IS THERE A DOUBLE INNERVATION OF THE TENSOR TYMPANI MUSCLE IN HUMANS?

ANTONIUS C. KIERNER, MD
VIENNA, AUSTRIA

REGINA MAYER, MTA
VIENNA, AUSTRIA

OLIVER ADUNKA, MD
FRANKFURT, GERMANY

The middle ear muscles and their function have not yet been fully explored. The statement of Lawrence, for example, that the tensor tympani muscle of humans might have a dual innervation has never been proven or disproven. The question is of great interest; in our opinion, it represents one of the key questions in the putative afferent feedback loop of the middle ear muscles in humans. A light microscopic study was performed on 16 tensor tympani muscles taken from 11 cadavers. Six muscles were taken out in toto and stained according to the modified method of Sihler. The remaining 10 muscles were dehydrated and embedded in paraffin. In 5 of these muscles, complete transverse serial sections were made on a microtome at 7 µm and alternately stained by silver impregnation, S-100 protein immunohistochemistry, and ferric oxide. In the remaining 5 muscles, complete longitudinal serial sections were made at 10 µm. These sections were alternately stained by the methods of Cason and Maskar. Neither the surgical microscopic investigation nor the light microscopic investigation revealed any innervation to the human tensor tympani muscle other than the one arising from the mandibular branch of the trigeminal nerve. Our findings, apart from the fact that they clearly refute an unproven hypothesis, might represent another small step toward understanding the innervation of the tensor tympani muscle.

KEY WORDS — innervation, middle ear, tensor tympani muscle.

INTRODUCTION

Searching the literature of the past 30 years, one has the impression that the human middle ear muscles have been almost forgotten. Surprisingly, the fact that neither the morphology nor the functions of these muscles have yet been fully understood is almost totally ignored by otolaryngologists. Our findings on proprioceptors in the middle ear muscles of humans have motivated us to focus on the source of innervation to the human tensor tympani muscle.[1] The statement of Lawrence[2] that the tensor tympani muscle might have a dual innervation has never been proven or disproven. This neglect is even more surprising since, according to the literature, this sort of double innervation of one middle ear muscle would be unique among mammals.[3] Apart from that, the question demands interest because, in our opinion, it may be one of the keys to the putative afferent feedback loop of the middle ear muscles in humans.[4]

The aim of this study, therefore, was to add another piece to the puzzle and reinvestigate the hypothesis of Lawrence that there is a double innervation of the human tensor tympani muscle. A suitable technique for questions like this is Sihler's stain. This method was first described more than 100 years ago and was more recently revived by Wu and Sanders,[5] who used it to clarify the innervation of the intrinsic laryngeal muscles. More recently, we obtained convincing results using Sihler's stain to elucidate the innervation of the trapezius muscle in humans.[6]

MATERIALS AND METHODS

A light microscopic study was performed on 16 tensor tympani muscles taken from 11 human cadavers of both sexes donated to the Institute of Anatomy, University of Vienna. The cadavers had been fixed by perfusion through the femoral artery with a mixture of 4% phenolic acid and 0.5% formaldehyde as is standard for dissection methods. The tensor tympani muscles were dissected free and taken out in toto under a surgical microscope by the first author. Then they were post-fixed in 4% paraformaldehyde

SIHLER'S STAIN

Maceration: fixed specimens were macerated in solution containing 4 mL 3% hydrogen peroxide + 2,000 mL 3% potassium hydroxide for approximately 3 weeks

Decalcification: macerated specimens were immersed in 250 mL glacial acetic acid + 250 mL glycerin (100%) + 1,500 mL 1% aqueous chloral hydrate (solution 1) for 2 to 4 weeks

Staining: decalcified specimens were immersed in 250 mL Ehrlich's hematoxylin + 250 mL glycerin (100%) + 1,500 mL 1% aqueous chloral hydrate (solution 2) for 2 to 3 weeks

After staining, specimens were again transferred to solution 1 for approximately 6 hours

For neutralization, specimens were then immersed in 0.05% lithium carbonate for approximately 1 hour

Finally, specimens were immersed in increasing concentrations of glycerin daily and preserved in 100% glycerin with some thymol crystals

From the Department of Otorhinolaryngology, University Hospital Frankfurt, Frankfurt, Germany (Kierner, Adunka), and the Institute of Anatomy, University of Vienna, Vienna, Austria (Mayer).

CORRESPONDENCE — Antonius C. Kierner, MD, Schumanngasse 81, A-1170 Wien, Austria.

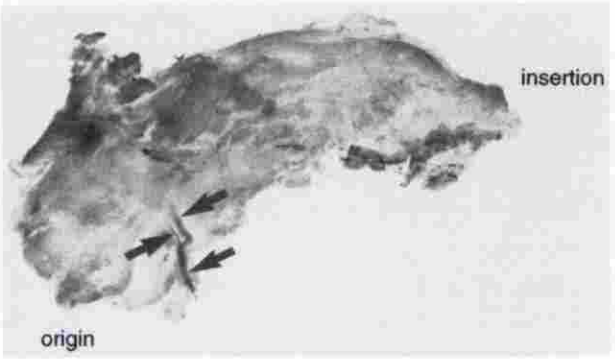

Fig 1. Complete tensor tympani muscle made translucent and stained according to Sihler's technique. Arrows point to only nerve passing into muscle.

for at least 4 weeks. Afterward, 6 muscles were stained according to the modified method of Sihler[7] (see Table) as described by Wu and Sanders,[5] and the remaining 10 muscles were dehydrated and embedded in paraffin. In 5 of these muscles, complete transverse serial sections were made on a microtome at 7 μm and alternately stained by silver impregnation, S-100 protein immunohistochemistry, and ferric oxide.[1] In the remaining 5 muscles, complete longitudinal serial sections were made at 10 μm. These sections were alternately stained by the methods of Cason and Maskar.[8] Specimens of which no complete section series could be obtained were not included in this study. Photographs were taken of representative cases, as well as exceptional cases.

