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OTOLOGY

Histological identification of nasopharyngeal mechanoreceptors

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Abstract The auditory tube plays a fundamental role in regulating middle ear pressure. A "system" sensitive to a pressure gradient between the middle ear and the ambient environment is necessary. The presence of mechanoreceptors in the middle ear and the tympanic membrane has been studied, but the presence of these receptors in the nasopharyngeal region remains unclear. The aim of this study is to confirm the presence of pressure sensitive corpuscles in the nasopharynx. An experimental study was conducted on five fresh and unembalded human cadavers. The pharyngeal ostium of the auditory tube and its periphery was removed in one piece by video-assisted endonasal endoscopy. Samples were fixed in formaldehyde solution, embedded in paraffin, and cut. Slides were analyzed by HES (Hematoxyline Eosine Safran) coloration, by S100 protein and neurofilament protein immunostaining. Encapsulated nerve endings were researched and identified by slides analysis. Eight samples were included in our study. On seven samples, Ruffini corpuscles were identified in the mucosa of the posterior area of the pharyngeal ostium, with a higher concentration in the pharyngeal recess and in the posterior nasopharyngeal wall. Our study identified nasopharyngeal mechanoreceptors that could detect the nasopharyngeal pressure and, by extension, the atmospheric pressure. These findings support the theory of the neuronal reflex arc of isobaric system of the middle ear, based on the existence of a "system" sensitive to a pressure gradient between the middle ear and the ambient environment. Understanding of this system has been helpful in the diagnosis and management of middle ear diseases.

Keywords Auditory tube · Eustachian tube · Sensory receptors · Mechanoreceptors · Nasopharynx · Middle ear pressure

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Introduction

The auditory tube (Eustachian tube) has at least three physiologic functions with respect to the middle ear: protection from nasopharyngeal sound pressures and secretions, clearance into the nasopharynx of secretions produced within the middle ear, and ventilation of the middle ear to equilibrate air pressure in the middle ear with atmospheric pressure [1]. The isobaric system of the middle ear (ISME) and the role of auditory tube have been described for several times now [1–3]. In normal tubal function, intermittent active opening of the auditory tube contributes to maintain nearly ambient pressure in the middle ear. Understanding of this system has been helpful in the diagnosis and management of middle ear diseases.

Reflex hypothesis to explain active tube opening due to pressure equilibration function is based on the existence of a "system" sensitive to a pressure gradient between the middle ear and the ambient environment. The identification

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of mechanoreceptors (pressure sensors) in the middle ear, the tympanic membrane and the nasopharynx has been studied for several years. Freeman and Wyke classified mechanoreceptors in four categories [4]. The type I, or Ruffini corpuscle, is a globular or ovoid corpuscle with thin capsule and average size of 100×40 μm. The type II, or Pacini corpuscle, is a cylindrical or conical corpuscle with thick, lamellate capsule and average size of 280×120 μm. The type III, or Golgi corpuscle, is a fusiform corpuscle with thin capsule and average size of 600×100 μm. And the type IV is unmyelinated free nerve endings with average size of 0.5–1.5 μm [4].

The human middle ear contained Pacini corpuscles [5, 6]. A histological study identified encapsulated nerve endings that corresponded to mechanoreceptors in the pars tensa of a human tympanic membrane [7]. These receptors would be sensitive to the stretching of the tympanic membrane and could therefore involve in the control of middle ear pressures. Actually, physiological studies showed dysfunctions of active tube opening when local anesthesia was performed on the tympanic membrane [8, 9]. But these results are controversial, and the mechanoreceptors on the tympanic membrane seem to have a minor effect on auditory tube functions [10].

In 1985, Guindi identified the nerve endings in the nasopharynx [11]. Free nerve endings have been described, and encapsulated receptors were present in the sub-epithelial tissue of the pharyngeal recess (fossa of Rosenmüller) that looked like Golgi corpuscles. A reflexogenic mechanism initiated by mechanoreceptors in the nasopharynx would provide an anatomical basis for part of the pressure sensor mechanism responsible for regulation of the middle ear pressure. Indeed, a physiological study by Esteve et al. showed a loss of active tube opening when local anesthesia was performed in the nasopharynx [9].

