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Author(s)	SUZUKI, Noriyo
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Motor Control of the Ciliary Activity in the Frog's Palate¹⁾

By

Noriyo Suzuki

Zoological Institute, Hokkaido University
(With 4 Text-figures and 4 Tables)

It has been confirmed by Phole (1931), Seo (1931) and Lucas (1933) that nerves modify the rate of ciliary movement in the palate of the frog. Furthermore, the study by Lucas (1935) revealed that the fibres in the palatine nerve of the frog are responsible only for activation of ciliary movement and not for its inhibition. In addition, the cessation of ciliary movement has been frequently observed in both the intact animal and isolated preparations under unstimulated conditions and it was thought to be autonomous inherent property of ciliated cells of the palate (Lucas, 1935, Seo, 1937 and 1938). And Seo (1931) also showed that closely correlated reflex exists between points of stimulation of the tongue surface and correspondingly located responding points on the palate.

On the other hand, the pharmacological studies have been made to find a relationship between acetylcholine and the ciliary activity. Ishikawa and Ohzono (1931) reported that the ciliary activity of the frog's palate was increased by acetylcholine and this effect was prevented by atropine, and they suggested that the parasympathetic nervous system is concerned with the control of the ciliary activity. The effect of acetylcholine on the ciliary activity has been confirmed by Kordik, Büllbring and Burn (1952). They reported that lower concentrations of acetylcholine and eserine increased the ciliary activity, while higher concentrations of both depressed it.

In the autonomic nervous system it is fairly well established that in mammals the correlation between the stimulation frequency and the effector response forms an exponential curve (Rosenblueth, 1932; Celander, 1954; Folkow, 1955).

The present experiment was undertaken to examine whether characteristics of the autonomic nervous system also exist in the palatine nerve-cilia system of the frog and what kind of humoral system is involved in this system, by means of electrophysiological and pharmacological methods.

Material and Method: The Japanese common frog, *Rana nigromaculata*, was used

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throughout this experiment. In order to observe the effect of electrical stimulation of the palatine nerve on the ciliary activity, the nerve-cilia preparation (Seo, 1931) was made. The glossopharyngeal nerve was cut as short as possible to reduce the complication. The palatine nerve bundle was carefully dissected free from the connective tissue in length of 3-4 mm from the proximal cut end, which was electrically stimulated by means of a pair of Ag-AgCl electrodes. Rectangular pulses were delivered from an electronic stimulator (Nihon Koden, Model-MSE 3). The ciliary activity was observed under the dissecting microscope with the high magnification and the rate of movement of a standard object on the ciliated surface of the palate was measured. The time required for the movement of a piece of aluminium foil (ca. 1 mm²; 0.1 mg) covering 4 mm-distance on the palatal surface was measured, and the rate of transport (mm/10 sec.) by cilia was calculated.

The procedures to observe the effect of drugs on the ciliary excitation caused by electrical stimulation of the palatine nerve, was as follows: The first rate determination was made in the control solution (Ringer's solution); the second determination was made after electrical stimulation of the palatine nerve; the isolated preparation was then transferred into the experimental solution (Ringer's solution in which the autonomic drug was dissolved), the equilibration period of 10 minutes was allowed in the experimental solution, and then the third rate determination was made before and after electrical stimulation.

The efferent impulses of the palatine nerve was recorded as follows: The head part was cut off from the frog body and it was fixed on a cork plate at the bottom of the moist chamber, in supine position with the mouth open, and the lower jaw was also fixed on the plate. The palatal mucous membrane was cut and the palatine nerve fibres was then freed from the surrounding tissue. The fibres of the palatine nerve were separated and the number of fibres was reduced to a few under a binocular microscope using a pair of watch maker's forceps. The palatine nerve fibres were lifted up by a platinum leading electrode into the liquid paraffin covering over Ringer's solution in which the frog head was immersed. And a piece of silver plate (10 mm × 10 mm) as an indifferent electrode was placed to the dorsal part of the head. The nerve impulses were amplified by R-C coupling amplifier, and displayed on the dual beam cathod-ray oscilloscope (Nihon Koden, Type VC-6).

