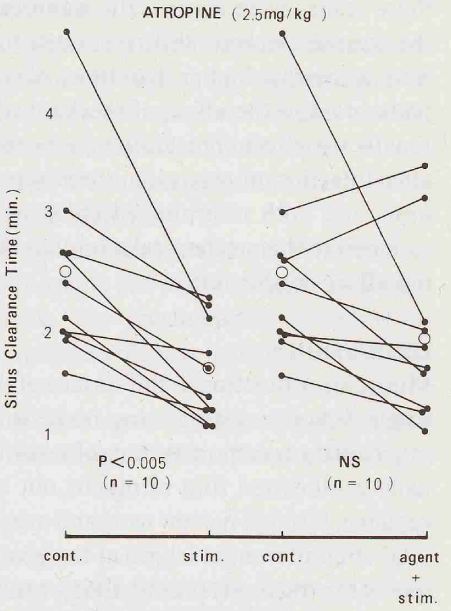

Figure 6. Effect of 2.5 mg/kg of atropine on the mechanical stimulation.

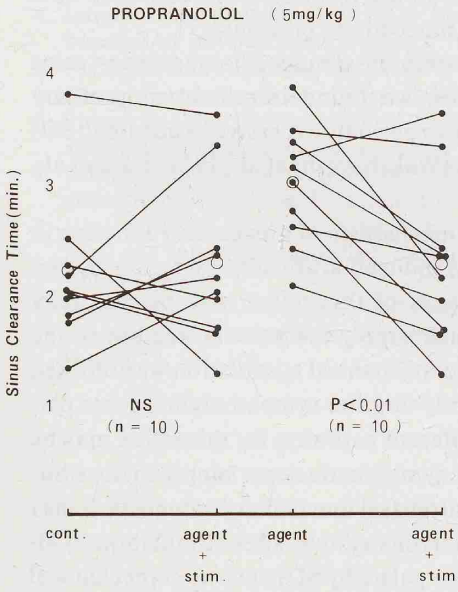

Figure 7. Effect of 5 mg/kg of propranolol on the mechanical stimulation.

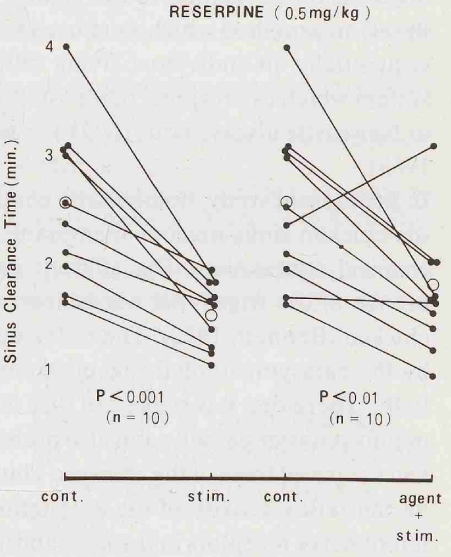

Figure 8. Effect of 0.5 mg/kg of reserpine on the mechanical stimulation.

these values were statistically significant ($p < 0.01$, $n = 10$) by the paired t-test, the treated chickens still responded to the mechanical stimulation. The values were somewhat higher than those of stimulation alone, so propranolol only partially blocked the effect of mechanical stimulation (Figure 7).

Finally the effect of reserpine on the mechanical reflex was evaluated. One hour after injection of reserpine, there was no significant change in sinus clearance time, and both reserpinized chickens (0.5 mg/kg) and untreated chickens responded to the mechanical stimulation (Figure 8). Thus reserpine had no blocking effect on this reflex.

DISCUSSION

Mucociliary function at the surface of the respiratory mucosa is a most important single factor in maintaining homeostasis. There are two truths in relation to mucociliary transport system of respiratory tract. In the upper airway, a continually synthesized film of mucus out of the sinuses transport as a normal conveyance, through narrow ostia and over turbinate and septa. In the lower airway, a total circumferential length at the level of the terminal bronchioles is about 600 fold of the trachea (Hilding, 1965). Mucociliary clearance rate increase many-fold from the periphery to the main bronchus (Iravani and Melville, 1976). The increase of mucociliary clearance rate from distal to proximal airways definitely plays a major role in the normal transport of mucus. Consistent variation of the mucous component and the transport rate in the respiratory mucosa may be a major factor in relation to the change of mucociliary clearance.

Based on a method which were used to monitor nasal mucociliary transport rates sequentially in individual living chickens, we found intranasal mucociliary system which can respond homeostatically to periodic excessive exposure to SO_2 or Newcastle disease virus (NDV) or both (Wakabayashi et al., 1977; Ukai et al., 1983).

In the present study, homeostatic control mechanism of mucociliary function in the chicken sinus mucous membrane was induced artificially by means of mechanical stimulation. The afferent pathways of this reflex may be maxillary branch of the trigeminal nerve fibers which supply the palatine regions of the chicken (Bennett, 1972). The reflex due to mechanical stimulation was blocked by the parasympatholytic agents completely and the sympatholytic agents partially. Therefore, it is suggested that the efferent pathways for this reflex may be mainly parasympathetic and also probably sympathetic nerve supply to the sinus and postnasal fossa of the chicken. Thus, intranasal mucociliary homeostasis may be the reflex activity of the autonomic nervous system after stimulation of afferent nerve receptors in the nose and/or the palate by SO_2 or virus or mechanical stimulation.

ZUSAMMENFASSUNG

Die Annahme, dass die homeostatische Regulierungsmechanismen die mukoziliäre Funktion der Mukosa mit Flimmerhaaren beherrschen, wurden dadurch veranlasst, dass diese Funktion künstlich mittels mechanisches Stimulus bewirkt wurde. Die Kante der rechten Palatumspaltes wurde mechanisch durch leichte Berührung mit Sektionsnadel stimuliert, und die Sinusausräumungszeit sofort nach dem Beginn der mechanischen Reizung registriert.

Der mechanische Stimulus bewirkte die Beschleunigung der mukoziliären Strömung des Sinus. Die Wirkung der Nervenblockers auf das mechanische Stimulieren erklärte, dass Parasympathikolytika das mechanische Stimulieren blockierten, während sympathikolytische Agenzien nicht vollständig seine Reaktion blockierten.

Diese Ergebnisse weisen, dass die mukoziliäre Ausräumungsrate durch den Reflex der parasympathischen und zum Teil sympathischen Nervenfaser reguliert werden könne, hin.

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