To our knowledge, no study validates Guindi study. This study focused on the histological identification of nasopharyngeal mechanoreceptors. The aim of this study was (1) to confirm the Guindi work by identifying pressure sensitive corpuscles in the nasopharynx, and (2) to make a map of their distribution.

Materials and methods

An anatomical–histological study was conducted on five fresh (less than 48 h post mortem) and unembalmed (without any formalin preparation) human cadavers that had been donated to science (2 males and 3 females aged from 82 to 88 years) through the thanatopraxis service at our University (Research and Teaching Unit). All post-mortem human subjects (PMHS) were obtained and treated in accordance with the ethical guidelines approved by our Faculty of Medicine, and all PMHS testing and handling procedures were approved by the Ethical Committee of our Faculty of Medicine. Otoscopic examination of the ear was performed.

Experimental protocol

The pharyngeal ostium of the auditory tube and its periphery was removed in one piece according to the following protocol. The surgical procedure was performed by video-assisted endonasal endoscopy with a 0°-endoscope (diameter of 4 mm and length of 16 cm) and dedicated instruments. The macroscopic integrity of the pharyngeal ostium of the auditory tube and its periphery was controlled. After dislocating of the inferior turbinate, an inferior turbinectomy of exposition was performed. Mucosal, submucosal and muscular planes were incised along a circular way centered by pharyngeal opening of the auditory tube (10 mm anteriorly, 20 mm posteriorly, 15 mm superiorly and 15 mm inferiorly). Torus of auditory tube, pharyngeal recess and lateral part of posterior nasopharyngeal wall were included in the sample. One-piece excision was performed from back to front. The nasopharyngeal muscles were cut as well as the cartilaginous part of auditory tube. The sample free of all joint was removed. The sample was numerated, oriented, photographed (Fig. 1) and immediately immersed in a 4 % formaldehyde solution.

Histological analysis

The pharyngeal ostium resections were sampled perpendicularly to the auditory tube axis and embedded in paraffin. Serial sections of three 3.5 μm slides were performed every 200 μm using a microtome (RM2155, Leica®) and collected on coated slides (SuperFrost Plus®, Menzel-Glaser). In each series, the first slide was dedicated to morphological analysis and stained with HES
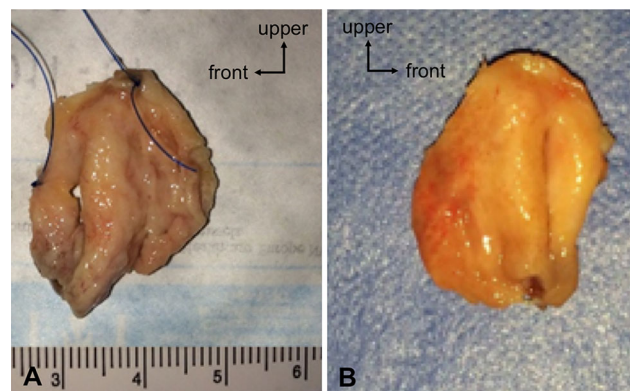

Fig. 1 Pictures showing the pharyngeal ostium of auditory tube and its periphery removed in one piece before fixation in a 4 % formaldehyde solution. **a** *Right* sample; **b** *Left* sample

(Hematoxyline Eosine Safran). The two others were immunostained with an antibody against S100 protein (Clone RP035, dilution 1/150 Diagnostic Systems Pleasanton CA), a specific marker for Schwann cells marker and neurofilament protein (Clone 2F11, dilution 1/20, Diagnostic Systems Pleasanton CA), an axon marker on a Ventana Benchmark XT (Ventana Tuscon AZ) using ultraview kit® according to the manufacturer instruction. Slides were first examined using an optical microscope (Eclipse® E800, Nikon), connected to a high-resolution color digital camera (DXM 1200, Nikon). Slides were digitized using a bright filed Scanscope XT scanner (Apério, Leica). To highlight mechanoreceptors, virtual slides series (HES, PS100 and neurofilament staining) were superimposed using synchronization function of Calopix® software (Calopix®, Tribvn France). After checking the quality of slides, mechanoreceptors were researched and identified according to the classification of Freeman and Wyke [4].

