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SCIENCE DIRECT

Developmental Brain Research 157 (2005) 192 – 200

DEVELOPMENTAL BRAIN RESEARCH

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Research report

Spatiotemporal patterns of neuronal programmed cell death during postnatal development of the gerbil cochlea

Stephen M. Echteler[a,b,c,*], Thomas Magardino[b,c], Matthew Rontal[b,c]

[a]Abramson Research Building, Room 510D, The Children's Hospital of Philadelphia, 34th St. and Civic Center Boulevard, Philadelphia, PA 19104-4318, USA
[b]Auditory Development Laboratory, The Center for Childhood Communication, The Children's Hospital of Philadelphia, Philadelphia, PA 19104-4318, USA
[c]Department of Otorhinolaryngology, Head and Neck Surgery, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

Accepted 25 April 2005
Available online 4 June 2005

Abstract

During early postnatal development, afferent neurons of the cochlear (spiral) ganglion progressively refine their projections to auditory hair cells so that, by hearing onset, most cochlear nerve fibers innervate a single hearing receptor. One mechanism that might contribute to these changes in cochlear innervation is the programmed cell death (apoptosis) of developing neurons within the spiral ganglion. In the present study, we used the TUNEL method and morphological criteria to identify apoptotic cells within the spiral ganglion of the Mongolian gerbil during the first week of postnatal life when afferent projections to the cochlea are actively refined in this species. The locations of individual apoptotic spiral ganglion cells were mapped onto three-dimensional reconstructions of the entire ganglion for an age-graded series of gerbils to produce the first high-resolution, spatiotemporal maps of apoptotic ganglion cell death for the postnatal cochlea. We observed a significant increase in apoptosis in the spiral ganglion from postnatal day (P) 4 through P6. During this time, the most intense apoptotic activity occurred in regions of the spiral ganglion providing innervation to the lower middle and apical turns of the cochlea. The time course and regional variation of programmed cell death within the developing gerbil spiral ganglion are discussed in terms of the postnatal refinement of cochlear innervation and its possible functional significance for hearing in gerbils.

Theme: Development and regeneration
Topic: Sensory systems

Keywords: Spiral ganglion; Apoptosis; Gerbil; Development; TUNEL

1. Introduction

Within many regions of the developing nervous system, one mechanism by which precise neural circuits are constructed is through programmed cell death (apoptosis) of neurons projecting to 'inappropriate' targets [28]. The mammalian organ of hearing, the cochlea, is well suited for examining this process because its pattern of innervation is among the most precise observed for any sensory or motor system [16,19,37]. The position of an auditory receptor (hair cell) along the length of the cochlea correlates with the sound frequency to which it is most sensitive; hair cells at the base of the cochlea respond best to high-frequency sounds, those at successively more apical locations respond best to progressively lower frequencies [1]. The topography of this frequency-place map must be conveyed accurately to the brain by neurons of the cochlear (spiral) ganglion for normal sound perception and discrimination to occur. Spiral ganglion neurons accomplish this task by projecting to hair cells with extraordinary precision; most neurons restrict their innervation to a single hair cell [2,4,16,19].

This precision in cochlear innervation is not evident in newborn mammals but arises gradually during postnatal development prior to hearing onset [8,30,36]. Refinement

* Corresponding author. Fax: +1 215 590 5202.
E-mail address: echteler@mail.med.upenn.edu (S.M. Echteler).

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doi:10.1016/j.devbrainres.2005.04.004

into mature cochlear innervation patterns could result from remodeling of terminal arbors by afferent cochlear neurons, the elimination of neurons with inappropriate projections from the developing spiral ganglion through apoptosis, or the combination of both mechanisms. Developmental remodeling of cochlear neuron arbors has been inferred from age-related decreases in the number of hair cells contacted by individual auditory nerve fibers prior to hearing onset in cats [30], hamsters [35,36], and gerbils [8]. In addition, cell counting studies have shown a decrease in the number of spiral ganglion cells during postnatal development in rats [34] and gerbils [9], and qualitative reports of apoptotic cell death within the developing spiral ganglia have been published for rats [24] and mice [26]. Significantly, however, the time course and regional distribution of apoptotic cell death within the developing spiral ganglion have not been quantified for any mammal.

