Searching for Fibers of Facial Nerve Origin in the Tympanic Plexus: A Tracer Study

Timpanik Pleksusta Facial Sinir Kökenli Liflerin Aranması: Bir İşaretleme Çalışması

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Abstract: This study investigated a possible connection between the facial nerve and/or its chorda tympani branch, and the tympanic plexus with the aim of defining some of the innervation of the middle ear mucosa. The study involved 20 locally bred male and female Wistar albino rats that were 2.5 to 3-months old and weighed 200-300 g.. Horseradish peroxidase, a retrograde axonal tracer, was injected into the middle ear mucosa of each rat, and leakage from the injection points was carefully removed by wiping with gauze pads or suctioning with a micropipette. The total amount of horseradish peroxidase solution that remained in the middle ear mucosa was approximately 3-4 µl. At 24-72 hours after this application, the animals were anesthetized and perfusion-fixed, and their geniculate ganglion, facial nerve, chorda tympani, and superior cervical ganglion were removed bilaterally under magnification. The tissues were then frozen, histochemically processed, and examined. Labeled neuron cell bodies were observed in the ipsilateral superior cervical ganglion, but there were no labeled neurons or axons in the geniculate ganglion, facial nerve, or chorda tympani. The experiment failed to show any connection between the facial nerve, its chorda tympani branch, and the tympanic plexus.

Key words: Chorda tympani, geniculate ganglion, middle ear mucosa innervation, rat, retrograde tracing

Özet: Bu çalışmanın amacı, fasiyal sinirin ve korda timpani dalının pleksus timpanikus ile olan bağlantılarını değerlendirip; bunların orta kulak mukozası inervasyonundaki önemini değerlendirmektir. Bu çalışma için her iki cinsten, yerel olarak temin edilmiş; 2.5-3 aylık, 200-300 gram ağırlığında Wistar albino sıçanlar kullanıldı. Retrograd bir aksonal işaretleyici olan horseradish peroxidase, sıçanların orta kulak mukozasına enjekte edildi ve enjeksiyon noktalarından olan sızıntı dikkatli bir şekilde spançla silinip veya pipetle aspire edilip; orta kulak mukozasında kalan horseradish peroxidase'ın toplam 3-4 mikrolitre olması sağlandı. Bu uygulamadan 24-72 saat sonra hayvanlar anestezi altında perfüzyonla tespit edildiler ve genikülat ganglionları, korda timpanileri, superior servikal ganglionları ve fasiyal sinirleri büyütme altında çıkarılıp, histokimyasal işleme tabi tutulduktan sora incelendiler. Her ne kadar uygulama ile bazı hayvanlarda aynı taraftaki superior servikal ganglionda işaretli hücreler görülse de; söz konusu hayvanların genikülat ganglion, fasiyal sinir ve korda timpanilerinde hiç bir işaretli sinir lifi veya hücresi gözlenmedi. Çalışmamızda fasiyal sinirden köken alan ve orta kulak mukozasına giden hiç bir sinir lifi gösterilememiştir; bunun nedeni pleksus timpanikus ile fasiyal sinirin dalları arasında herhangi bir bağlantının yokluğu olabilir.

Anahtar kelimeler: Genikülat ganglion, korda timpani, orta kulak mukozası inervasyonu, Retrograd işaretleme, sıçan

INTRODUCTION

The latest version of a classical human anatomy atlas contains one new figure: a sketching of the "tympanic plexus" (a network containing sensory fibers that supply the middle and external ear) with some fine connections from the facial nerve named the "ramus communicans (cum plexu tympanico)" (13). It is proposed that a few sensorial tympanic branches of the facial nerve join the tympanic plexus after passing through the petrous bone from the geniculate ganglion, where their cell bodies are found. "These fibers are embryological remnants of the second pharyngeal pouch. Apart from supplying the mucosa, they may extend to the tympanic membrane and external meatus; thus explaining the occurrence of vesicles there in focal herpes." (9)

Classical neuroanatomic data based on observations made using magnification in human cadavers and retrograde tracer techniques indicate that the main trunk of the tympanic plexus is Jacobson's nerve (18). This nerve arises from the glossopharyngeal nerve, penetrates the floor of the tympanum, and courses superiorly across the promontory to project to the tympanic plexus and anastomose with the lesser petrosal nerve (1,5,11,14).

Within the middle ear (ME) mucosa that covers the promontorium is a fine plexiform network of nerves, the abovementioned tympanic plexus, which supplies sensory fibers to the middle and external ear as well as preganglionic secretomotor fibers to the petrosal nerves (18). The sympathetic components of the ME mucosa innervation arise from the caroticotympanic nerve branch of the internal carotid sympathetic plexus, which originates mainly in the ipsilateral superior cervical ganglion and partly in the ipsilateral pterygopalatine ganglion, and anastomoses with Jacobson's nerve anteriorly (4, 6,7,8,9,11,12,14,16).

The chorda tympani branches off the facial nerve approximately 6 mm above the stylomastoid foramen. It then ascends anteriorly within a canal to penetrate the posterior wall of the tympanic cavity via its posterior canaliculus, near the posterior border of the medial aspect of the tympanic membrane and level with the upper end of the handle of the malleus. Passing anteriorly between the fibrous and mucous layers of the membrane, the nerve crosses medial to the handle of the malleus and re-enters the bone via its anterior canaliculus at the medial end of the tympanic fissure. The neuronal cell bodies are located

in the geniculate ganglion, and receive taste inputs from the anterior two-thirds of the tongue. The chorda tympani also includes some parasympathetic fibers that synapse in the submandibular ganglion and innervate the submandibular and sublingual salivary glands (18).

