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Review

Nitric oxide – A versatile key player in cochlear function and hearing disorders

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ABSTRACT

Nitric oxide (NO) is a signaling molecule which can generally be formed by three nitric oxide synthases (NOS). Two of them, the endothelial nitric oxide synthase (eNOS) and the neural nitric oxide synthase (nNOS), are calcium/calmodulin-dependent and constitutively expressed in many cell types. Both isoforms are found in the vertebrate cochlea. The inducible nitric oxide synthase (iNOS) is independent of calcium and normally not detectable in the un-stimulated cochlea. In the inner ear, as in other tissues, NO was identified as a multitask molecule involved in various processes such as neurotransmission and neuromodulation. In addition, increasing evidence demonstrates that the NO-dependent processes of cell protection or, alternatively, cell destruction seem to depend, among other things, on changes in the local cochlear NO-concentration. These alterations can occur at the cellular level or within a distinct cell population both leading to an NO-imbalance within the hearing organ. This dysfunction can result in hearing loss or even in deafness. In cases of cochlear malfunction, regulatory systems such as the gap junction system, the blood vessels or the synaptic region might be affected temporarily or permanently by an altered NO-level. This review discusses potential cellular mechanisms how NO might contribute to different forms of hearing disorders. Approaches of NO-reduction are evaluated and the transfer of results obtained from experimental animal models to human medication is discussed.

Contents

Abbreviations: ABR, auditory brainstem response; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ATP, adenosine triphosphate; DAF, 4,5-diaminofluorescein diacetate; dB, decibel; eNOS, endothelial nitric oxide synthase; EP, endocochlear potential; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; GABA, γ-aminobutyric acid; GR, glucocorticoid receptor; h, hour; HPLC, high performance liquid chromatography; IHC, inner hair cell; iNOS, inducible nitric oxide synthase; L-NAME, N^G-nitro-L-arginine methyl ester; L-NMMA, NG-monomethyl-L-arginine monoacetate; LPS, lipopolysaccharide; MD, Ménière's disease; NADPH, nicotinamide adenine dinucleotide phosphate; NMDA, N-methyl-D-aspartate; nNOS, neural nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; OHC, outer hair cell; ROS, reactive oxygen species; RNS, reactive nitrogen species; SPL, sound pressure level; BH_4, Tetrahydrobiopterin; TLR-4, Toll-like receptor 4.

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General aspects of NO-formation

Nitric oxide (NO) is a widespread signaling molecule with important roles in physiological and pathological processes in numerous biological systems, including the vertebrate cochlea [1,2]. This molecule is highly diffusible, has a low molecular weight, is uncharged, and is soluble in both aqueous and hydrophobic environments [3]. It can freely permeate membranes and diffuses over its half-life span of seconds in and out of a cell and organelles such as mitochondria. The average molecular velocity of a molecule with the mass of NO was determined to be ~400 m/s at room temperature [2].

In various regions of the cochlea, numerous fine-tuned NO-dependent processes occur constantly. Thus, NO is widely involved in pathways of signal transduction and ion regulation to control cochlear homeostasis. The up- and down-regulation of NO-dependent processes after various natural and ototoxic impacts can either support processes of cell protection or lead to cell destruction, depending on the level of NO-concentration. Therefore, it is of particular importance to collect comprehensive information about the regional and temporary alterations in NO-production in cases of inner ear dysfunction. An increased knowledge about the underlying processes of NO-formation is a prerequisite for the implementation of new therapeutic approaches in human inner ear diseases.

Generally, NO-formation can be enzyme-independent through direct disproportion or reduction of nitrite to NO under acidic or ischemic conditions [4,5] or enzyme-dependent by the three nitric oxide synthases (NOS). The endothelial nitric oxide synthase (eNOS) and the neural nitric oxide synthase (nNOS) were found to be constitutively expressed in the un-stimulated cochlea [6–11]. An expression of the inducible nitric oxide synthase (iNOS) is basically not found in the inner ear [12,13], but its expression can be induced by different triggers such as intensive noise exposure [14–16], application of ototoxic substances like gentamicin [17] or cisplatin [18,19], lipopolysaccharides (LPS) [12,13,16,20] and conditions of ischemia/reperfusion [21]. Furthermore, iNOS-expression was found in the cochlea after experimental induction of hydrops [22]. Nevertheless, it has to be mentioned that there are few reports about the constitutive expression of iNOS in the un-stimulated cochlea [16,23]. These findings are still under debate for the inner ear.

