

DISTRIBUTION AND CLINICAL SIGNIFICANCE OF THE AUTONOMIC NERVOUS SYSTEM IN THE HUMAN NASAL MUCOSA

Y. Nomura and T. Matsuura

*From the Department of Otolaryngology, University of Tokyo, and the Department of
Otolaryngology, Kudanzaka Hospital, Tokyo, Japan*

(Received July 5, 1971)

Abstract. Biopsied specimens from the human inferior concha were studied histochemically to elucidate the function of the cholinergic nerve fiber, adrenergic nerve fiber, and myoepithelial basket cell and capillary. There are rich cholinergic nerve fibers in the inferior concha. They terminate mainly in the nasal gland and the blood vessels. Lacking in adrenergic nerve fiber, the nasal gland is therefore cholinergic. When the cholinergic nerve is over-stimulated by triggers, such as allergic or other reactions, the nasal gland and vessel work as a functional unit and produce profuse nasal secretion. Studies on human specimens reveal that the greater superficial petrosal nerve, vidian nerve and sphenopalatine ganglion are cholinergic in nature. The anterior ethmoid nerve also contains abundant cholinergic fibers. The nerve fibers in the vidian nerve are mainly cholinergic. The rationale for vidian neurectomy for allergic rhinitis is discussed.

Clinical and experimental observations have established that the mucous membrane of the nasal cavity is under control of the autonomic nervous system. According to Higbee (1949), vasoconstriction is a dominant effect resulting from stimulation of the sympathetic nerve fibers. On the other hand, the parasympathetic nervous system is continuously making adjustments to atmospheric conditions and constitutional states in an effort to maintain the nasal membrane in a normal state. The sphenopalatine ganglion is parasympathetic and relays vasodilator and secretory fibers to the nasal mucosa.

The characteristic symptoms of allergic rhinitis are rhinorrhea, nasal obstruction and

sneezing, the first two of which are due to the predominant manifestation of parasympathetic nerve stimulation. Dramatic relief from the symptoms is obtained by sectioning the vidian nerve, which is the main route of the parasympathetic nerve fibers to the nasal mucosa.

This paper describes the distribution of the autonomic nerve fibers in the human inferior concha, the cholinergic fiber component in the vidian nerve and other related nerves, and the rationale for vidian neurectomy for allergic rhinitis is discussed.

The following study consists of laboratory and clinical observations.

MATERIAL AND METHODS

A piece of the inferior concha was obtained by conchotomy from 20 patients who were suffering from vasomotor rhinitis. The acetylcholinesterase, alkaline phosphatase and catecholamine were stained using histochemical techniques, for the locating of the cholinergic nerve fibers, capillary and myoepithelial cells, and the adrenergic nerve fibers, respectively.

The vidian nerve, the greater superficial petrosal nerve, the sphenopalatine ganglion, the anterior ethmoid nerve and maxillary nerves were obtained from cadavers and stained with acetylcholinesterase.

The histochemical techniques employed are described below.

