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Research Report

Neuroglobin immunoreactivity in the human cochlea

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ABSTRACT

Neuroglobin (Ngb) is an oxygen-binding protein with a demonstrated role in endogenous neuroprotective mechanisms. It has been shown to function as a scavenger for reactive oxidizing species thereby assisting in cellular defense against oxidative stress. In the present study, we characterized the presence of Ngb in the human cochlea. Immunohistochemical staining was performed on formalin fixed celloidin human cochlea sections obtained from human temporal bones, using affinity purified polyclonal antibodies against Ngb. Thirty-six temporal bones were analyzed, 15 with normal otologic histories and 21 diagnosed with different inner ear pathologies. Ngb immunoreactivity (Ngb-IR) was consistently expressed in the neurons of spiral ganglia (SG) and supporting cells of the organ of Corti. There was a significant decrease of Ngb-IR in SGNs from specimens with inner ear pathologies when compared to normal specimens. In contrast, Ngb-IR in the organ of Corti did not show significant changes between pathological and normal specimens. The differential pattern of Ngb expression in these cochlear structures suggests that Ngb may participate in defense mechanisms in inner ear pathologies where oxidative stress is involved.

1. Introduction

The generation of reactive oxygen species (ROS) by oxidative stress plays a central role in the development of inner ear disease (Calabrese et al., 2010; Schacht et al., 2012). Oxidative stress in a physiological setting occurs when there is an imbalance between production and destruction of ROS, resulting in excessive bioavailability of ROS (Lopez et al., 2009). Sensory hair cells of the cochlea are very susceptible to oxidative stress due to excessive production of ROS (Kidd and Bao, 2012) in diseases like Meniere's disease, age-related hearing loss (presbycusis), noise induced hearing loss, and iatrogenic ototoxic damage. Antioxidant defense mechanisms present in the inner ear may prevent further deterioration and

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exogenous modulation of this defense may be a potential target for therapeutic influence (Kopke et al., 2002).

The modulation of endogenous protective mechanisms is a promising strategy for the development of novel treatments against neurodegenerative and otologic disorders (Abi-Hachem et al., 2010; Shibata and Raphael, 2010; Yu et al., 2013). Neuroglobin (Ngb) is a tissue globin mainly expressed in the central and peripheral nervous systems of mammals with a potential role in regulating oxidative stress pathways (Burmester et al., 2000; Greenberg et al., 2008, Reuss et al., 2015). In the mouse cerebral cortex, Ngb expression is upregulated by neuronal hypoxia *in vitro* and by focal cerebral ischemia *in vivo* (Sun et al., 2001). Neuronal function after hypoxia is worsened when Ngb expression is inhibited with an antisense olygodeoxynucleotide. Conversely, neuronal survival is enhanced by Ngb overexpression (Sun et al., 2001). Ngb is highly expressed in the retina (100-fold higher than in the brain) (Schmidt et al, 2003). The effect of Ngb overexpression in the retina was recently examined (Chan et al., 2012) *in vivo* using a Ngb-transgenic mouse model. The authors found that Ngb overexpression *in vivo* plays a neuroprotective role in retinal ischemia by preventing mitochondrial oxidative stress leading to decreased activated caspase-3 and apoptosis. Altogether these findings suggest that Ngb may be a potential therapeutic option to mitigate neuronal damage from pathologies that produce oxidative stress (Fetoni et al., 2009).

Neuroglobin immunoreactivity and mRNA expression was described in the rat cochlea (Lopez et al., 2010; Reuss et al., 2015) and cerebella (Beltran-Parrazal et al., 2010). In the rat cochlea Ngb, was localized in the spiral ganglia neurons (SGNs), supporting cells of the organ of Corti, and fibrocytes of the spiral ligament (Lopez et al., 2010). Ngb expression (mRNA and protein) was decreased in the rat SGNs after mild carbon monoxide exposure, suggesting an association between Ngb and oxidative stress (Lopez et al., 2010). Using immunohistochemistry, *in situ* hybridization and quantitative real time PCR, Reuss et al. (2015) recently described the expression of Ngb in the rat and mouse peripheral (cochlea) and central auditory system, and in the human superior olivary complex.