Results

Our study was conducted on five human cadavers. Otoscopic examination of the ear was normal in all cases after removal of earwax. Ten samples of pharyngeal ostium of the auditory tube and its periphery were collected. Two samples were excluded, one because testing method, and one because removal failure. Consequently, 8 samples were included in our study, 5 on the right side and 3 on the left side, corresponding to 40 fragments (5 fragments by samples) and 120 slices (3 slices by fragments). The mean size of the fragments was 2.8 cm (2.1–3.9).

Histological analysis

Histological and immunohistochemical analysis were reported in Table 1.

Table 1 Immunohistochemical analysis of samples

Sample N°	Side	Mechanoreceptors by immunostaining of S100 protein	Mechanoreceptors by immunostaining of neurofilament protein
1	R	+ (Posterior)	+ (Posterior)
2	R	+ (Posterior)	+ (Posterior)
3	R	− (Marker failure)	− (Marker failure)
4	L	+ (Posterior)	+ (Posterior)
5	L	+ (Posterior)	+ (Posterior)
6	R	+ (Posterior)	+ (Posterior)
7	L	+ (Posterior)	+ (Posterior)
8	R	+ (Posterior)	+ (Posterior)

L left, R right

Under microscope, cadaveric tissue had a well-preserved morphology and immunogenicity, only one sample (sample n°3) had a marker failure.

Immunohistochemical analysis by immunostaining of S100 protein and neurofilament protein showed the presence of encapsulated nerve endings that looked like mechanoreceptors. On eight samples, seven presented nerve endings and corpuscules. All encapsulated nerve endings identified as mechanoreceptors were positive with S100 protein and neurofilament protein (Fig. 2).

The structure of the corpuscles was round or oval with diameters that were about 100 μm, and contained a number of axon terminals with Schwann cell processes, thin capsule and amorphous materials in the intercellular space (Fig. 2). The histological appearance of these corpuscles was similar to Ruffini corpuscles [4]. No element similar to Pacini or Golgi corpuscles has been highlighted [4].

These corpuscles were located superficially, in the mucosa. Only the posterior part of the sample presented corpuscles, corresponding to the posterior periphery of the pharyngeal ostium. The anterior part of the opening did not present corpuscle. Nerve endings and corpuscles were identified in the posterior part of the sample, with a higher concentration in pharyngeal recess and posterior nasopharyngeal wall.

These features appear to be according in transmitting mechanical forces and are comparable to the function of mechanoreceptors that could detect the nasopharyngeal pressures and, by extension, the atmospheric pressures.

Discussion

We aimed to confirm the Guindi study by identifying pressure sensitive corpuscles in the nasopharynx and to make a map of their distribution [11]. A histological study was conducted on eight fresh and unembalmed samples of the pharyngeal ostium of the human auditory tube and its periphery. The major findings of this work were the histological identification of Ruffini corpuscles in the posterior portion of pharyngeal ostium of auditory tube, and their higher concentration in the pharyngeal recess and the posterior nasopharyngeal wall. These mechanoreceptors could detect the nasopharyngeal pressures and, by extension, the atmospheric pressures.

Reflex hypothesis to explain active tube opening due to pressure equilibration function is based on the existence of a "system" sensitive to a pressure gradient between the middle ear and the ambient environment.

Eden et al. reported the hypothesis concerning the tympanic plexus "reflex arc" [12–14]. They provided anatomic evidence of afferent and efferent pathways