In this study, we have investigated apoptotic cell death within the spiral ganglion of the Mongolian gerbil during the first week of postnatal life when afferent projections to the cochlea are actively refined [8] and the number of cochlear neurons decreases by 30% [9]. Cells undergoing apoptosis were identified by distinctive morphologic features such as cellular shrinkage and condensation of nuclear chromatin (reviewed in [40]), as well as by fragmentation of DNA (a hallmark of apoptosis) visualized by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) method [11]. Cell counting and three-dimensional reconstruction methods were then used to create the first quantitative, high-resolution, spatiotemporal maps of apoptotic cell death within the developing spiral ganglion. We report that apoptotic cell death increased significantly in the gerbil spiral ganglion during the first week of postnatal life, varied in intensity along the frequency axis of the cochlea, and manifested frequently in cells located within the periphery of the spiral ganglion.

2. Materials and methods

2.1. Animals

A breeding colony was established from mated pairs of Mongolian gerbils, *Meriones unguiculatus*, obtained from a commercial supplier (Charles River Laboratories; Boston, MA). The colony was checked twice daily for births at 0800 and 1700 h. Only pups born during this 9-h period were used in this study and the day of birth was designated as postnatal day zero (P0). All litters were culled to 5 animals, as this resulted in a more homogeneous growth distribution of the remaining pups. Pup weight and length were measured daily during the first 3 weeks after birth. These data (not shown) were used to select animals that fell within one standard deviation from mean body weight and length curves. Gerbil pups were euthanized by an overdose of sodium pentobarbital (180 mg/kg body weight) administered by intraperitoneal injection. Following cessation of respiration, gerbils were decapitated and both cochleae were rapidly dissected from the temporal bone. A pathway for flow of fixative was created by removing the stapes and opening a small hole in the apex of the cochlear capsule. The specimen was then fixed in 4% (w/v) paraformaldehyde for 2 to 4 days. No more than 10 min elapsed between decapitation and immersion in fixative. All procedures involving animals in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of the Joseph Stokes, Jr. Research Institute at the Children's Hospital of Philadelphia, protocol number: 2001-8-220.

2.2. TUNEL labeling

After fixation, cochleae were dehydrated through a graded series of ethanol and embedded in paraffin. Serial sections were cut at 6-μm intervals along the mid-modiolar axis throughout the entire cochlea. Every fifth section containing a cross-section of the spiral ganglion was assayed for the presence of apoptotic cells using the protocol and reagents supplied within the ApopTag Plus Peroxidase kit (Intergen; Purchase, NY). Tissues from each age group were processed together for TUNEL staining to reduce variations in labeling that might result from minor differences in protocol.

2.3. Cell counting

Cochlear sections that included the spiral ganglion were first traced through a drawing tube attached to a Nikon Microphot-FX microscope. First, a tracing at 100× magnification was made for the purpose of creating serial reconstructions (below) and then a tracing at a total magnification of 350× was completed in order to count cells.

TUNEL-labeled and unlabeled ganglion cell profiles were traced within each cochlear section. For unlabeled profiles, only ganglion cells with a well-defined nucleus containing at least one nucleolus were included. The number of TUNEL-labeled and unlabeled spiral ganglion cell profiles was then totaled for each cochlea and multiplied by 5 to account for intervening sections. An 'apoptotic index' was calculated by dividing the number of TUNEL-labeled cell profiles by the sum of all neuronal cell profiles and multiplying the result by 100. Statistical analyses were performed by using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test for simple main effects.

Since our purpose was to quantify the percentage difference in the apoptotic index with age and cochlear location rather than to provide absolute numbers of normal or apoptotic spiral ganglion cells, we did not adjust our counts for possible over-counting of spiral ganglion cells with multiple nucleoli. We have previously shown, however, that the percentage of such cells does not change