All experiments were carried out at room temperature (16-23°C).

Results

Stimulating effect of the palatine nerve: In the isolated nerve-cilia preparation the ciliary movement became slower and slower or stopped in a certain position of the palate but it continued gently in the most parts of the palate. In this state, the direct mechanical stimulation of the ciliated cells or electrical stimulation of the palatine nerve caused an activation of the ciliary movement.

A single shock caused a constant activation period of ciliary movement. The ciliary movement was enhanced for 3.9-4.0 sec. and then returned to the initial level. The solid line of Fig. 1 shows the intensity-time curve of the response caused by the single shock. The threshold value slightly varied in different preparations but all of them showed the hyperbolic curve. Thus, the excitation of all-or-none fashion was observed on the nerve-cilia preparation.

When the repetitive stimulation was applied to the palatine nerve the ciliary movement was also activated as long as stimulation continued. When the stimulus

was stopped the ciliary activity slowly returned to the initial level. The dotted line of Fig. 1 shows the intensity-time curve of the response caused by repetitive stimulation in the same preparation. In this experiment the stimulus frequency was fixed at 7 pulses/sec. The chronaxies in the former curve and in the latter were 2.0 msec. and 1.2 msec., respectively, the former one was 1.65 times longer. Thus, the repetitive stimulation was more effective on the ciliary activity than the single shock.

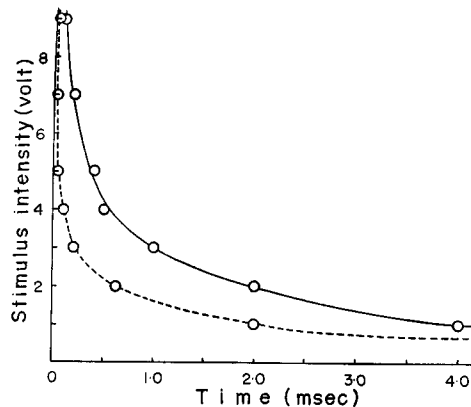


Fig. 1. Intensity-time curve of the palatine nerve-cilia preparation. Solid line: single shock. Dotted line: repetitive stimulation (The stimulus frequency was fixed at 7 pulses/sec.).

Furthermore, the experiment for the relationship between the stimulus frequency and the ciliary activity was quantitatively made. Fig. 2 shows the relationship in which the ciliary activity is relatively presented as the rate of particle transport by cilia. The initial rate of particle transport by cilia before electrical stimulation was measured as the control. In this figure the stimulus strength and duration are fixed at 5 volts and 1 msec., respectively. Each stimulation period was 10 sec., and it was long enough to allow the response to reach its maximum.

Marked effects appeared at lower frequencies of stimulation, where the ciliary activity increased almost linearly with the stimulus frequency up to 5-7 pulses/sec., beyond which no further enhancement was observed by increasing frequency of stimulation. In Fig. 2, the maximal response of the ciliary activity is 2.2-2.6 times of the control activity. And the effect of repetitive stimulation was a function of the stimulus frequency but did not depend on the stimulus strength or duration.

Previously, Seo (1931) reported that closely correlated reflex exists between stimulation points on the tongue and correspondingly located responding points on the palate in the intact frog. This was also confirmed in the present experiment

and the enhancement of the ciliary activity caused by mechanical stimulation of the tongue surface was observed to be 1.4–1.8 times greater than the control ciliary activity. Judging from the characteristics of the exponential curves, as is shown in Fig. 2, the increase in ciliary activity would be obtained if the frequency of palatine nerve discharge increased as much as 1-2 impulses/sec.

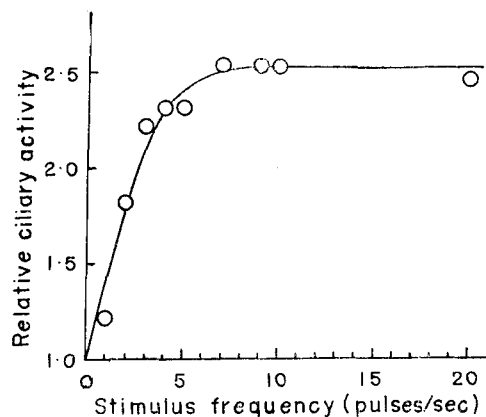


Fig. 2. Relationship between the stimulus frequency and ciliary activity. The ciliary activity was measured as the rate of particle transport by cilia. The relative rates are plotted in the ordinates as the rate is 1.0 in the normal activity.

