BRAIN RESEARCH 1373 (2011) 25–38

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BRAIN RESEARCH

Research Report

Responses of pigeon vestibular hair cells to cholinergic agonists and antagonists

Gang Q. Li[1], *Manning J. Correia*[*]

Departments of Otolaryngology, Neuroscience and Cell Biology, University of Texas Medical Branch at Galveston, 301 University Boulevard, Galveston, TX 77550-1063, USA

ARTICLE INFO

Article history:
Accepted 6 December 2010
Available online 11 December 2010

Keywords:
Pigeon
Acetylcholine
Muscarinic receptor
Nicotinic receptor
Vestibular
Hair cells

ABSTRACT

Acetylcholine (ACh) is the major neurotransmitter released from vestibular efferent terminals onto hair cells and afferents. Previous studies indicate that the two classes of acetylcholine receptors, nicotinic (nAChRs) and muscarinic receptors (mAChRs), are expressed by vestibular hair cells (VHCs). To identify if both classes of receptors are present in VHCs, whole cell, voltage-clamp- and current-clamp-patch recordings were performed on isolated pigeon vestibular type I and type II HCs during the application of the cholinergic agonists, acetylcholine and carbachol, and the cholinergic antagonists, D-tubocurarine and atropine. By applying in different combinations, these compounds were used to selectively activate either nAChRs or mAChRs. The effects of nAChR and mAChR activation on HC currents and zero electrode current potential (V_z) were monitored. It was found that presumed mAChR activation decreased both inward and outward currents in both type I and type II HCs, resulting in either a depolarization or hyperpolarization. Conversely, nAChR activation mainly increased both inward and outward currents in type II HCs, resulting in a hyperpolarization of their V_z. nAChR activation also increased outward currents in type I HCs resulting in either a depolarization or hyperpolarization of their V_z. The decrease of inward and outward currents and the depolarization of the V_z in type I pigeon HCs by activation of mAChRs represents a new finding. Ion channel candidates in pigeon vestibular HCs that might underlie the modulation of the macroscopic ionic currents and V_z by different AChR activation are discussed.

[*] *Corresponding author*. Fax: +1 4109 772 1715.
E-mail address: manningcorreia@yahoo.com (M.J. Correia).

Abbreviations: ACh, acetylcholine; AChRs, acetylcholine receptors; BK, big conductance I_{KCa}; BSA, bovine serum albumin; CCh, carbachol; DIC, differential interference contrast; DMPP, 1,1,dimethyl-4-phenylpiperazinium iodide; EGTA, ethylene glycol-bis(b-aminoethyl ether)-N,N,N9,N9-tetraacetic acid; FBS, fetal bovine serum; HEPES, N-2-hydroxyethylpiperazine-N9-2-ethanesulfonic acid; HCs, hair cells; I_{KCa}, calcium-activated potassium current; $I_{KI} \equiv I_{K,L}$, a potassium current found in almost all type I hair cells that activates at low voltages, activates rapidly, and slowly inactivates at a V_h of ~60 mV; I_{IRK1}, inwardly rectifying potassium current—(Kir2.1); $KMeSO_4$, potassium methanesulfonate; mAChRs, muscarinic acetylcholine receptors; nAChRs, nicotinic acetylcholine receptors; NE, normal external; PKC, protein kinase C; SCCs, semicircular canals; SK, small conductance—I_{KCa}; TC, tubocurarine chloride hydrate; VHCs, vestibular hair cells; V_h, voltage clamp holding potential; V_z, zero electrode current potential; wk, week

[1] Current address: Department of Otology & Laryngology, Harvard Medical School, Eaton-Peabody Laboratories, Massachusetts Eye and Ear Infirmary, 243 Charles St., Boston, MA 02114, USA.

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doi:10.1016/j.brainres.2010.12.016

1. Introduction

Vertebrate vestibular organs possess two kinds of hair cells, so-called type I and type II. Birds, mammals, and reptiles (amniotes) have both type I and type II HCs, whereas fish, frogs, and other anamniotes only express type II HCs (Yan et al., 1991; Lysakowski, 1996). Pigeon and gerbil type I and type II hair cells can be distinguished by their shape, innervation, and several of their ionic currents (Correia and Lang, 1990; Rennie and Correia, 1994; Ricci et al., 1997a). Similar observations have been made in turtle (Brichta and Goldberg, 2000; Brichta et al., 2002). Pigeon vestibular hair cells receive both the pre-synaptic (efferent) and post-synaptic (afferent) innervation. Afferent bouton nerve terminals innervate pigeon type II hair cells, whereas nerve calyces surround type I hair cells (Li et al., 2007; Correia et al., 1985). Pigeon efferent fibers originate in the reticular formation and brainstem (Eden and Correia, 1982; Schwarz et al., 1981) and course peripherally to innervate type I and type II hair cells, afferent calyces of type I hair cells, and bouton afferents of type II hair cells (Li et al., 2007). Therefore, it is possible that efferent activation modulates the hair cell-afferent response both presynaptically (on HCs) and postsynaptically (on afferent terminals). In different animal models, it has been shown that efferent activation can either increase and/or decrease afferent firing activity (Dickman and Correia, 1993; Furukawa, 1981; Dechesne and Sans, 1980; Goldberg and Fernandez, 1980; Hartmann and Klinke, 1980; Holt et al., 2006; Rossi et al., 1980). Thus, the predominant efferent neurotransmitter, acetylcholine may act on different types of acetylcholine receptors present on both HCs and afferent terminals to produce the different changes in vestibular afferent firing rates.

