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Expression of bFGF and NGF and their receptors in chick's auditory organ following overexposure to noise

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Abstract

Growth factors are known to activate signaling cascades for DNA replication; they participate in the regulation of cell differentiation and are required as positive signals for cell survival. Thus, many of them may be regarded as potential candidates stimulating regeneration processes in the inner ear. We analyzed the expression of basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) and their receptor (bFGFR and NGFR)-like immunoreactivity in chick basilar papillae, along with bFGF and NGF mRNA expression. The evaluation was made 1 and 5 days after exposure to wide-band noise with two increasing levels of acoustic energy.

For both factors, the immunoreactivity was shown predominantly in the middle part of basilar papilla, in noise-exposed, but not control birds. It was localized in the cytoplasm of hair cells, nuclei of supporting cells and cytoplasm of ganglion cells. Strong immunoreactivity of bFGFR and NGFR was found both in control and noise-exposed animals, with the cell localization similar to that of growth factors. The increase in mRNA expression for bFGF and NGF was found in noise-exposed animals only after lower exposure to noise, on day 5 after exposure ($p < 0.01$). A lack of increased expression after higher exposure could be excused by larger damage of hair cells followed by the increase of mRNA for β-actin to which the results were referred.

The results suggest bFGF and NGF involvement in postinjury regeneration of the basilar papilla.

Keywords: mRNA; Hair cells; Regeneration; Immunohistochemistry; Polyclonal antibody anti-CRF; Polyclonal anti-tyrosine kinase antibody

1. Introduction

The majority of disorders causing sensorineural hearing impairment are thought to be due to degeneration of hair cells, that are the sensory receptor cells of the inner ear epithelia. Hair cells can deteriorate after cochlear insult such as acoustic overstimulation or treatment with aminoglycoside drugs. In humans, this process is believed to be cumulative and irreversible. In contrast, in some lower species, the auditory system is better equipped to deal with injuries. Fish, amphibians and birds possess the capacity to repair injured sensory cells by regeneration and thus can maintain hearing function. The discovery of hair cell regeneration and further researches provide new optimism that there may be a treatment for hearing disorders in humans (Corwin and Cotanche, 1988; Cotanche, 1987; Kanzaki et al., 2002; Holley, 2003).

It has been found that in the mature avian cochlea (basilar papilla) new hair cells are produced to replace those that are lost, and that supporting cells within the sensory

Abbreviations: bFGF, basic fibroblast growth factor; bFGFR, basic fibroblast growth factor receptor; aFGF, acidic fibroblast growth factor; NGF, nerve growth factor; NGFR, nerve growth factor receptor; BDNF, brain-derived neurotrophic factor; GDNF, glial-cell derived neurotrophic factor; IGF1, insulin-like growth factor 1; IGF2, insulin-like growth factor 2; IGFR, insulin-like growth factor receptor; EGFR, epidermal growth factor receptor; NOF, neurite outgrowth factor; NT-3, neurotrophin-3; IR, insulin receptor; RARβ, retinoic acid receptor beta; RUR, retinoic acid receptor gamma; PCNA, proliferating cell nuclear antigen

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epithelia are the cellular precursors. The regeneration process follows the sequence of embryogenesis of chick basilar papilla (Adler and Raphael, 1995; Roberson et al., 1995, 1996). This process can occur de novo, as a result of mitosis (Girod et al., 1989; Raphael, 1992; Hashino et al., 1992, 1995; Adler and Saunders, 1995) or by transdifferentiation of not damaged cells into new hair cells. Mitosis can occur both in normal and damaged regions of basilar papilla (Girod et al., 1989; Hashino et al., 1992; Raphael, 1992).

During development neuronal survival, migration and differentiation can be influenced by a variety of signals mediated by diffusible trophic factors. Among them are neurotrophins, for example nerve growth factor (NGF), brain- derived neurotrophic factor (BDNF), glial-cell derived neurotrophic factor (GDNF), and growth factors, like acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), insulin-like growth factor 1 (IGF1), insulin-like growth factor 2 (IGF2) and others.