Fig. 2 Immunohistochemical examination of nasopharyngeal mechanoreceptors showed ending nerves (*gray arrow*) and corpuscles (*black arrow*) inside the mucosa of the posterior periphery of the pharyngeal ostium of the auditory tube. **a**, **c**, **e** Positive with S100 protein. **b**, **d**, **f** Positive with neurofilament protein
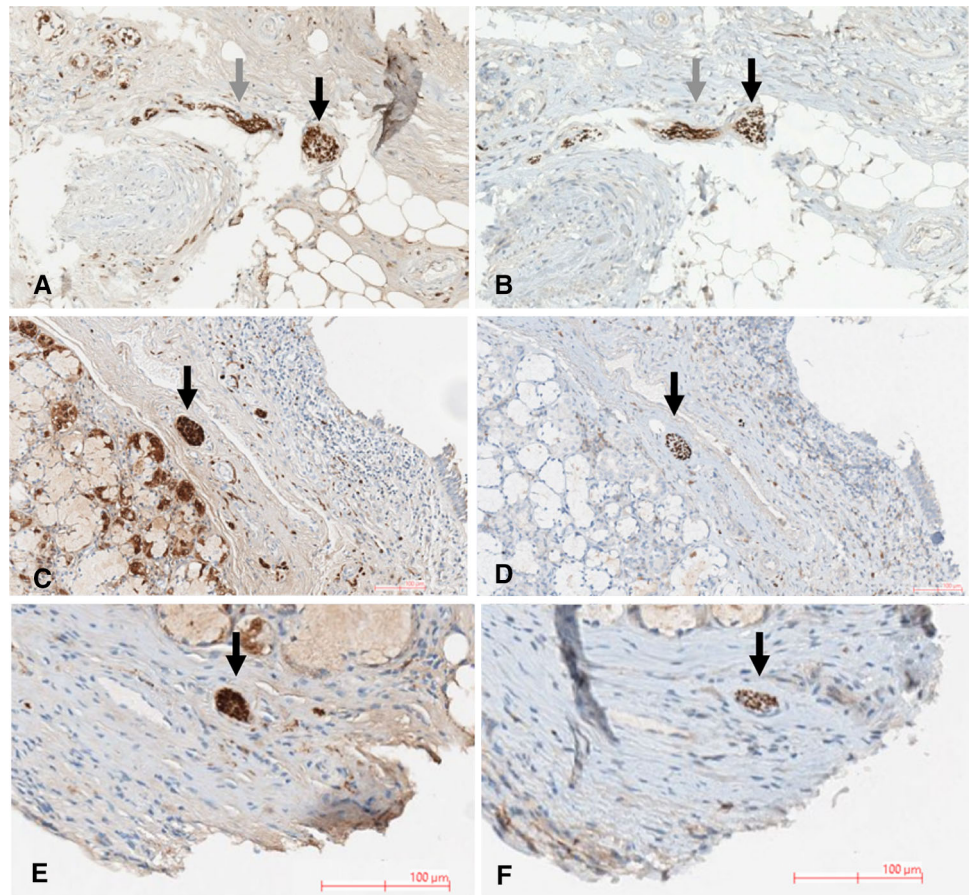
between the middle ear, the brain and the auditory tube in animal models. A neural tracer, horseradish peroxidase (HRP), was placed on the transected nerves of the tympanic plexus, and after a while HRP-labeled nerve terminal fields were observed in the nucleus of the solitary tract, which may represent the sensory pathways by which the middle ear aeration is monitored. Then, HRP was injected into the tubal muscles and HRP-labeled motor neurons were observed in both the ipsilateral trigeminal motor nucleus and nucleus ambiguous, which may represent the efferent pathways by which the middle ear aeration is regulated. They suggested that the polysynaptic pathways indicate a regulatory mechanism in which brain stem neurons both monitor and regulate the middle ear pressure [12–14].

In the middle ear and the tympanic membrane, pressure sensitive mechanoreceptors, able to collect the middle ear pressure, were identified in some studies.

Gussen's report indicated that the human middle ear and antrum contained Pacini corpuscles [5]. Lim et al. were in agreement with Gussen, and the corpuscles were located in the mucosa, most commonly in the tympanic antrum and epitympanic recess [6]. A histological study was performed from the second quadrant (pars tensa) of a human tympanic

membrane [7]. Nagai and Tono identified encapsulated nerve endings in both the sub-epidermal connective tissue and the lamina propria. The structure of the corpuscles was round or oval with diameters that were about 40 μm, and contained a number of axon terminals with mitochondria, Schwann cell processes and amorphous materials in the intercellular space. These features appear to be according in transmitting mechanical forces (stretching of the tympanic membrane) and are comparable to the function of mechanoreceptors that could detect middle ear pressure [7]. Indeed, physiological studies showed dysfunctions of active tube opening when local anesthesia was performed on the tympanic membrane [8, 9]. After topical anesthesia of the tympanic membrane, many of subjects felt some dull sensation on external ear pressures or stated that they could not detect the air coming into the tympanic cavity during the Vasalva maneuver. On 20 subjects, 13 ears needed more than 2 swallows and 4 ears failed to equalize middle ear pressure in spite of repeated swallows. As auditory tube function changed following anesthesia of the tympanic membranes, a neural connection between sensory receptors in the tympanic membrane and tubal muscles was suggested [8]. In 2001, a physiological study by Esteve et al. showed a dysfunction of active tube opening when local