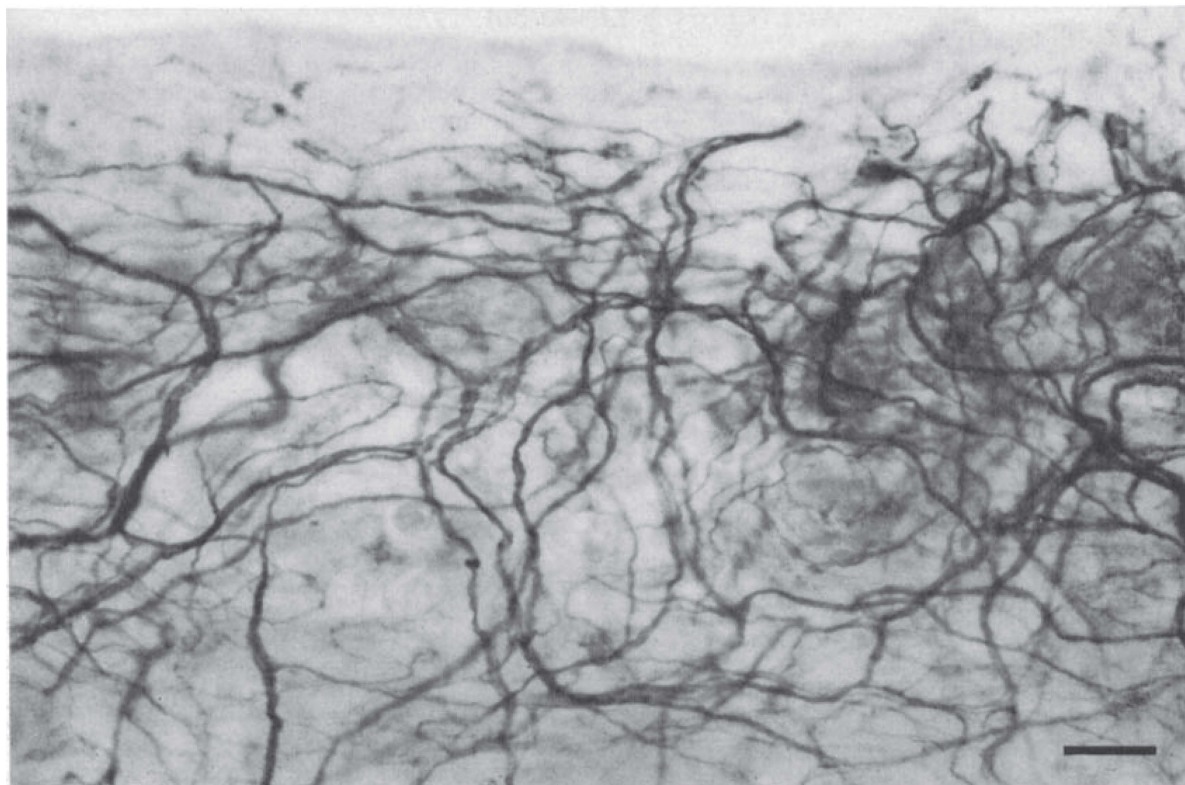


Fig. 1. Plexus of the cholinergic nerve in the human inferior concha. 1 mm thick specimen. AChE stain. 27-year-old male. Scale: 100 μ ($\times 25$).

Acetylcholinesterase

Karnovsky-Roots' method (1964) was applied in this study. The tissue was immediately fixed in a 10% solution of formalin-sucrose and cut into 10 μ frozen sections using a cryostat. Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates. A control specimen was stained in the above solution from which the substrates were omitted. The incubation time was 60–120 min at 37°C.

Alkaline phosphatase

The activity of the alkaline phosphatase was located in slices of the inferior concha. The naphthol AS-MX phosphate-fast blue RR method (a coupling azo dye method, Tomonaga et al., 1963) and Gomori-Takamatsu's calcium-cobalt method (Pearse, 1961) were employed for the locating of alkaline phosphatase. Control experiments were carried out by omitting naphthol AS-MX phosphate or

fast blue RR from the substrate solution of the coupling azo dye method and substituting equal parts of sodium barbiturate for β -glycerophosphate for Gomori-Takamatsu's method. The incubation time was 120–180 min at 37°C.

Catecholamine

The adrenergic nerve fibers in the nasal mucosa were demonstrated using a histochemical technique developed by Falck (1962). This technique makes the chemical mediator of the adrenergic fibers directly observable. A small piece of biopsied inferior concha was immediately chilled in isopentane with dry ice and freeze-dried. The tissue was treated with paraformaldehyde vapour, then embedded in paraffin, sectioned, and observed under a fluorescence microscope.

A case of allergic rhinitis was taken under study. For the examination of the role of

