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Erratum

Hyposmotic stimulation-induced nitric oxide production in outer hair cells of the guinea pig cochlea ☆

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Abstract

Nitric oxide (NO) production during hyposmotic stimulation in outer hair cells (OHCs) of the guinea pig cochlea was investigated using the NO sensitive dye DAF-2. Simultaneous measurement of the cell length and NO production showed rapid hyposmotic-induced cell swelling to precede NO production in OHCs. Hyposmotic stimulation failed to induce NO production in the Ca^{2+}-free solution. L-N^G-nitroarginine methyl ester (L-NAME), a non-specific NO synthase inhibitor and gadolinium, a stretch-activated channel blocker inhibited the hyposmotic stimulation-induced NO production whereas suramin, a P2 receptor antagonist did not. *S*-nitroso-*N*-acetylpenicillamine (SNAP), a NO donor inhibited the hyposmotic stimulation-induced increase in the intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) while L-NAME enhanced it. 1*H*-[1,2,4]oxadiazole[4,3*a*]quinoxalin-1-one, an inhibitor of guanylate cyclase and KT5823, an inhibitor of cGMP-dependent protein kinase (PKG) mimicked effects of L-NAME on the Ca^{2+} response. Transient receptor potential vanilloid 4 (TRPV4), an osmo- and mechanosensitive channel was expressed in the OHCs by means of immunohistochemistry. 4α-phorbol 12,13-didecanoate, a TRPV4 synthetic activator, induced NO production in OHCs.

These results suggest that hyposmotic stimulation can induce NO production by the $[Ca^{2+}]_i$ increase, which is presumably mediated by the activation of TRPV4 in OHCs. NO conversely inhibits the Ca^{2+} response via the NO-cGMP-PKG pathway by a feedback mechanism.

Keywords: Cochlea; DAF-2 DA; Hypotonic stimulation; Nitric oxide; Outer hair cell; Transient receptor potential vanilloid 4

Abbreviations: OHCs, outer hair cells; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentrations; TRPV4, transient receptor potential vanilloid 4; NO, nitric oxide; nitric oxide synthase, NOS; nNOS, neuronal nitric oxide synthase; eNOS, endothelial nitric oxide synthase; IHCs, inner hair cells; PSS, physiological standard solution; DAF-2DA, 4,5-diaminofluorescein diacetate; ROIs, regions of interest; L-NAME, *l*-N^G-nitroarginine methyl ester; $[K^+]_i$, intracellular K^+ concentrations; EGTA, ethylene glycol-bis(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid; SNAP, *S*-nitro-*N*-acetylpenicillamine; ODQ, oxadiazolo(4,3-a)quinoxalin-1-one; DMSO, dimethylsulfoxide; PKG, cGMP-dependent protein kinase; 4α-PDD, 4α-phorbol 12,13-didecanoate (4α-PDD)

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☆ This paper is now published including the color figures which were inadvertently reproduced in black and white in the previous version (Hearing Research 227/1–2 (May 2007), pp. 59–70). The Publisher apologizes for any inconvenience caused.

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1. Introduction

The mammalian outer hair cells (OHCs) are mechanical effectors of the cochlea. The motility of OHCs is divided into a fast and a slow motility (Zenner et al., 1985; Brownell et al., 1985; Zenner, 1986; Ashmore, 1987). The slow motility was considered to be a possible adaptive mechanism in the cochlea (Zenner, 1986). Slow motile responses can be divided into an active calcium-dependent motility and passive calcium-independent shape changes (Dulon and Schacht, 1992). The calcium-dependent slow motility is based on activation of actin–myosin systems (Zenner, 1986; Dulon et al., 1990; Kalinec et al., 2000). In contrast, osmotic effect induced by hyposmotic stimulation causes a slow shape change which is accompanied by a cell swelling independent of calcium and actin–myosin systems (Dulon et al., 1987, 1988).

Harada et al. (1994) showed that the cell swelling induced by hyposmotic stimulation was accompanied by an increase in the intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in OHCs. This $[Ca^{2+}]_i$ increase may subsequently activate metabolic processes including phosphorylation in OHCs. Previous study showed that hyposmotic stimulation results in magnitude and gain increase of electromotility (Sziklai and Dallos, 1997). Previous study also showed that direct perfusion of hypotonic solution into the perilymph spaces affects the summating potential and the compound action potential (Klis and Smoorenburg, 1994). Those studies suggested that the ionic environment and the changes in osmolarity of the inner ear may therefore affect the OHC motility, thereby varying the sensitivity of the inner ear to sound. They also suggested that hyposmotic swelling of OHCs may have physiological or pathological significance even in normal or impaired hearing of cochlear origin.