Two types of nAChRs are present on auditory and vestibular hair cells. One is defined by the association of the α9 and α10 nAChR subunits. It is selectively blocked by strychnine, tropisetron, and α-bungarotoxin. The other nAChR is defined by its sensitivity to the nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (Holt et al., 2003). Activation of α9/α10 nAChRs in mammalian auditory hair cells (Elgoyhen et al., 1994; Elgoyhen et al., 2001) hyperpolarizes the hair cell membrane through the downstream activation of SK-I_{KCa} currents (Yuhas and Fuchs, 1999) on the hair cell's membrane, which subsequently inhibits afferent firing (Art et al., 1982). On the other hand, the activation of the DMPP-sensitive nAChR in frog produces a rapid depolarization of semicircular canal (SCC) hair cells, probably through the classical nicotinic receptor action (Holt et al., 2003).

The details of activation of cholinergic muscarinic receptors are less well worked out. Indirect evidence suggests that mAChR activation may involve different transduction pathways leading to depolarization or hyperpolarization of frog type II VHCs (Guth and Norris, 1996). The hyperpolarization pathway is believed to incorporate the phosphatidylinositol pathway ultimately resulting in the activation of I_{KCa} currents (Shigemoto and Ohmori, 1991; Yoshida et al., 1994; Guth and Norris, 1996). I_{KCa} currents are present in pigeon type I (Rennie and Correia, 1994) and type II (Lang and Correia, 1989) VHCs. The depolarization pathway and the effector are still unknown. However, it is widely recognized that activation of mAChRs modulates a number of ionic currents directly or indirectly resulting in membrane depolarization in a variety of tissues (Caulfield, 1993; Inoue and Yoshii, 1992; Jones, 1993). Five strong candidate currents include a potassium M (muscarinic) current, I_M, (Adams et al., 1982; Brown and Adams, 1980); an outwardly rectifying delayed rectifier current, I_{KD} (Harvey and Hume, 1989; Janssen, 1996); an inwardly rectifying potassium current I_{IRK1} (Jones, 1993, 1996, 1997, 2003; Rossignol and Jones, 2006), a L-type I_{Ca} (Fuchs and Murrow, 1992; Guth and Norris, 1996; Holt et al., 2003; Pemberton and Jones, 1997; Wikstrom et al., 1998); and a calcium-activated potassium current, I_{KCa} (Kong et al., 2005). A variant of I_M, (I_{KI}), and I_{KCa} are present on pigeon type I hair cells (Rennie and Correia, 1994; Rennie et al., 2001). I_{IRK1}, I_{KCa}, L-type I_{Ca}, and I_{KD} are expressed on pigeon type II VHCs (Correia et al., 2004; Masetto and Correia, 1997; Zampini et al., 2008; Lang and Correia, 1989).

The purpose of the present investigation was threefold: (1) to determine if there are functional cholinergic receptors on pigeon type I and type II vestibular hair cells, (2) to determine if the application of cholinergic agonists produces hyperpolarization and/or depolarization of type I and type II VHCs, and (3) to try to parse mAChR activation from nACHR activation in type I and type II hair cells by monitoring changes in membrane currents and voltages in VHCs while using a combination of cholinergic agonists and antagonists.

2. Results

2.1. *Pharmacological studies of type II VHCs*

During both ruptured voltage clamp and current clamp recordings described below, the total number of type II HCs activated by acetylcholine was 22.1% (38/172).

2.1.1. *Voltage clamp responses during voltage step protocol*

During the voltage step protocol (Fig. 1A), the application of 100 μM ACh *increases* outward currents (●) at voltage potentials of –60 mV and +40 mV and inward current (●) at –120 mV (Fig. 1A) relative to control currents (■). Conversely, application of a mixture of tubocurarine (TC) with ACh *decreases* both inward (●) and outward (●) currents (Fig. 1B). Under both pharmacological conditions, both outward and inward currents (▲) return towards control values after drug washout with normal external (NE) solution (Fig. 1C). Since 10 μM TC has been shown to block DMPP (Holt et al., 2003) and α9/α10 nAChRs (Holt et al., 2001; Elgoyhen et al., 2001; Verbitsky et al., 2000), we assume that the current (●) in Fig. 1B results from the activation of mAChRs whereas that in Fig. 1A results from activation of nAChRs. The above conclusion assumes that both nAChRs and mAChRs are simultaneously expressed on the same HC and that TC selectively antagonizes nAChRs.

Not all type II HCs had the same response to ACh application. Among 10 type II HCs that responded to ACh applications, 8 showed an *increase* in outward current (520.3 ± 399 pA with the range from 200 pA to 1400 pA) at the +40 mV potential while 7 showed an *increase* of the inward current (125.7 ± 32.3 pA with the range from 70 pA to 170 pA) at the –120 mV potential. Other HCs demonstrated a *decrease* of the outward current (n = 2, 165.6 ± 31.1 pA) and a *decrease* of the