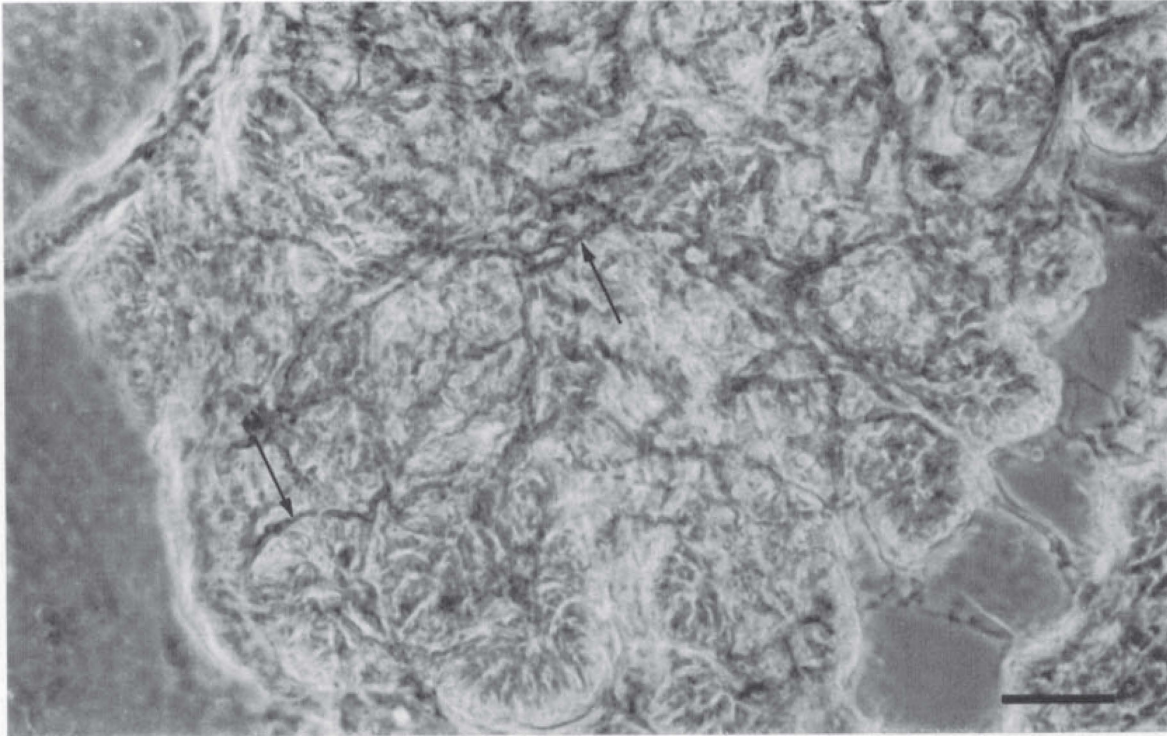


Fig. 2. Cholinergic nerve fiber (arrows) around the nasal glands. Human inferior concha. AChE stain. Phase contrast. 51-year-old male. Scale: 50 μ ($\times 63$).

cholinergic nerve in producing symptoms of allergic rhinitis, the patient's symptoms were evaluated before and after vidian neurectomy using a provocation test.

RESULTS

Acetylcholinesterase activity

A precipitate of brown color (copper ferrocyanide-Hatchett's brown) indicated the location of acetylcholinesterase (AChE) activity in the specimen. Stained nerve fibers are indicative of the cholinergic, probably parasympathetic nature in the inferior concha (Fig. 1). Control specimens were free of the reaction. Butyrylcholinesterase (BChE) activity was practically negative in the nerve fibers of the inferior concha. The cholinergic nerve fiber terminates mainly on the nasal gland, the artery and the arteriole, and to lesser extent on the sinusoid. It intermingles and twines with mucous glands as well as

serous glands (Fig. 2). The precise site of its termination was not known. However, there is a possibility that the nerve fiber terminates in the myoepithelial basket cell of the gland. There were no cholinergic fibers directed to or terminating in the ciliated epithelium including the goblet cells.

Cholinergic fibers were found in the greater superficial petrosal nerve and the vidian nerve. The cross section of the vidian nerve at the midportion usually showed a single bundle of nerve fibers of a diameter of about 0.5–1 mm. This was based on observation of 12 vidian nerves. However, at the posterior opening of the vidian canal the nerve fibers were divided into two bundles (Fig. 3). It is obvious that two bundles consisting of mainly cholinergic nerve fibers united into a bundle at the midportion of the vidian nerve and emerged from the canal after a rearrangement of the nerve fibers. There were some ganglion cells scattered in the vidian nerve. The perikarya of