Growth factors are known to activate signaling cascades for DNA replication; they participate in the regulation of cell differentiation and are required as positive signals for cell survival. Thus, many of them may be regarded as potential candidates stimulating regeneration processes in the inner ear. The transforming growth factor β and neurite outgrowth factor (NOF) are taking part in the embryonic development of chick basilar papillae (Wu and Oh, 1996; Kajikawa et al., 1997) while nerve growth factors (NGF) – in the development of cochlear and vestibular ganglion neurons (Bernd et al., 1994). Their receptors were found in otocyst and spiral ganglion cells of chick and rat embryos (Escandon and Chao, 1990; Ylikoski et al., 1993; Zuniga et al., 1993; Bernd and Li, 1999). Neurotrophic factors, such as NGF, BDNF, and neurotrophin-3 (NT-3) may also play an important role in the development of auditory neurons of chickens (Avila et al., 1993) and rats (Lefebvre et al., 1990). NGF is probably a mitogen for spiral ganglion cells (Bernd et al., 1994), is involved in genesis and differentiation of neurons during embryogenesis (Escandon and Chao, 1990; Ylikoski et al., 1993), enhances the survival of neurons in chick embryos (Bartlett, 1997).

Since several growth factors are involved in embryogenesis, it has been investigating whether this growth promoting substances could exert protective effects on sensory cells and promote their regeneration. Some factors and their receptors expression was investigated in the inner ear epithelia in both chicks and mammals (Lee and Cotanche, 1996; Chardin and Romand, 1997; Kajikawa et al., 1997; Pickles and van Heumen, 1997). The results of these studies revealed that the receptors for several growth factors, namely epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), insulin-like growth factor receptor (IGFR), insulin receptor (IR), retinoic acid receptor beta (RARβ), retinoic acid receptor gamma (RUR), and basic fibroblast growth factor (bFGF), are present in both normal and noise-damaged basilar papillae.

The previous studies indicate that both aFGF and bFGF are potent mitogens (Brindle, 1993), that bFGF stimulates capillary endothelial cell proliferation and has a role in development of nervous system (Luo et al., 1993; Quian et al., 1997; Alvarez et al., 1998; Yokoyama et al., 1997). It has been recognized that bFGF is involved in proliferation of neuronal progenitor cells (DeHamer et al., 1994), neuronal development (Ghosh and Greenberg, 1995; Gensburger et al., 1987), neurite outgrowth (Williams et al., 1994), enhancement of neuronal survival (Schmidt and Kater, 1993), repair response after injury (Gomez-Pinilla et al., 1992) and amelioration of trauma-induced cochlear nerve degeneration (Sekiya et al., 2003). All these data suggest that this factor is a good candidate if considering hair cell regeneration processes in the inner ear, therefore should be further investigated.

Another group of factors, neurotrophins (including BDNF, NT-3, and NGF) is known to play a role in the survival of injured auditory neurons in mammals in vitro and in vivo (Duan et al., 2002). NGF exerts its effects through tyrosine kinase TrkA receptor. Recent data demonstrate the presence of TrkA receptor in the adult rodent organ of Corti, suggesting that NGF may exert specific functions in the peripheral auditory system and may promote mature auditory neuronal survival (Dai et al., 2004). However, its expression after exposure to ototoxic factors or noise has not been yet investigated.

The purpose of the study was to assess the immunolocalization of basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) along with the expression of mRNA for bFGF and NGF in chick basilar papillae after different exposures to noise, and to determine the localization of receptors for bFGF and NGF.

2. Materials and methods

2.1. Animals

The experiment was performed in total on 140 one-day-old White Leghorn chicks (*Gallus domesticus*). One hundred experimental chicks were exposed to wide band, steady state industrial noise, while 40 control birds were kept in a room where the noise level did not exceed 60 dB SPL.

All experimental methods and procedures, involving the use of animals were approved by the Animal Care Use Committee of the Institute of Occupational Medicine in Lodz conforming to NIH guidelines for the human treatment of laboratory animals.

2.2. Noise exposure

The white noise produced in the weaving mill was registered on magnetic tape with the measuring system consisting of a condenser microphone (type 4155 Brüel and Kjær), sound level meter (type 2231 Brüel and Kjær), and a tape recorder (type 7005 Brüel and Kjær). During the experiment, noise was reproduced with a tape recorder (type RS-TR 333 Technics with an auto-reverse option) and an amplifier (Luxman Stereo, type LV-120) with two loud speakers (Ultra Tonsil ZG). During the exposure, the birds (maximum 15 in number) were housed in a basket (40 cm × 30 cm × 50 cm) in sound-proof chamber, 1.2 m wide and 2.5 m high. The loudspeakers were located above the