RESULTS

Neither the preparation under the surgical microscope nor the light microscopic investigation of the whole muscles (Sihler's stain; Fig 1) or of the complete section series (Fig 2) step by step revealed any innervation to the human tensor tympani muscle other than the one arising from the mandibular branch of the trigeminal nerve. In all specimens, one single nerve was clearly stained and could be easily followed into the muscle (Figs 2 and 3). With none of the three different methods used did we detect any additional nerve fibers passing into the muscle.

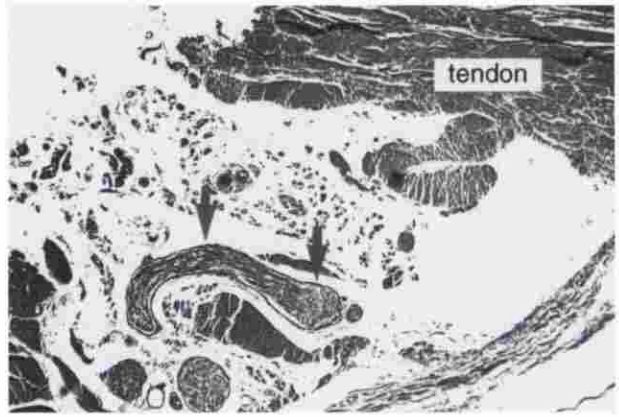

Fig 3. Transverse section of tensor tympani muscle with silver impregnation. Arrows point to nerve passing into muscle.

CONCLUSIONS

This is, as far as we know, the first exact histologic investigation of the innervation of the tensor tympani muscle in humans. The advantage of the techniques described herein is the combination of macroscopy and microscopy with additional serial sectioning. Thereby, all nerves or similar structures could be followed into the muscles by steps not larger than 7 μm. Apart from the fact that this method, in our opin-

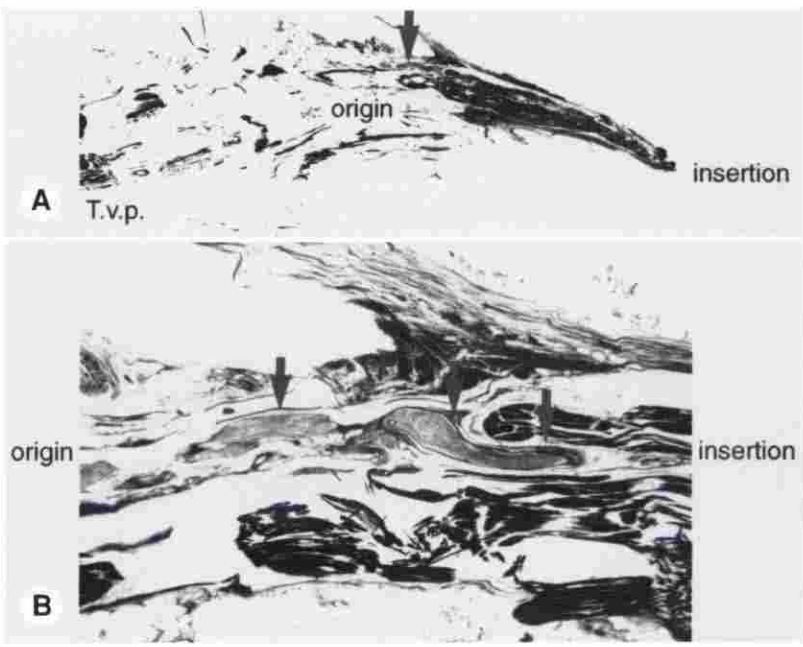

Fig 2. Longitudinal section along axis of tensor tympani muscle (Maskar's staining). Arrows point to nerve passing into muscle. **A)** T.v.p. — tensor veli palatini muscle. **B)** Magnification of A.

ion, almost totally excludes bias, our findings can hardly be compared, since the literature on this very special topic is sparse.

Almost all anatomy textbooks state that the human tensor tympani muscle is innervated by a branch of the mandibular nerve (via the nerve to the medial pterygoid muscle) that traverses the otic ganglion.[9-11] Yet, whether or not fibers from the ganglion intermingle here with the tensor tympani nerve,[9] or even if there is a branch of the ganglion joining the nerve,[10] is still discussed controversially. Because the human middle ear muscles seem to be widely forgotten, no one has reinvestigated this anatomic dogma. Therefore, it was undoubtedly of merit for Lawrence[2] to reinvestigate this question. Yet, we believe that Lawrence was confused by the tympanic plexus, which, of course, covers parts of the tensor tympani muscle within the middle ear cavity. Furthermore, he failed to investigate his own findings in greater detail, for example, by means of serial sectioning, and therefore stated that the nerve fibers he found passed into the muscle, although he could not prove this hypothesis.

Our findings, apart from the fact that they clearly disprove an unproven hypothesis, might represent another small step forward toward understanding the innervation of the tensor tympani muscle and thereby help to elucidate the muscle's afferent feedback loop. Understanding the tensor tympani innervation in detail, moreover, might provide further information on the muscle's functions.

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