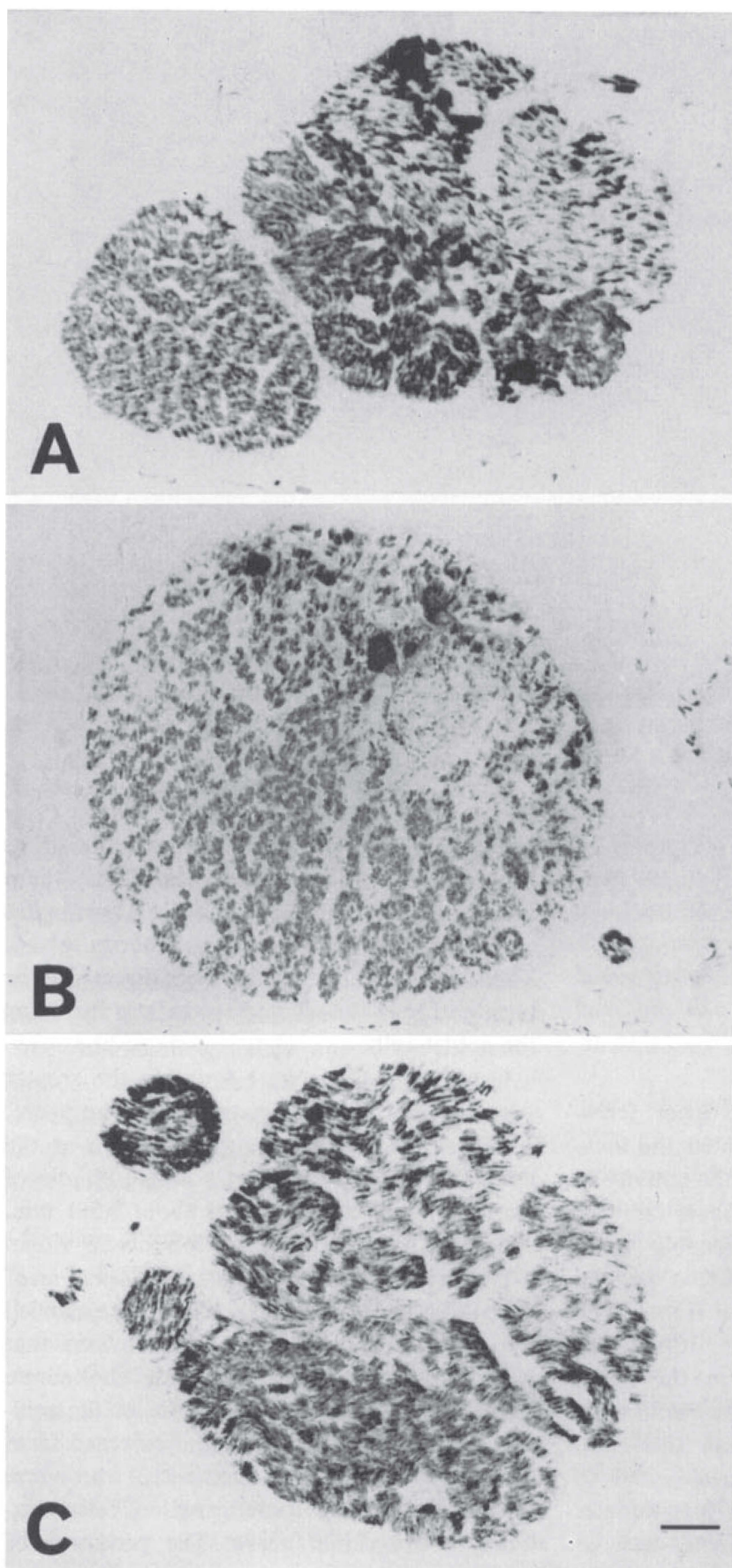


Fig. 3. Cholinergic nerve fibers in the vidian nerve. *A*, distal portion; *B*, midportion, three ganglion cells are seen; *C*, proximal portion. Notice that the bundles of the vidian nerve unite and separate when passing through the vidian canal. Unstained fibers are contained. 34-year-old female. Scale: 100 μ ($\times 25$).



Fig. 4. Ganglion cells and nerve fibers in the sphenopalatine ganglion. AChE stain. 26-year-old male. Scale: 100 μ ($\times 25$).

the sphenopalatine ganglion was stained with both AChE and BChE substrate solutions (Fig. 4). Nerve fibers in the ganglion reacted negatively to BChE. The anterior ethmoid nerve contained abundant cholinergic nerve fibers, while the maxillary nerve contained less cholinergic fibers (Fig. 5).

Alkaline phosphatase activity

The reaction deposit was blue resulting from the coupling azo dye method and black from Gomori-Takamatsu's method. Control specimens were free from the reaction. The capillary was made clearly visible by alkaline phosphatase staining. There are two capillary networks in the inferior concha; the subepithelial capillary network and the periglandular capillary network. There was a stained structure which encircled the gland. This suggested the presence of myoepithelial basket cells (Fig. 6). However, there is the possibility that the margins of the cells were stained by alkaline phosphatase.

Adrenergic nerve fiber

Adrenergic nerve fibers fluoresced when seen under a fluorescence microscope. The ar-

teriole of the inferior concha were richly endowed with adrenergic nerves. The nasal gland was almost devoid of adrenergic nerve fibers, although fluoresced nerve fibers were occasionally seen near the gland (Fig. 7).