anesthesia were performed on the tympanic membrane in one voluntary subject [9]. But these results are controversial and not compatible with the study of Songu et al. [10]. Actually, in a population of 95 ears, the mechanoreceptors in the middle ear and on the tympanic membrane were blocked by topical administration of lidocaine hydrochloride. The auditory tube functions were evaluated by manometric tests. When the mechanoreceptors in the middle ear were blocked in patients with a chronic perforation, a dysfunction of the auditory tube was significantly observed in 37 of 40 ears. When the mechanoreceptors in both the middle ear and the medial surface of the tympanic membrane were blocked in patients with subjective tinnitus treated by intratympanically administrated lidocaine, a dysfunction of the auditory tube was significantly observed in eight of ten ears. And when the mechanoreceptors in the lateral surface of the tympanic membrane were blocked in patients with normal otoscopic findings, a dysfunction of the auditory tube was observed in 4 of 45 ears. The mechanoreceptors established in the middle ear might possibly have an effective role in the auditory tube function where the mechanoreceptors on the tympanic membrane seem to have a minor effect [10]. With elasticity and histology different from the rest of the membrane, pars flaccida has been suggested as a very sensitive and fast reacting part of the tympanic membrane providing pressure equilibrium to the middle ear in the range of natural levels of atmospheric pressure fluctuations [15, 16]. This part of the tympanic membrane could act as a baroreceptor, initiating the physiological reactions in the body in the course of adaptation to the changing levels of atmospheric pressure fluctuations.

> The principle of isobaric system of the middle ear (ISME) is based on the existence of pressure sensitive mechanoreceptors, able to collect the middle ear pressure but also the atmospheric pressure in the upper airway.

A reflexogenic mechanism initiated by mechanoreceptors in the nasopharynx would provide an anatomical basis for part of the pressure sensor mechanism responsible for regulation of the middle ear pressure. In a study of the human nasopharyngeal innervation, Kanagasuntheram et al. reported a predominance of free nerve endings and a few organized non-encapsulated endings, the latter only in sub-epithelial tissue [17]. By Bodian and modified Bielschowsky-Gros silver techniques, the different receptors were more common in the caudal part of the nasopharynx lined by stratified squamous epithelium. In 1985, a histological study by Guindi identified the nerve endings in the nasopharynx with gold chloride using Gairn's method [11]. This work was performed from patients undergoing adenoidectomy or biopsy examination of the post-nasal space

for the exclusion of neoplasia. Free nerve endings have been described, and encapsulated receptors were present in the sub-epithelial tissue of the pharyngeal recess, especially near the tubal elevation. They resembled the Golgi-Mazzoni corpuscules described by Ruffini and represented a simple variety of Krause's end bulbs. In 2001, a physiological study by Esteve et al. showed a loss of unilateral active tube opening when local anesthesia were performed on the unilateral pharyngeal ostium of the auditory tube in the nasopharynx in one voluntary subject [9].

> In our histological study, Ruffini corpuscles were identified in the nasopharynx, with a higher concentration in the pharyngeal recess and in the posterior nasopharyngeal wall.

Guindi localized mechanoreceptors in the pharyngeal recess, especially near the tubal elevation [11]. But this work was performed from patients undergoing adenoidectomy without visual control and orientation of these samples. Consequently, the location of corpuscles in the nasopharynx appears to be unclear. Our study was conducted by video-assisted endonasal endoscopy to better control the anatomical location of the samples and their orientation. Thus, our location of the mechanoreceptors aggregation sites was more accurately.

Our study was conducted on five fresh (less than 48 h post mortem) and unembalmed (without any formalin preparation) human cadavers to limit the deterioration of tissues. However, even if the samples quality was satisfactory, the handling was more difficult than living tissue handling. Actually, one sample was excluded because failure of removal, and one sample (sample n°3) did not presented nerve endings and corpuscles by marker failure, probably due to a technical fault during immunostaining.