To the best of our knowledge, Ngb expression has not been described in the human cochlea. The purpose of this study was to investigate the distribution of Ngb in the normal human cochlea and changes in Ngb immunoreactivity (Ngb-IR) in the pathological cochlea using immunohistochemistry on archival temporal bones.

Table 1 – Temporal bones used in this study (normal hearing).

TB side	Age	Gender	PMT	Type of hearing reported
1L	8.5	F	10	Normal
2L	10	M	18	Normal
3R	18	M	9	Normal
4L	32	M	12	Normal
5R	50	M	9	Normal
6L	52	F	11	Normal
7L	55	M	10	Normal
8L	55	M	12	Normal
9L	55	F	14	Normal
10R	59	F	16	Normal
11R	67	M	17	Normal
12R	67	F	10	Normal
13L	67	F	8	Normal
14R	71	F	13	Normal
15R/L	72	F	18	Normal

Table abbreviations: TB: temporal bone; R: right side; L: left side; Age: in years, M: male; F: female; PMT: Post mortem time in hours. Normal: indicate normal hearing and balance.

Table 2 – Temporal bones used in this study (inner ear disease).

TB side	Age	Gender	PMT	Type of hearing loss (HL) reported
1L	10	M	6	Moderate HL
2L	10	F	4	Pronounced HL (OM)
3L	42	F	5	Moderated HL (vertigo)
4R	44	M	16	Bilateral HL
5L	46	M	12	Bilateral HL
6L	52	M	17	HL (Meniere's disease)
7L	62	M	12	Decreased hearing
8L	63	F	18	Moderated HL (OM)
9L	64	M	14	Moderated HL
10L	65	M	12	HL (inner ear degeneration)
11L	69	F	9	HL (Meniere's disease, otosclerosis)
12R	73	M	5	Bilateral HL (Tinnitus)
13L	74	F	12	Severe HL
14R	75	M	4	Nerve deafness
15L	76	F	9	Cochlea and saccule degeneration
16R	79	M	10	HL (Meniere's disease)
17R	80	F	10	HL (Kanamycin ototoxicity)
18R	81	F	9	Bilateral HL (vertigo)
19L	82	F	12	Bilateral Sensory neural HL
20R	85	M	18	Decreased hearing
21R	95	F	10	Pronounced HL (Meniere's disease)

TB: temporal bone; R: right side; L: left side; Age: in years, M: male; F: female; PMT: Post mortem time in hours. Normal: indicate normal hearing and balance. OM: otitis media.

2. Results

2.1. *Ngb-IR in the normal human cochlea*

Ngb-IR was visualized by indirect immunohistochemistry using horseradish peroxidase and the chromogen diaminobenzidine. We used tissue sections from 15 patients with normal hearing (Table 1), and 21 with hearing loss caused by different conditions (Table 2). Fig. 1a and b shows Ngb-IR in the organ of Corti and SGNs (middle region of the cochlea) from a 50-year-old male (normal hearing). Ngb-IR was seen in the cytoplasm of Deiter's and inner and outer pillar cells, but cell nuclei were not reactive (Fig. 1a). The Hensen, Claudius, and inner and outer hair cells were not immunoreactive to Ngb. The stria vascularis and spiral ligament showed no Ngb-IR (not shown). Ngb-IR was seen in the cytoplasm of most of the SGNs, but not in their nuclei (Fig. 1b). Large and small sized neurons of the SGN were immunoreactive to Ngb. Satellite cells that surround SGNs were not immunoreactive to Ngb antibodies. This pattern of immunoreactivity in the organ Corti and SGNs was similar at the apical middle and basal portion of the cochlea. Fig. 1a′ and b′ shows a consecutive section of the organ of Corti and SGNs (from the