Effects of some autonomic drugs: Effects of autonomic drugs on the cilio-excitation caused by the electrical stimulation of the palatine nerve were studied in the palatine nerve-cilia preparation. Repetitive electrical stimulation (5 volts, 1 msec, 7 pulses/sec.) was applied to the palatine nerve for 20–30 seconds. The moving rate of particles on the cilia were measured 3–5 times during stimulation in each experimental solution and the average rate of particle transport by cilia

Table 1. Effect of acetylcholine chloride on ciliary activity

Concentration (g/ml.)	Rate of particle transport before stimulation	Rate of particle transport after stimulation	Relative rate of particle transport (the rate before stimulation =1.0)
Control (Ringer)	2.4	4.6	1.9
1×10^{-7}	2.5	5.0	2.0
1×10^{-6}	2.5	5.0	2.0
1×10^{-5}	0.9	3.6	4.0
1×10^{-4}	1.0	1.7	1.7
1×10^{-3}	1.7	1.7	1.0

was calculated.

Acetylcholine chloride. The concentration effect of acetylcholine chloride on the ciliary activity was investigated varying the concentration from 1×10^{-7} to 1×10^{-3} g/ml. The lower concentrations increased a little the rate of particle transport comparing with the control Ringer's solution but the higher concentrations decreased it. The results are summarized in Table 1, in which the effect of electrical stimulation is clearly observed in the concentration range from 1×10^{-7} to 1×10^{-5} g/ml. and the relative rate of transport (as the rate of transport before stimulation = 1.0) is 2.0-4.0. But in the higher concentrations (1×10^{-4} to 1×10^{-3} g/ml.) the effect of electrical stimulation is markedly blocked, and the relative rate of transport remains 1.0-1.7.

Table 2. Effect of eserine salicylate on ciliary activity

Concentration (g/ml.)	Rate of particle transport before stimulation	Rate of particle transport after stimulation	Relative rate of particle transport (the rate before stimulation = 1.0)
Control (Ringer)	1.3	3.1	2.4
1×10^{-3}	0.8	0.8	1.0

Eserine salicylate. The effect of electrical stimulation was blocked at the concentration of 1×10^{-3} g/ml. eserine (Table 2). The blocking effect of acetylcholine and eserine was reversible and the initial rate of particle transport was restored after washing with fresh Ringer's solution for 10 minutes.

Atropine sulfate. The blocking effect of atropine occurred at the concentration 1×10^{-5} to 1×10^{-4} g/ml. (Table 3). The effect of atropine continued longer than those of higher concentration of acetylcholine and eserine, and the ciliary activity was hardly restored to the normal.

Table 3. Effect of atropine sulfate on ciliary activity

Concentration (g/ml.)	Rate of particle transport before stimulation	Rate of particle transport after stimulation	Relative rate of particle transport (the rate before stimulation = 1.0)
Control (Ringer)	1.3	3.1	2.4
1×10^{-6}	0.9	2.3	2.6
1×10^{-5}	1.2	1.2	1.0
1×10^{-4}	0.6	0.6	1.0

d-Tubocurarine chloride. The transmission of the neuro-muscular junction is blocked by d-tubocurarine chloride at the concentration of about 10^{-6} g/ml. But in this platine nerve-cilia preparation, the relative rate of transport by cilia at the concentration of 1×10^{-3} g/ml. was 1.6 and the blocking effect of tubocurarine on the neuro-cilia transmission was not remarkable. The rate transport in each tubocurarine solution before the electrical stimulation was lower than that of the control Ringer (Table 4).