Case Report

A 34-year-old female complained of paroxysmal sneezing and rhinorrhea from which she had suffered for several years. The symptoms lasted for several hours daily immediately after awaking in the morning, and showed no seasonal changes. Skin testing showed a positive reaction to house dust. A diluted solution of house dust elicited profuse rhinorrhea and sneezing when applied on the mucosa of the inferior concha. Desensitization of house dust was attempted without effect. On September 25, 1970, a left vidian neurectomy was performed using a transantral approach. This brought about a dramatic relief from the symptoms. The application of the house dust solution on the mucous membrane of the inferior concha on the non-operated side elicited the usual symptoms while application to the operated side failed to bring about sneezing

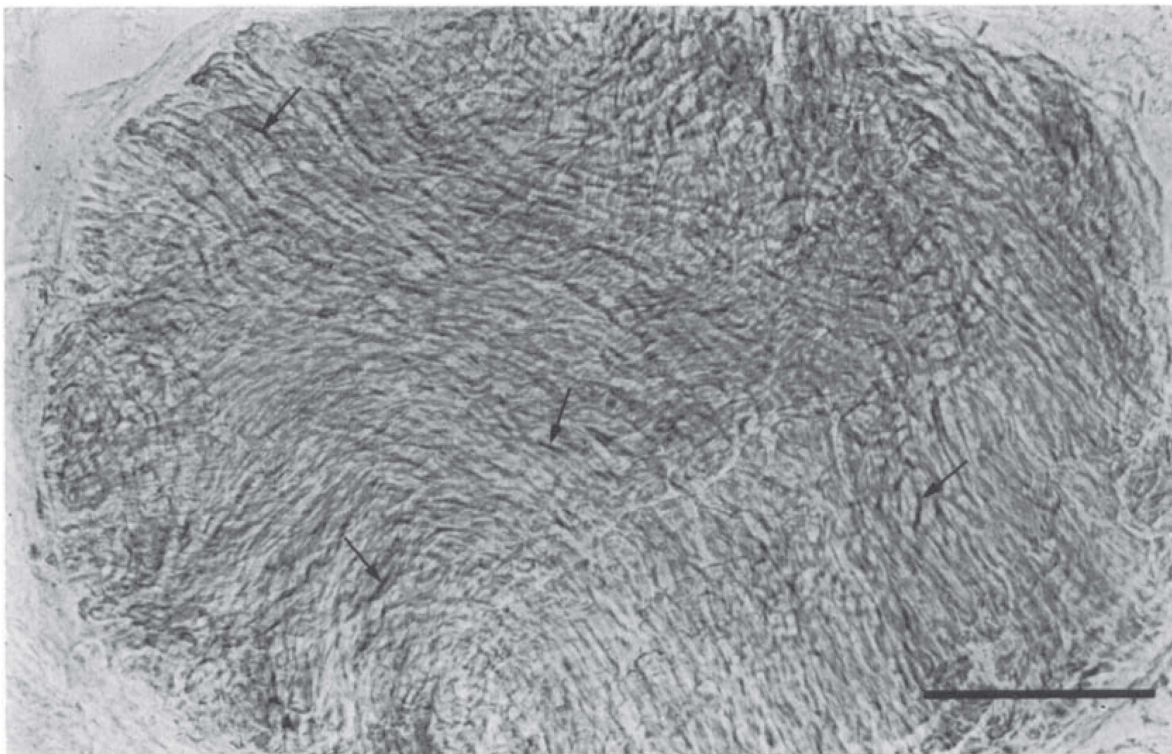


Fig. 5. Abundant cholinergic fibers (arrows) in the anterior ethmoid nerve. 51-year-old male. Scale: 100 μ ($\times 63$).

and rhinorrhea. Skin testing showed a positive reaction to house dust.

DISCUSSION

The present study is based on the concept of the neurohumoral theory of the autonomic nervous system. Generally speaking, cholinergic fibers of the autonomic nervous system are pre- and post-synaptic fibers of the parasympathetic nerve and the presynaptic fiber of the sympathetic nerve. The adrenergic fiber is the postsynaptic fiber of the sympathetic nerve. There are also cholinergic sympathetic fibers, such as those to the sweat glands of the skin. These fibers can be identified by staining with acetylcholinesterase and catecholamine. Cholinesterase may be divided into two types: specific acetylcholinesterase and non-specific cholinesterase. Acetylcholinesterase is capable of hydrolysing acetylthiocholine,

whereas cholinesterase hydrolyses esters of choline other than acetyl more rapidly than acetylcholinesterase. In order to demonstrate cholinesterase, it is necessary to use butyrylthiocholine instead of acetylthiocholine as a substrate (Bancroft, 1967).

The nasal glands produce mucus which protects the nasal mucosa by forming a mucous blanket. Overproduction of mucus results in nasal discharge.

