Available online at www.sciencedirect.com
SCIENCE DIRECT®
Hearing Research 202 (2005) 209–221
HEARING RESEARCH
www.elsevier.com/locate/heares

Effect of cochlear integrity on cochlear nucleus neuron glucose metabolism in aged adult broiler chickens

Susan E. Smittkamp [a,b], Dianne Durham [b,*]

[a] Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66160, USA
[b] Department of Otolaryngology-Head and Neck Surgery, University of Kansas Medical Center, 3901 Rainbow Blvd, MS 3010, Kansas City, KS 66160, USA

Received 2 September 2004; accepted 21 October 2004
Available online 7 December 2004

Abstract

Abrupt removal of excitatory input is devastating to post-synaptic neurons in normally functioning sensory systems. In both mammalian and avian auditory systems, abrupt temporary or permanent experimental deafferentation stimulates a cascade of changes in central auditory structures that can result in neuron death. Effects of naturally occurring progressive deafferentation on central auditory structure and function have not been fully described. Extensive naturally occurring cochlear damage is found in some aged chickens, despite their regenerative capacity, providing the opportunity to examine the effects of this type of deafferentation on the avian cochlear nucleus (nucleus magnocellularis, NM).

Previous evaluation of NM oxidative metabolism using cytochrome oxidase histochemistry revealed that naturally occurring cochlear damage results in down-regulated metabolism in corresponding regions of NM. It is unknown how progressive hair cell damage and loss affects NM glucose uptake. Here, NM glucose metabolism is assessed using 2-deoxyglucose uptake as a marker for metabolic activity in the presence of normal, mildly damaged, severely damaged, and totally damaged cochlear hair cells. Results indicate that while severe and total cochlear damage significantly decrease NM oxidative metabolism, only total damage results in significantly decreased NM glucose metabolism. Results are discussed in the context of functional reorganization and trophic support.

Keywords: Aging; Auditory; Chicken; 2-Deoxyglucose; Nucleus magnocellularis

1. Introduction

Sensory neurons are dependent on excitatory input for maintenance and survival (LeVay et al., 1980; Dietrich et al., 1985; Westrum and Bakay, 1986; Churchill et al., 1998; Horton and Hocking, 1998; Mandairon et al., 2003). The abrupt removal of afferent input sets off a cascade of changes in central nervous system structure and function that can culminate in cell death. In the auditory system, abrupt deafferentation has been shown to result in, among other things, an immediate cessation of action potentials, a marked decrease in glucose uptake, changes in cell metabolism and protein synthesis,

Abbreviation: 2DG, 2-deoxyglucose; ANOVA, analysis of variance; BDNF, brain derived neurotrophic factor; CO, cytochrome oxidase; DPOAE, distortion product otoacoustic emissions; GAP-43; growth associated protein-43; IACUC, Institutional Animal Care and Use Committee; KUMC, University of Kansas Medical Center; NIDCD, National Institute for Deafness and other Communication Disorders; NM, nucleus magnocellularis; NOF, neurite outgrowth factor; NT3, neurotrophin 3; NT4, neurotrophin 4; NT5, neurotrophin 5; OD, optical density; PBS, phosphate buffered saline; PLSD, protected least significant difference; SEM, scanning electron microscopy; Trk B, tyrosine kinase receptor B; Trk C, tyrosine kinase receptor C

* Corresponding author. Tel.: +1 913 588 6731; fax: +1 913 588 6708.
E-mail address: ddurham@kumc.edu (D. Durham).

0378-5955/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.heares.2004.10.009

and neuron death (Koerber et al., 1966; Born and Rubel, 1985; Durham and Rubel, 1985; Hashisaki and Rubel, 1989; Hyde and Durham, 1990; Born et al., 1991; Sie and Rubel, 1992; Tierney et al., 1997; Edmonds et al., 1999; Mostafapour et al., 2000; Wilkinson et al., 2002).

Abrupt deafferentation of the auditory system is clinically atypical, however, usually occurring only in cases of trauma or surgical necessity. Most cases of hearing loss, particularly presbycusis, arise due to progressive deafferentation, usually occurring through the damage and subsequent loss of cochlear hair cells. The cochlear pathology characteristic of presbycusis has been well documented (Johnsson and Hawkins, 1972; Bess and Strouse, 1996). However, the effects of long-term progressive cochlear damage on central auditory structures have yet to be fully characterized. The discovery of extensive cochlear hair cell damage and loss in aging chickens (Durham et al., 2002; Smittkamp et al., 2002) provides the opportunity to examine the effects of this type of deafferentation on the auditory brainstem, as well as the loss and recovery of excitatory input through their regenerative capacity (Cotanche, 1999; Smolders, 1999).

