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Hearing Research 200 (2005) 29–37
HEARING RESEARCH
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Smooth muscle in the annulus fibrosus of the tympanic membrane in bats, rodents, insectivores, and humans

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Received 9 April 2004; accepted 9 September 2004
Available online 18 October 2004

Abstract

The annulus fibrosus and its attachment to the bony tympanic ring were studied in a series of mammals. In the pallid bat, *Antrozous pallidus*, there is an extensive plexus of large interconnected blood sinuses in the part of the annulus that borders the tympanic bone. The spaces between the sinuses are packed with smooth muscle cells. Most of the cells have a predominately radial orientation; they extend from the bony tympanic sulcus to a dense collagenous matrix (apical zone) where radially oriented fibers of the pars tensa are confluent with the annulus. The muscles and vessels constitute a myovascular zone. A structurally similar myovascular zone is also present in the European hedgehog. In rodents, the annulus lacks the large interconnected blood sinuses but many small vessels are present. Smooth muscle is concentrated in the broad area of attachment of the annulus to the tympanic bone. In the gerbil, smooth muscle seems to be concentrated in the central part of the width of the annulus where it is attached to bone and radiates toward the tympanic membrane. In humans collections of radially oriented smooth muscle cells were found in several locations. The smooth muscle in all species studied appears to form a rim of contractile elements for the pars tensa. This arrangement suggests a role in controlling blood flow and/or creating and maintaining tension on the tympanic membrane.

Keywords: Tympanic membrane; Tympanic annulus; Annulus fibrosus; Smooth muscle

1. Introduction

In the mammalian ear, the annulus fibrosus is the thickened circumferential rim of the pars tensa of the tympanic membrane. It is an area into which the collagenous matrix of the tympanic membrane extends to become directly or indirectly attached to the inner surface of the tympanic bone (ring). This surface usually has a circumferential concavity called the sulcus tympanicus. Recent studies have shown that the annulus of the rat is associated with contractile elements called myofibroblasts (Kuijpers et al., 1999) or smooth muscle in mustached bats (Henson and Henson, 2000a,b). In the latter, the muscle tissue is closely associated with an elaborate plexus of blood vessels, which has previously been described as a cavernous plexus (Bondy, 1907; Henson, 1961). In these animals the muscle fiber orientation is predominately radial. The attachment of smooth muscle fibers to extensions of the collagenous

Abbreviations: CM, cochlear microphonic; EDTA, ethylene diamine tetra-acetic acid; H&E, hematoxylin and eosin; LM, light microscope; LSM, laser scanning microscope; TEM, transmission electron microscope; μm, micrometers

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0378-5955/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.heares.2004.09.004

matrix of the tympanic membrane, and to the bony tympanic sulcus suggests that it may play a role in applying tension to the tympanic membrane. Drugs commonly used to produce contraction or relaxation in isolated smooth muscle preparations have been applied to the tympanic membrane of gerbils. This resulted in dose dependent changes in cochlear microphonic (CM) thresholds with maximum changes that were usually in the range of 5.0–7.5 dB for frequencies tested: 2.16, 4.47 and 9.11 kHz (Yang and Henson, 2002).

The purpose of this investigation was to determine if smooth muscle is a common feature of the annulus in mammals. Preliminary reports on this subject have been published in Abstracts of the Association for Research in Otolaryngology (Henson and Henson, 2000a,b, 2002; Henson et al., 2001a,b, 2002).

2. Methods

2.1. Animals

The tissues examined were from representatives of four mammalian orders.

Order	Genus–species	Common name	Number studied
Insectivora	*Erinaceus europaeus*	European hedge hog	1 ear
Chiroptera	*Antrozous palidus*	Pallid bat	2 ears
Rodentia	*Meriones unguiculatus*	Mongolian gerbil	8 ears
	Rattus ratus	Laboratory rat	2 ears
	Mus musculus	Laboratory mouse	4 ears
Primates	*Homo sapiens*	Human	4 ears

The gerbils and mice were maintained in captivity. Their care and use was approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill (animal assurance numbers: 01-014 0-A, gerbils: 01-074 0-B, mice). The ears of the pallid bat were received as anatomic specimens from Dr. Zoltan Fuzessery, University of Wyoming, Laramie. The hedgehog material was in the form of thick, 70 μm serial sections prepared for an earlier study (Henson, 1961). The human tissue was obtained from four adult patients ranging in age from 56 to 71 years. These patients all had sensorineural hearing loss due to benign tumors of the VIIIth nerve (acoustic neuromas). The translabyrinthine approach to the tumor required removal of the tympanic membrane. No middle ear pathology was observed in three of the patients, but one had a small myringosclerotic plaque in the posterior part of the tympanic membrane. In addition, four sets of H&E stained, serial sections of the human temporal bone were examined. These are part of the temporal bone bank collection at the Wake Forest University School of Medicine. The sections had a thickness of 20 μm and every fifth section was mounted and stained. The material studied was from a 49-year-old white male (#910468L), a 58-year-old white male (#981542R), a 57-year-old white female (#981562L) and a one-year-old white female infant (#90312L).

2.2. Isolation and processing of tissue

When live animals were used, they were deeply anesthetized by an intramuscular injection of ketamine (40 mg/kg) and then killed by decapitation. The annulus was isolated by removing the pinna and trimming the ear canal down to the level of the tympanic membrane. The parts of the bulla surrounding the membrane could then be dissected away and the membrane, attached malleus and surrounding tympanic ring removed as a unit and placed in fixative. Human tissue was removed during surgery and immediately immersed in fixative. For light and transmission electron microscopy, the fixative consisted of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.15 M sodium phosphate buffer, pH 7.4. Tissue was fixed for 12–24 h in the cold (4 °C) and decalcified in 0.1 M EDTA in 0.15 M sodium phosphate buffer in a microwave oven according to the method of Madden and Henson (1997). Specimens were then osmicated, dehydrated, embedded in epoxy resin, sectioned and stained according to standard TEM procedures.

Since the annulus forms a nearly complete ring, sections must be close to true cross-sections to interpret the orientation of the muscle cells and collagen fibers. When possible, the ring was cut into two segments prior to embedding; the blocks were then reoriented to provide cross-sectional profiles.

2.2.1. Light, transmission and confocal microscopy

For light microscopy, blocks were sectioned at 1–2 μm and stained with toluidine blue. For TEM, specimens were sectioned at 70 nm and viewed on a Zeiss EM910 transmission electron microscope (LEO Electron Microscopy, Inc., Thornwood, NY) at an accelerating voltage of 80 kV.

For confocal microscopy, Bouin's solution was used as a fixative. Tissue processing and staining with Mallory trichrome followed the method of Baird and Henson (1961). Thick (75 μm) sections were examined with a Zeiss LSM 210XX confocal laser scanning microscope using the 514-nm line of an argon ion laser and an emission 575–645 nm band pass filter. The wavelengths employed resulted in excitation of the aniline blue in the