A gold chloride using Gairn's method was employed by Guindi to highlight pressure sensitive corpuscles [11]. This method can provide excellent staining of myelinated axons and sensory nerve endings, but several modifications have been made by many investigators since 1985 [18]. This method remains long, complex and difficult. Consequently, we preferred using immunohistochemical analysis by immunostaining of S100 protein, a marker for Schwann cells, and neurofilament protein, an axon marker. These analyses have already demonstrated their efficiency in the search of mechanoreceptors on human tissues [19, 20].

In our study, myelinated nerve endings organized as corpuscles were identified and were similar to Ruffini corpuscles [4]. Mainly located in the dermis, these mechanoreceptors are deeply located, sensitive to pressure, tonic and no adaptive. They are sensitive to the stretching of the skin. These receptors are the majority of joint receptors, fixed to ligaments. They are both dynamic and static. They are activated under specific angle near the

extreme positions of the member. They are provided with multiple terminal branches of the nerve fiber and are in close contact with the collagen fibers from the dermis and entering the corpuscles by its two poles. The pressure on the surface is transmitted to the collagen fibers and stretches the central nervous peloton [21]. The Ruffini corpuscle is a slow receptor, sensitive to pressure and stretching, that informs about position and installation speed of the stimulus. Its reception area is large with fuzzy edges. No element similar to Pacini or Golgi corpuscles has been highlighted in our study.

Receptor count and density measure were not realized because we performed staggered cuts in the lining of each sample. The corpuscles were concentrated in pharyngeal recess and posterior nasopharyngeal wall. The anterior part of the pharyngeal ostium did not present any corpuscle, as supposed by Guindi [11]. Further explorations with analyses of whole sample were necessary to realize receptor count and density measure.

Our results support the theory described in the scientific literature about the "neuronal reflex arc" of isobaric system of the middle ear (ISME).

The "neuronal reflex arc" of ISME is based on the existence of a sensitive "system" to a pressure gradient between the middle ear and the ambient environment and an effector "system" to pressure equilibration [9, 10, 12–14]. The overall regulation has been suggested to be based on a neural feedback control similar to respiratory control and related to similar centers in the nucleus of the solitary tract of the brainstem [14]. The afferent plexus of the "neuronal reflex arc" should be mechanoreceptors in three pressure sensitive locations to regulate the middle ear pressures. The first site had already been discovered in the middle ear whose function was to collect endotympanic pressure. The second site at the tympanic membrane, especially pars flaccida, collect a pressure gradient between endo and exotympanic pressures. Our study confirmed location of the third site in the nasopharynx whose role is to collect pressures of the upper aerodigestive tract and so reflect atmospheric pressure. The efferent plexus of the "neuronal reflex arc" should be tubal muscles to tube opening and vascularization of the mastoid mucosa to middle ear gas pressure regulation. In normal middle ear, when a pressure gradient between the middle ear and the ambient environment is detected, two distinct and combined patterns contribute to maintain nearly ambient pressures in the middle ear: (1) auditory tube opening with steep intermittent changes in pressure against 0 Pa, and (2) mastoid-related changes in pressure, which were gradual and appeared in both negative and positive directions, and which could transverse 0 Pa into opposite pressures [22]. These gradual pressure changes by gas exchange are suggested to be related to the

perfusion of the mastoid mucosa. ISME dysfunctions could be responsible for middle ear diseases [9, 23]. Understanding of this system has been helpful in the diagnosis and management of middle ear diseases.

Conclusion

In our histological study, type I mechanoreceptors, also known as Ruffini corpuscles, were identified in nasopharynx. Their aggregation sites were the pharyngeal recess and the posterior nasopharyngeal wall. These mechanoreceptors could detect the nasopharyngeal pressures and, by extension, the atmospheric pressures. Our findings support the theory of the "neuronal reflex arc" of ISME, based on the existence of a "system" sensitive to a pressure gradient between the middle ear and the ambient environment. Understanding of this system has been helpful in the diagnosis and management of middle ear diseases.

Compliance with ethical standards

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