The nasal gland is controlled by the autonomic nervous system (Fig. 8). Secretory impulses to the nasal gland are carried by post-synaptic fibers of the sphenopalatine ganglion, where the presynaptic nerve fibers make synapses. The cell body of the presynaptic fiber is in the superior salivatory nucleus. The fiber which runs through the intermediate nerve of the facial nerve, the greater superficial petrosal nerve and the vidian nerve along with the sympathetic deep petrosal nerve, finally establish synaptic contact with

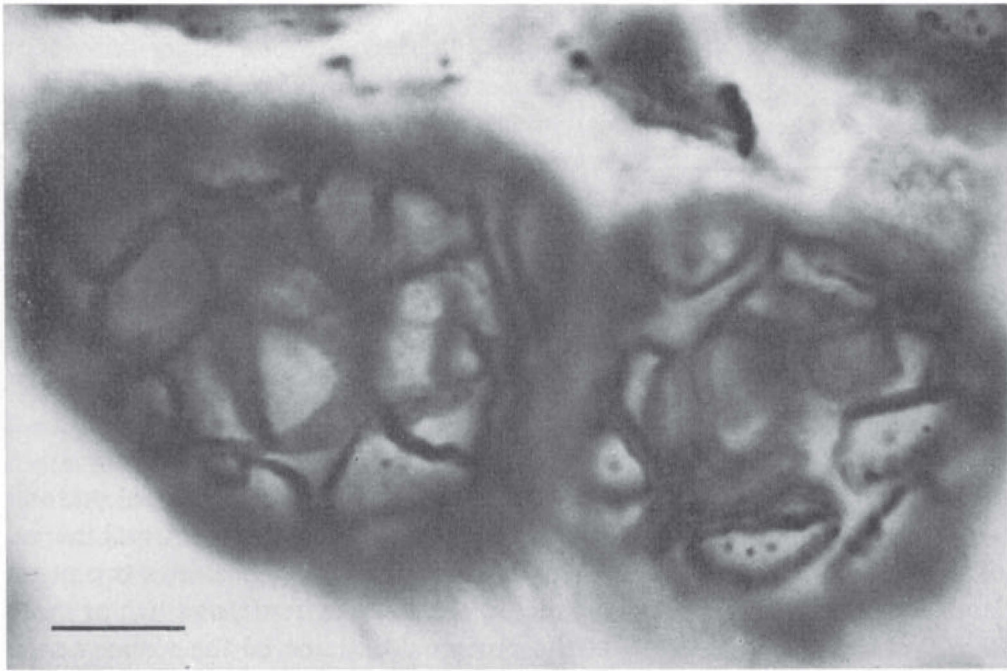


Fig. 6. Presence of myoepithelial basket cell. Long processes encircle two glands. Gomori-Takamatsu's alkaline phosphatase stain. 23-year-old male. Scale: $10\ \mu$ ($\times 400$).

the perikarya of postsynaptic neurons in the sphenopalatine ganglion. We have demonstrated with human patients that the secretory nerve fibers are cholinergic. So far, the adrenergic nerve fiber of the nasal gland has not

been demonstrated (Dahlström & Fuxe, 1965; Nishimura, 1970). The present study confirms this finding. Consequently, we conclude that cholinergic innervation is dominant in the functioning of the nasal gland. Nevertheless,