The constitutively expressed NOS-isoforms and iNOS differ, not only in the way of regulation (calcium/calmodulin-dependent regulation of the constitutively expressed isoforms vs. calcium-independent regulation for iNOS), but also in the amount of NO-production. The constitutively expressed NOS-enzymes produce NO in nanomolar concentrations [3], whereas iNOS-induction results in micromolar NO-concentration [24].

The enzymatic activity of all three NOS-isoforms is tightly controlled by the availability of the substrates arginine and oxygen and the co-factors nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), tetrahydrobiopterin (BH_4) and flavin adenine dinucleotide (FAD) [25,26].

An increase in NO-production within a tissue might occur either via an increase in NOS-activity by an already existing set of enzymes or by the up-regulation of the NOS-expression of the different isoforms, thus increasing the number of enzymes. The increase in NOS-activity and NOS up-regulation both result in an increased NO-concentration, followed by the activation of different enzymes cascades in a dose-dependent manner [3].

In summary, NO is an important signal molecule within the cochlea and it can be produced by the three different isoforms of NOS resulting in distinct local concentrations.

Functional entities of the vertebrate cochlea

In order to understand the role of alterations in NO-concentration and its influence on the complexity of cellular interactions within the cochlea, a short overview about the anatomy and the main functional properties of the hearing organ is necessary. The vertebrate cochlea can be divided roughly into three separate entities: (1) the sensory epithelium composed of the organ of Corti, (2) the lateral wall and the Limbus-area which are responsible for cochlear ion-regulation and -homeostasis, and (3) the fluid compartments (Fig. 1). The main task of the organ of Corti is the transformation of fluid movements into nerve signals. To fulfill this job, the organ of Corti contains sensory cells, the inner and outer hair cells (IHC and OHC), and different non-sensory (supporting) cells, such as Hensen cells, Deiters' cells and pillar cells. (Fig. 1; [27]). The lateral wall is located near the bony capsule and consists of the stria vascularis and the spiral ligament (Fig. 1). In the stria vascularis, the marginal cells control potassium release into the endolymph, whereas the fibrocytes in the spiral ligament ensure the potassium recycling from the organ of Corti [28,29]. By ion delivery into the endolymph, the lateral wall ensures the generation of the endocochlear potential (EP) and the maintenance of a stable ion concentration within the Scala media. In addition, the Limbus-area near the modiolus was found to contribute to potassium recycling. The potential difference between the endolymph and the hair cell cytoplasm is in a magnitude around +80 mV to +100 mV relative to perilymph. The potential difference between the endolymph (low in Na^+ and Ca^{++} but rich in K^+) and the intracellular fluid in hair cells is the driving force for the potassium current. The ions enter the hair cells at the apical cell pole and activate the nerves at the basolateral cell sides [30].

Methodological approaches for NO-localization within the cochlea

Nitric oxide was localized by different methodological approaches within the cochlea, ranging from the subcellular level to the complete cochlear entities such as the lateral wall or the organ of Corti.

At the subcellular level, strong signals of the fluorescent dye 4,5-diaminofluorescein diacetate (DAF) probing NO-production were identified in certain cellular spots within the outer hair cells [6]. These regions corresponded to areas where numerous mitochondria were identified by electron microscopic analyses several years ago [31]. Recently, higher amounts of the NO-related signals were identified in mitochondria in comparison to the NO-fluorescence signal in the cytosol using the same DAF-fluorescence-technique [32].

At the cellular level, NO was detected using DAF-2 in living cells [6,32,33] and in fixed material [33,34]. In addition, alterations in