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Role of cochlear integrity in cochlear nucleus glucose metabolism and neuron number after cochlea removal in aging broiler chickens

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Abstract

In the chicken auditory system, cochlear nucleus (nucleus magnocellularis, NM) neurons receive their only excitatory input from the ipsilateral cochlea. Cochlea removal (CR) results in an immediate decrease in NM neuron electrical activity, followed by death of ~30% of NM neurons. Previous work showed a decrease in NM activity and subsequent loss of NM neurons in all chicks. Egg layer adults showed NM neuron loss after CR, while neuron number remained stable in broiler adults. This suggested that effects of CR on NM were age- and breed-dependent. We now know that most aging egg layer chickens maintain largely normal cochleae throughout adulthood. Some exhibit cochlear damage with age. The converse is true of broiler chickens. Most aging broiler chickens display cochlear degeneration, with some maintaining normal cochlear anatomy throughout adulthood. The presence of extensive cochlear damage may alter the effect of CR on NM, leading to the described differences.

Here, we examine the effect of unilateral CR on NM glucose metabolism and neuron number in 2, 30, 39, and 52 week-old broiler chickens found to have normal cochleae. Chickens with damaged cochleae were excluded. Using 2-deoxyglucose uptake to evaluate bilateral NM glucose metabolism, we found significantly decreased uptake ipsilateral to CR at each age examined. Bilateral cell counts revealed significant neuron loss ipsilateral to CR at each age examined. This suggests that NM glucose metabolism decreases and subsequent neuron death occurs in aging broiler chickens when a normal cochlea is removed. The status of the cochlea must play a role in the effect of deafferentation on NM glucose metabolism and neuron survival. The effect of CR appears to be dependent upon neither age nor breed, but upon cochlear integrity instead.

Keywords: Auditory; Avian; Deafferentation; Nucleus magnocellularis

1. Introduction

The permanent loss or temporary deprivation of excitatory input can be devastating to normally functioning sensory systems. Afferent regulation is crucial for the maintenance and survival of post-synaptic neurons in the somatosensory (Dietrich et al., 1985; Churchill et al., 1998; Xu and Wall, 1999; Jain et al., 2000; Woods et al., 2000), visual (LeVay et al., 1980; Wong-Riley and Carroll, 1984; Jeffery and Parnavelas, 1987; Chino et al., 1992; Chino, 1995; Yinon et al., 1995; Horton and Hocking, 1998), olfactory (Westrum and Bakay, 1986; Capurso et al., 1997; Leung and Wilson, 2003; Mandairon et al., 2003), and auditory (Born and Rubel, 1985; Pasic and Rubel, 1989; Rubel et al., 1990; Born et al., 1991; Lippe, 1991; Pasic and Rubel, 1991; Sie and Rubel, 1992; Edmonds et al., 1999; Mostafapour et al., 2000) systems of mammals and avians.

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In the chick auditory system, cochlea removal (CR) results in an immediate decrease in cochlear nucleus (nucleus magnocellularis or NM) neuron activity followed by death of approximately 30% of NM neurons (Born and Rubel, 1985; Born et al., 1991). Previous work has shown this deafferentation-induced neuron death to be age- and breed-dependent (Born and Rubel, 1985; Edmonds et al., 1999). After CR, egg layer and broiler chicks exhibited NM neuron death while only egg layer adults exhibited a loss. Neuron number remained stable in broiler adults. Thus, the response to CR was thought to be dependent on the age and breed of the birds. Cochlear anatomy was not examined in these studies. Recently, we found that most aging commercially raised broiler chickens exhibit progressive cochlear damage, while most aging commercially raised egg layer chickens maintain normal cochlear anatomy throughout adulthood (Durham et al., 2002).

The survival of NM neurons is dependent upon excitatory input from the eighth nerve and cochlear hair cells (Born and Rubel, 1985; Edmonds et al., 1999). The abrupt interruption of afferent activity is thought to be the signal responsible for NM neuron death. However, if slow, progressive cochlear degeneration is occurring, the cochlear hair cells may gradually cease providing excitatory input over a period of many weeks. It is known that absolute NM neuron number does not change in aging birds likely to have cochlear damage (Edmonds et al., 1999), so perhaps NM neurons can adapt to slowly decreasing afferent input. In this situation, it seems unlikely that CR will interrupt eighth nerve activity and initiate the cascade of changes that results in neuron death. The response to removal of a damaged cochlea may thus differ from the response to removal of a normal cochlea. Therefore, the discrepancy in cochlear anatomy may play a role in the differing response to CR seen in adult egg layer and broiler chickens in the Edmonds et al. (1999) experiment.

As mentioned above, many aging broiler chickens undergo progressive cochlear degeneration. However, some of these birds maintain normal cochlear anatomy throughout adulthood. Using these normal birds, we investigate the role of cochlear integrity on the effect of CR on NM glucose metabolism and neuron number.

2. Materials and methods

A total of 28 broiler hens (Cobb or Ross strain) served as experimental subjects. All birds were obtained from a commercial supplier (ConAgra, Batesville, AR). The birds were divided among the ages of 2 weeks ($n=6$), 30 weeks ($n=5$), 39 weeks ($n=9$), and 52 weeks ($n=8$). Two week-old chicks were obtained as day-old hatchlings and raised in the University of Kansas Medical Center (KUMC) animal care facility. Thirty, 39, and 52 week-old adults were raised in the commercial facility until the ages indicated. Upon arrival at KUMC, these chickens were housed communally or in separate cages with free access to food and water for up to 2 weeks before sacrifice. All animal procedures were approved by the KUMC Institutional Animal Care and Use Committee (IACUC).

2.1. Surgery and histology

All animals underwent right CR according to the method of Durham and Rubel (1985). Briefly, animals were anesthetized with intramuscular injections of Xylazine (3.2 mg/kg) and Ketamine (80 mg/kg). 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 window. The basilar papilla was exposed using suction and removed with forceps. 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.

Prior to sacrifice, birds received an intramuscular injection of [^{14}C]2-deoxyglucose (2DG) (30 μCi/kg) (American Radiolabeled Chemicals, St. Louis, MO) and exposure to an augmented auditory environment (music played in the laboratory, as described by Park et al. (1999)) for 45 min to provide general auditory stimulation. 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 remaining 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 16 μm sections were thaw-mounted onto Superfrost Plus slides (Fisher Scientific, St. Louis, MO). One series was washed in PBS to remove radioactivity, hydrated, stained with thionin, dehydrated, and coverslipped using DPX (BDH Laboratories). The second series was stained for cytochrome oxidase (CO). Slides were incubated at 40° C in a solution containing 4 g sucrose, 60