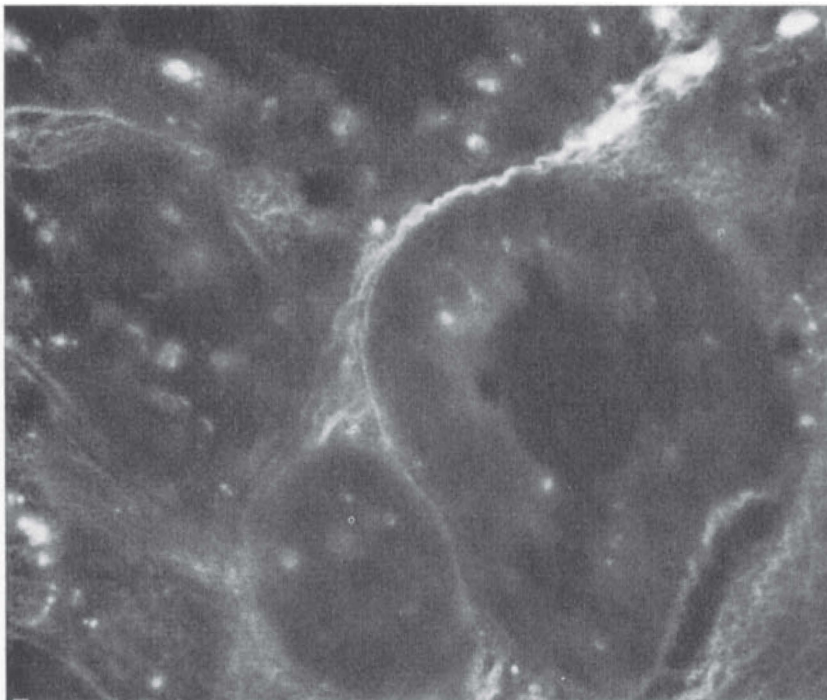


Fig. 7. A fluorescent nerve fiber around the nasal gland of the human inferior concha. 20-year-old female ($\times 160$).

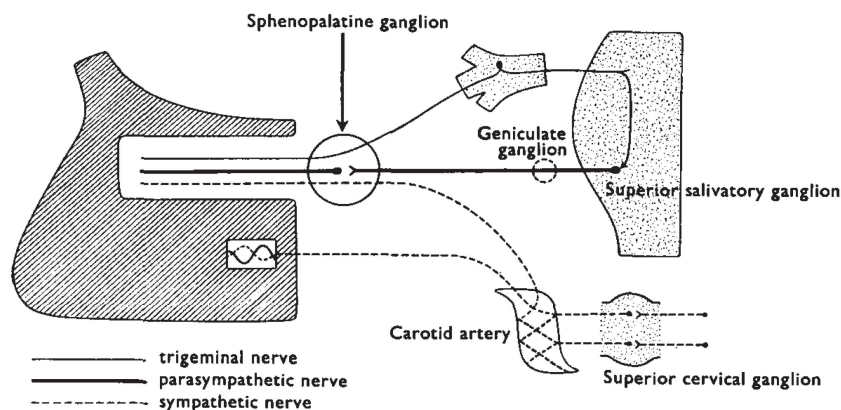


Fig. 8. Schema of the innervation of the nasal mucosa.

we cannot disregard the presence of sympathetic fibers.

The destination of the cholinergic fiber is mainly the nasal gland and blood vessels, as Ishii reported in 1970. Yet electronmicroscopic studies of the nasal gland have not ascertained the location of the cholinergic nerve fiber endings. The present study suggests the possibility that the cholinergic fiber terminates in the myoepithelial basket cell. It is present in secretory units of either the mucous or the serous type. The cell has a central body and many long cytoplasmic processes that encircle and grasp the secretory unit. Its cytoplasm seems contractile as in electronmicroscope studies which reveal myofibrils. Leeson (1956) by demonstrating the activity of alkaline phosphatase in rat submaxillary gland indicates that the cell is selectively stained histochemically. Its function appears to squeeze the gland so as to extrude the secretion. This function is probably connected with the rich periglandular capillary network. When the cholinergic nerve is stimulated, the arterioles in the concha dilate, permeability through the capillary wall changes, the uptake of serum constituents through the basement membrane into the gland increases, secretion is formed, and this is finally squeezed from the gland.

The vidian nerve is said to contain both sympathetic and parasympathetic fibers. According to Fenton & Larsell (1928), and Larsell (1942), instead of one nerve there are two nerves in the vidian canal. One is the

continuation of the greater superficial petrosal nerve, the other is the deep petrosal nerve. The present study also demonstrates two nerve bundles at the distal extreme, and two or more at the proximal extreme of the vidian nerve. However, they are stained by acetylcholinesterase, indicating that the bundles are cholinergic. Furthermore, two AChE-positive bundles are found to be united into a large bundle at the midportion of the vidian nerve. By histochemistry it was demonstrated that the vidian nerve is mainly cholinergic, although a small number of fibers mixed with the vidian nerve remained unstained. There is, therefore, an interlacing of nerve fibers in the vidian canal and the canal is seen to be not merely a channel for passing nerve fibers. The separation and rearrangement of nerve fibers take place here. From the present findings, there is a possibility that the vidian nerve consists of parasympathetic cholinergic and sympathetic cholinergic fibers.

Golding-Wood (1970) causally classified chronic vasomotor rhinitis into two types: atopic and non-atopic (cholinergic). Histamine and acetylcholine are the chemical mediators in the atopic and cholinergic types respectively. Golding-Wood does not think it difficult to distinguish between these types.