Transient receptor potential vanilloid 4 (TRPV4) is a Ca^{2+}-permeable channel, and it has been recently proposed to be an osmo- and mechanosensitive channel (Liedtke et al., 2000; Strotmann et al., 2000). A very recent study demonstrated the functional expression of TRPV4 to be involved in the hypotonic stimulation-induced Ca^{2+} influx in OHCs of the mouse cochlea (Shen et al., 2006a). These authors suggested that TRPV4 may function as an osmo- and mechanosensory receptor in OHC (Shen et al., 2006a). They also suggest that TRPV4 may play an important role in the active function of OHCs in cochlear micromechanics.

It is well-known that nitric oxide (NO), a gaseous membrane permeate messenger, is an important signaling molecule synthesized by NO synthase (NOS) in the nervous system (Garthwaite and Boulton, 1995; Prast and Philippu, 2001). The neuronal isoform (nNOS) and the endothelial isoform (eNOS) of NOS are constitutive and Ca^{2+}-dependent whereas the inducible NOS is Ca^{2+}-independent (Griffith and Stuehr, 1995). Previous studies showed both nNOS and eNOS to be expressed in the guinea pig cochlea, including OHCs (Franz et al., 1996; Gosepath et al., 1997). Previous studies revealed evidence of intracellular NO production in several types of cells in the guinea pig cochlea using NO-sensitive dye DAF-2 (Shi et al., 2001; Takumida and Anniko, 2001; Shen et al., 2003; Yukawa et al., 2005; Shen et al., 2005).

There is also increasing evidence that NO influences the mechanism of cellular Ca^{2+} homeostasis in several cell types by either a positive or negative feedback mechanism (Clementi and Meldolesi, 1997). The effects of NO are involved in the NO-cGMP- the cGMP-dependent protein kinase (PKG) signaling pathway in the sensory system of olfaction (Breer and Shepherd, 1993) and vision (Cudeiro and Rivadulla, 1999). A recent study also showed ATP-induced NO to affect the intracellular Ca^{2+} mobilization via the NO-cGMP-PKG pathway by a feedback mechanism in the inner hair cells (IHCs), OHCs, and spiral ganglion neurons of the guinea pig cochlea (Shen et al., 2005; Yukawa et al., 2005; Shen et al., 2006b). Immunocytochemical data also supports the presence of these signaling molecules in the cochlea (Michel et al., 1999; Takumida et al., 2000). As a result, NO may play an important role in the inner ear function (Takumida and Anniko, 2002).

Recent studies have shown hyposmotic stimulation to induce nitric oxide production in endothelial cells (Kimura et al., 2000, 2004). They thus suggested that NO may play an important role in regulating the volume and mechanical stress to the cell membrane in those cells.

However, the role of NO in cellular signaling transduction of OHCs for hypotonic response has yet to be demonstrated. We therefore investigated whether hyposmotic stimulation can induce NO production in OHCs. We also investigated the effect of NO on the hyposmotic stimulation-induced Ca^{2+} signaling in OHCs.

2. Materials and methods

2.1. Preparation of outer hair cells

This study was reviewed by the Committee for Ethics for Animal Experiments of Kansai Medical University, and was carried out under the Guidelines for Animal Experiments of Kansai Medical University. All experiments also conformed to the international guidelines on the ethical use of animals.

OHCs were isolated from the guinea pig cochlea as described previously (Harada et al., 1993). In brief, albino guinea pigs weighing 250–360 g with good Preyer's reflexes were deeply anesthetized and then decapitated. Each bulla was opened and placed into physiological standard solution (PSS) containing (mM): NaCl, 150; KCl, 5; $MgCl_2$, 1; D-glucose, 5; HEPES, 10; $CaCl_2$, 2, adjusted to a pH 7.4 and 300 mOsm. The organ of Corti was then carefully separated by mechanical dissociation, and gently transferred into an experimental chamber with a micropipette. The cells were then mechanically isolated with gentle trituration using a micropipette. No enzymatic treatment was used in the present study. The volume of the chamber was approximately 300 μl. The isolated OHCs were allowed to settle down and adhere to the bottom of the