Previous work has shown that, alone or in combination with age, cochlear damage results in down-regulated oxidative metabolism in regions of NM (nucleus magnocellularis, avian homologue of mammalian anteroventral cochlear nucleus) that correspond tonotopically to regions of cochlear damage (Smittkamp et al., 2003; Smittkamp and Durham, 2004). These studies used cytochrome oxidase (CO) histochemistry to evaluate NM metabolism, a method that, because intracellular CO levels do not fluctuate rapidly, primarily assesses long-term metabolic activity. The effect of cochlear damage on short-term metabolic activity is unknown. Intracellular glucose levels do fluctuate rapidly, as cell activity demands rise and fall. Here, we evaluate NM metabolism using incorporation of a radiolabeled glucose analogue (2-deoxyglucose, 2DG) as a marker for short-term metabolic activity in the presence of varying degrees of cochlear damage.

2. Materials and methods

A total of 17 broiler hens (Cobb or Ross strain) served as experimental subjects. All birds were obtained from a commercial supplier (ConAgra: Batesville, AR) at 19 ($n = 11$) and 67 ($n = 6$) weeks of age and raised to the age of approximately 70 and 80 weeks, respectively, in the University of Kansas Medical Center (KUMC) animal care facility. Upon arrival at KUMC, these chickens were housed communally or in separate cages with free access to food and water. All animal procedures were approved by the KUMC Institutional Animal Care and Use Committee (IACUC).

2.1. Surgery

As part of other experiments, all animals underwent electrophysiological assessment of distortion product otoacoustic emissions (DPOAEs). Briefly, animals were anesthetized with intramuscular injections of Xylazine (3.2 mg/kg) and Ketamine (80 mg/kg) and placed in a sound-proof booth. An infant probe was inserted in the ear canal and advanced until an air-tight fit was achieved. DPOAE thresholds were then determined bilaterally using a Tucker Davis Technology system, according to the method of Lichtenhan et al. (in press).

Additionally, some animals underwent unilateral placement of an osmium tetroxide-infused gelfoam pledget on the round window. Animals were anesthetized with intramuscular injections of Xylazine and Ketamine as described above. The external ear was treated with Lidocaine before 2 incisions were made to increase visibility of the tympanic membrane. An incision was then made in the tympanic membrane and the columella was removed, exposing the oval and round windows. An incision was made in the round window membrane and the pledget was placed inside the duct. Pledgets were removed after 1 h or left in place until sacrifice. Before animals came out of anesthesia, the incision in the external ear was closed using cyanoacrylate glue and the surgical site was cleaned of blood before animals were returned to cages. Animals survived 7 or 9 days post-op. No measurements were made in NM ipsilateral to this experimental manipulation.

2.2. Sacrifice and histology

Forty-five minutes prior to sacrifice, birds received an intramuscular injection of [^{14}C]2-deoxyglucose (2DG, American Radiolabeled Chemicals, St. Louis, MO) (45 μCi/kg) and exposure to an augmented auditory environment (music). Then the birds were deeply anesthetized with an intraperitoneal injection of Euthasol and decapitated. The brains were removed and frozen in heptane cooled with dry ice (−60 °C) and then stored at −80 °C. The cochleae were perfused with a solution of 3.5% glutaraldehyde in 0.1 M phosphate buffered saline (PBS) (Park et al., 1998).

Following perfusion, the cochlear ducts were exposed and the cochleae were prepared for scanning electron microscopy (SEM) according to an established protocol (Park et al., 1998). Briefly, the cochleae were post-fixed in 1% osmium tetroxide before dehydration in 70% ethanol. The sensory epithelia were exposed and the tegmentum vasculosa and tectorial membranes were removed via microdissection. The sensory epithelia were further dehydrated with a graded ethanol series before critical point drying and coating with gold palladium.

Frozen brains were sectioned coronally at −20°C on a cryostat (Jung Frigocut). Three one-in-four series of