From the present clinical study, the role of the cholinergic nerve fiber in producing symptoms of allergic rhinitis is obvious. Since the symptoms subsided after sectioning the vidian nerve, allergic reaction in the nasal mucosa is

only a trigger which probably stimulates the trigeminal nerve and/or afferent fibers first and this stimulus is eventually relayed to the cholinergic nerve. Whether the triggers are due to allergy, mechanical stimuli, thermal stimuli or whatever, the symptoms are produced by the cholinergic nerve system.

Golding-Wood (1970) stated that the allergic cases are usually successfully treated by desensitization and that the cholinergic cases are candidates for vidian neurectomy only when the symptoms are severe and intractable to conservative therapy. Not infrequently, desensitization fails to cure allergic patients in our clinic. Irrespective of whether they are allergic or cholinergic types, vidian neurectomy is suggested for those whose symptoms are so persistent and intractable that any conservative therapy fails to abate the symptoms. This is because the cholinergic nerve plays a predominant role in both allergic and cholinergic rhinitis. The functional relation between the autonomic nervous system and IgE in the nasal mucosa must be elucidated.

ACKNOWLEDGMENT

We would like to thank Dr K. Uono of Department of Internal Medicine, University of Tokyo, for the preparation of the adrenergic nerve stain, and Dr Y. Nakayama of the Children's Clinic, Kudanzaka Hospital, for running the skin tests.

ZUSAMMENFASSUNG

Die Schleimhaut der unteren Nasenmuschel bei Menschen wurde im lebenden Körper histochemisch gefärbt und die Verteilung der cholinergischen Nerven, die Verteilung der myoepithelialen Korbzellen und die Verteilung der Kapillaren untersucht. In der Schleimhaut der unteren Nasenmuschel gab es reichlich cholinergische Nervenfasern, die meistens in den Nasendrüsen und in den Gefäßen endeten. In den Nasendrüsen wurden keine adrenergischen, sondern cholinergische Nervenfasern beobachtet. Falls die parasympathischen Nerven durch Allergie oder durch irgend eine andere Ursache übermässig gereizt werden, so funktionieren Nasendrüsen und -gefäße als Einheit zusammen, dann erfolgt profuse Nasensekre-

tion. Nervus petrosus superficialis major, N. Vidianus und Ganglion sphenopalatinum bei Menschen sind von Natur aus cholinergisch. Nervus ethmoidalis anterior enthält auch reichlich cholinergische Nervenfasern. Die Nervenfasern des N. Vidianus sind hauptsächlich cholinergisch. Ferner erörtere der Verf. das vernunftgemässe Auftreten der Neurektomie von N. Vidianus für Nasenallergie.

REFERENCES

- Bancroft, J. D. 1967. *An introduction to histochemical technique*. Butterworths, London.
- Dahlström, A. & Fuxe, K. 1965. The adrenergic innervation of the nasal mucosa of certain mammals. *Acta Otolaryng* (Stockh.) 59, 65.
- Falck, B. 1962. Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. *Acta Physiol Scand*, Suppl. 197, 1.
- Falck, B., Hillarp, N.-A., Thieme, G. & Torp, A. 1962. Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J Histochem Cytochem* 10, 348.
- Fenton, R. A. & Larsell, O. 1928. The mechanism of pain transmission in certain types of otalgia. *Ann Otol* 37, 739.
- Golding-Wood, P. H. 1970. Vidian neurectomy and other transantral surgery—1970. *Laryngoscope* 80, 1179.
- Higbee, D. 1949. Functional and anatomic relation of sphenopalatine ganglion to the autonomic nervous system. *Arch Otolaryng* (Chic.) 50, 45.
- Ishii, T. 1970. The cholinergic innervation of the human mucosa. A histochemical study. *Pract Otorhinolaryng* (Basel) 32, 153.
- Karnovsky, M. J. & Roots, L. 1964. A "direct-coloring" thiocholine method for cholinesterases. *J Histochem Cytochem* 12, 219.
- Larsell, O. L. cited by Higbee, D.
- Leeson, C. R. 1956. Localization of alkaline phosphatase in the submaxillary gland of the rat. *Nature* 178, 858.
- Nishimura, T. 1970. Presented at the ninth annual meeting of Japan Rhinologic Society.
- Pearse, A. G. E. 1961. *Histochemistry, theoretical and applied*. Churchill, London.
- Tomonaga, M., Sasaki, T. & Okuzaki, M. 1963. Studies on alkaline phosphatase: 1. Use of the naphthol AS-MX phosphate-fast blue RR staining method. *Acta Haemat Jap* 26, 179.

Y. Nomura, M.D.
Dept. of Otolaryngology
University of Tokyo
Tokyo
Japan