Table 4. Effect of d-tubocurarine chloride on ciliary activity

Concentration (g/ml.)	Rate of particle transport before stimulation	Rate of particle transport after stimulation	Relative rate of particle transport (the rate before stimulation =1.0)
Control (Ringer)	2.4	6.7	2.8
1×10^{-5}	1.5	4.0	2.7
1×10^{-4}	1.1	3.1	2.8
1×10^{-3}	1.6	2.5	1.6

Recording of efferent impulses: The spontaneous impulses of the palatine nerve could be observed without application of the stimulus to the particular receptor organ. Figure 3 shows the efferent discharge of a single fibre of the palatine nerve. The frequency of the efferent discharge was not uniform and 1-2 impulses/sec in average.

It was also confirmed in the present experiment that the ciliary movement on the palate could be activated by mechanical stimulation of correspondingly located points on the tongue surface as was reported by Seo (1931). And so the relation between the efferent discharge of the palatine nerve and the stimulation of the

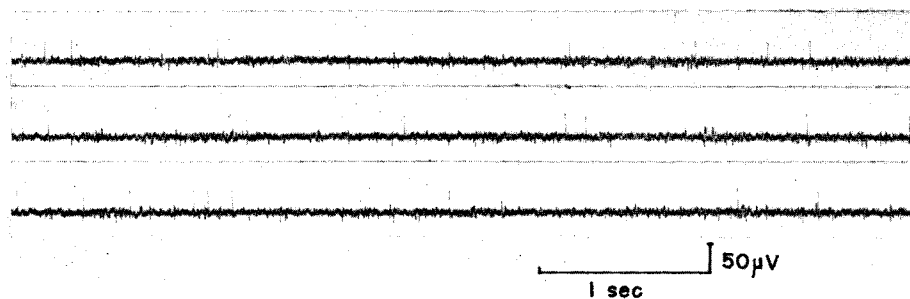


Fig. 3. Spontaneous discharge impulses of the single palatine nerve fibre. Calibration mark indicates $50\mu\text{V}$. and 1 second.

tongue surface was investigated. In this experiment the mechanical stimulation was applied to various points of the tongue surface by touch with the glass needle. There was an increase in impulse frequency recorded from the single fibre of the one side palatine nerve only when the definite points on the same side tongue surface was mechanically stimulated. The two examples of the recorded impulses from the single fibres are shown in the histograms of Fig. 4, A and B, where the impulse frequency is increased by application of the mechanical stimulus to the tip and middle part of the tongue, respectively. The latent period of the response was 1-2 sec. The impulse frequency was increased up to 4 impulses/sec. by a single touch stimulation.

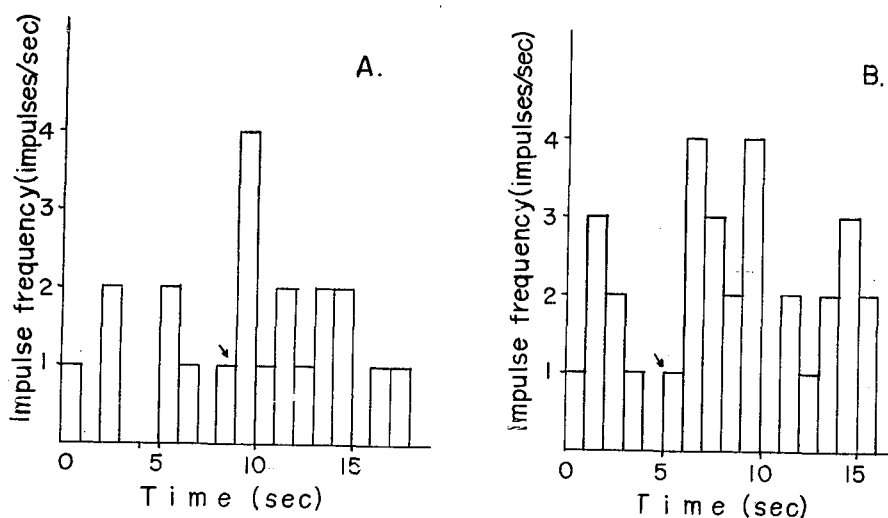


Fig. 4. An increase in impulse frequency caused by the stimulation of the tongue.
 A: The fibre which showed an increase in impulse frequency, when the same side tip of the tongue was stimulated.
 B: The fibre which showed an increase in impulse frequency, when the middle part of the same side of the tongue was stimulated.
 Arrows indicate the application points of the mechanical stimulation.