Since its introduction, the use of retrograde axonal transport of the macromolecular tracer horseradish peroxidase (HRP) has proven to be a reliable and sensitive method for mapping neural pathways. HRP labels both axons and cell bodies, thus pinpointing the origin of fibers that project to a given site within the central or peripheral nervous system. This glycoprotein enzyme is injected into the terminal area of the projection, where it is taken up at the synapses and transported in retrograde direction via the axoplasm, ultimately reaching the cell bodies (2,3,10).

Since the facial nerve passes close to the tympanic plexus, we thought it possible that some nerve fibers from the facial nerve and/or the chorda tympani might innervate the tympanic plexus and the ME mucosa. Since there is currently some controversy surrounding this subject, we sought to investigate with a tracer study.

MATERIALS AND METHODS

The investigation involved 20 locally bred male and female Wistar albino rats that were 2.5 to 3-months old and weighed 200-300 g. All work was conducted in accord with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health, Publication No: 86-23). Initially, all the rats were sedated with ether and were otoscopically examined for the presence of middle- or external ear disease. The 14 rats with disease-free middle ears were selected for the experiment. These animals were deeply anesthetized with an intramuscular injection of 5mg/kg xylazine and 44 mg/kg ketamine. Using operating loops (x3.5), 5 µl of 30% HRP (Type VI, Sigma, St. Louis, USA) in water was slowly introduced by pressure injection through a thin glass cannula into various points of the mucosa of the left ME cavity. This was accomplished by piercing the eardrum through a small hole made in the tympanic bulla. To reach the site, we made a postauricular incision and then retracted the sternocleidomastoid muscle to expose the tympanic bulla. Care was taken not to injure the tympanic plexus with inadvertent movements of the cannula. HRP leakage at the injection sites was carefully removed by wiping with

gauze pads or suctioning with a micropipette. The total amount of HRP solution that remained in the ME mucosa was estimated to be 3-4 µl. The openings were sealed using superglue (Cyanoacrylate, Loctite, UK) to prevent the dye from leaking out of the ME cavity into the surrounding tissue. The wounds were then sutured using 3/0 Prolene.

At 24-72 hours after HRP application, we re-anesthetized the animals and intracardially perfused them with a rapid bolus of isotonic saline solution followed by a fixative solution containing 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer at room temperature (pH 7.4). The geniculate ganglion, facial nerve, chorda tympani, and superior cervical ganglion were removed bilaterally under magnification. These tissues were placed in 0.1 M phosphate buffer containing 30% sucrose and then stored at 4 °C for 3-5 days. Frozen specimens were then cut into 30 µm-thick serial sections on the long axis. These were treated with tetramethylbenzidine in the presence of hydrogen peroxide, and were then counterstained with neutral red (pH 4.8), according to the method of Mesulam (10).

RESULTS

The control outcome was positive in only some of the animals in which HRP injected into the ME mucosa labeled the perikarya and dendrites of the superior cervical ganglion neurons ipsilateral to the injected site (the left side). Diffusion and escape of the dye is a well-known problem in tract-tracing studies. Even though we injected an adequate amount of HRP and there was no tracer contamination of the surrounding tissues, nine of the rats showed no HRP labeling in the superior cervical ganglion, possibly due to technical problems.

The superior cervical ganglia of the remaining five animals exhibited wide variation in the quantities of neurons that were labeled. None of these five rats had any labeled neurons or axons in their geniculate ganglia, facial nerves, or chorda tympanii.

DISCUSSION

There are numerous human anatomy reports of contributions to the tympanic plexus from the trigeminal and vagal nerves, and from the first four dorsal root ganglia (4,14,17). In addition, there is reported to be a filament that connects the auricular branch of the vagus nerve (containing fibers from the glossopharyngeal nerve) with the facial nerve, and sometimes with the chorda tympani (11).

When we checked the reference that was cited in one of the aforementioned human anatomy studies regarding this connection, it noted an anastomotic branch from the facial nerve connecting with Jacobson's nerve in 86% of cases, but no such connection in the remaining 14% (14). Some animal studies have reported that the tympanic plexus has "functional connections" with the chorda tympani, and a very fine connection with the geniculate ganglion, but the authors did not define these connections clearly or reliably (17). They may exist, however, and some recent animal studies showed that a few small nerve cell bodies in the geniculate ganglion and in the pterygopalatine ganglion (a parasympathetic ganglion) of the facial nerve were labeled after a retrograde tracer was introduced into the ME mucosa (12,16). Although animal experiments can shed light on these issues, species differences preclude confident extrapolation to humans.

In this study, although our tracer injections were adequate, we were not surprised to observe the lack of consistent HRP labeling in some rats. The reasons for this may be inappropriate timing of survival or technical problems during fixation. In the five animals that exhibited HRP labeling in the ipsilateral superior cervical ganglion, we observed no labeled neurons or axons in the geniculate ganglia, facial nerves, or chorda tympanii. The problem with such negative findings is that proving the absence of an entity is extremely difficult, if not impossible. With this type of result, the only deduction that can be made, assuming that the technique has been effective and that enough data have been gathered, is that the experiment failed to show any connection between the facial nerve, its chorda tympani branch, and the tympanic plexus.

Our results differ from those of some other reports in that we were unable to morphologically demonstrate that the ME mucosa contains any fibers of facial nerve origin (12,16). The reason for this discrepancy may be intraspecies or interindividual variation. It is also possible that these other studies showed "false positivity" that actually reflected direct uptake by nerve tissue that was damaged during injection, or that our results indicate "false negativity" in that HRP may not have been taken up by nerve fibers that were, in fact, present.

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