The present results concerning an increase in impulse frequency of the palatine nerve clarifies electrophysiologically the fact that an activation of the ciliary activity on the palate is elicited indirectly by the application of stimulus to the corresponding sensory organ. Thus, the efferent impulse discharge was confirmed to be concerned with the control to the ciliary activity in the palate.

Discussion

The present results concerning the frequency-response characteristics of the nerve-cilia preparation correspond very well with the findings in similar studies on the autonomic neuro-effectors in mammals (Folkow, 1952; Girling, 1952; Folkow and Hamberger, 1956). In the present experiment a resting tonic discharge in the palatine nerve fibres was 1-2 impulses/sec. and the estimated increase (1-2 impulses/sec., see p. 70) in the tonic activity elicited by electrical stimulation of the fibres agrees well with the experimental results (see p. 73) obtained from recording efferent impulses of the palatine nerve.

And the fact that the repetitive stimulation of the palatine nerve is more effective than a single shock on the ciliary response, shows that the summation of excitation in ciliated cells is necessary for the ciliary activation and a certain chemical transmitter substance may be involved in the transmission of nerve impulses.

Recently, Aiello and Guideri (1964, 1965) have shown that they could trace the ending structure of the nerve fibres which control the ciliary activity of gill filaments in the mussel, *Mytilus*, by new staining methods. But in the present experiment, the endings of the palatine nerve fibres which control the ciliary activity could not completely be traced, though the large branches could be observed by staining with osmic acid and methyleneblue. But the above mentioned results from the electrical stimulation suggest the existence of the real connections of fibre endings and ciliated cells in the palate. These connections seem to have a very fine structure.

In the isolated palate mucous membrane itself, the direct application of mechanical stimuli to the ciliated cells caused the activation of the ciliary movement. This fact suggests that the direct control system of the ciliary activity is present in the ciliated cells themselves except the nervous control. Thus, the nervous control of the ciliary activity seems to be secondary.

Kordik, Bulbring and Burn (1952) studied the ciliary activity in the frog pharynx and the isolated mucous membrane of the rabbit trachea. And they were able to show the presence of acetylcholine and histamine in the rabbit trachea and also found that the rabbit mucous membrane contained both choline acetylase and 'true' choline esterase by the pharmacological assay. Because there is no ganglion cell in the tracheal mucous membrane, acetylcholine was considered to be produced by non-nervous tissue and to control the ciliary activity as a local hormone. But the present experiment shows that the ciliary activation caused by electrical stimulation of the palatine nerve was blocked by acetylcholine, eserine and atropine except d-tubocurarine. These facts suggest that the cholinergic transmitter substance of the nerve is involved in the control of the ciliary activity in the frog's palate.

Summary

1. Electrical stimulation of the palatine nerve caused an enhancement of the ciliary activity. A single shock elicited an increase in the ciliary activity with a constant time course (3.9–4.0 sec.). The repetitive stimulation of the palatine nerve was more effective on an activation of the ciliary movement than the stimulation of a single shock.

2. The maximal response represented as the rate of particle transport by cilia was obtained at 5–7 pulses/sec. of the stimulus frequency and no more increase of the activity could be obtained by the stimuli at the frequencies higher than 7 pulses/sec.

3. The cilio-excitatory effect caused by the electrical stimulation was blocked by acetylcholine (1×10^{-3} g/ml.), eserine (1×10^{-3} g/ml.) and atropine (1×10^{-5} g/ml.) but not by d-tubocurarine.

4. The impulse frequency of the single palatine nerve was found to be 1–2 impulses/sec. in resting stage. An increase in impulse frequency of a single fibre was observed when the mechanical stimulation was applied to the tongue surface. A single touch stimulation caused an increase as much as 1–2 impulses/sec. in impulse frequency.

5. It is discussed that a cholinergic system is involved in the nervous control of ciliary activity in the frog's palate.

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