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The properties of ACh-induced BK currents in guinea pig type II vestibular hair cells

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Abstract

Molecular biological studies have demonstrated that both muscarinic receptor subtypes and nicotinic receptor subunits were located in mammalian vestibular sensorineural epithelium. However, the functional roles are still unclear, with the exception of the well-known α9-containing nicotinic ACh receptor (α9nAChR). In this study, the properties of acetylcholine (ACh)-induced currents were investigated by whole-cell patch clamp technique in isolated type II vestibular hair cells (VHCs II) of guinea pig. VHCs II displayed a sustained, non-inactivating current when extracellular application of ACh. ACh-induced currents restored gradually and it took about 60 s to get a complete recovery. ACh-induced current was not affected by extracellular Na^+, but strongly affected by extracellular K^+ and Ca^{2+}. Depletion of the intracellular Ca^{2+} stores by intracellular application of inositol 1,4,5-trisphosphate (IP3) or blocking of the release of intracellular Ca^{2+} stores by intracellular application of heparin failed to inhibit this current. ACh-induced currents were inhibited by nifedipine, Cd^{2+}, tetraethylammonium (TEA), charybdotoxin (CTX), iberiotoxin (IBTX), atropine and d-tubocurarine (DTC), respectively, but not by apamin. In conclusion, ACh stimulates a large conductance, Ca^{2+}-activated K^+ current (BK) in guinea pig VHCs II by activation of the influx of Ca^{2+} ions, which is mediated by an ACh receptor that could not be defined to be the odd-number muscarinic receptor.

Keywords: ACh receptor; Type II vestibular hair cells; Large conductance, Ca^{2+}-activated K^+ currents

1. Introduction

A preponderance of evidence has indicated that acetylcholine (ACh) is the principal inner ear efferent neurotransmitter among mammalians (for reviews, see Eybalim, 1993; Guth et al., 1998). Cholinergic efferent labyrinthine fibers have been found by investigation of acetylcholine-esterase (AChE) and choline acetyltransferase (ChAT) activities (Godfrey et al., 1984; Lopez and Meza, 1988). Our previous studies proved that ChAT-

Abbreviations: ACh, acetylcholine; VHCs II, type II vestibular hair cells; IP3, inositol 1,4,5-trisphosphate; TEA, tetraethylammonium; CTX, charybdotoxin; IBTX, iberiotoxin; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid; DTC, d-tubocurarine; BK, large conductance, Ca^{2+}-activated K^+ currents; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); AChE, acetylcholine-esterase; ChAT, choline acetyltransferase; OHCs, outer hair cells; α9nAChR, α9-containing nicotinic ACh receptor; SK, small conductance, Ca^{2+}-activated K^+ currents; EC50, half-maximal response; $[Ca^{2+}]i$, intracellular Ca^{2+} concentration; $[Ca^{2+}]o$, extracellular Ca^{2+} concentration; IP3R, IP3 receptor; DPOAE, distortion product otoacoustic emission; K_{Ca}, Ca^{2+}-activated K^+ currents

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like immunoreactivity localized in vestibular end-organ of rat (Kong et al., 1994), human (Kong et al., 1998) and mouse (Kong et al., 2002). Recent data suggested that the efferent cholinergic axodendritic and axosomatic synapses have a muscarinic component including the expression of m1, m2 and m5 muscarinic receptors in human vestibular periphery (Wackym et al., 1996; Ishiyama et al., 1997). The differential expression of α2-7, α9 and β2-4 nicotinic receptor subunits were demonstrated in the vestibular end-organs of the rat (Anderson et al., 1997). It has been proved that α9 (Hiel et al., 1996) and α10 nicotinic subunits (Elgoyhen et al., 2001) are expressed in adult rat vestibular hair cells.

Different from typical inhibitory synapse, synaptic inhibition in vestibular hair cells is suggested to be achieved by activation of neuronal nicotinic receptor that usually initiates excitatory postsynaptic response, similar to what is observed in outer hair cells (OHCs) (Fuchs and Murrow, 1992b; Nenov et al., 1996; Oliver et al., 2000). This inhibition is mediated by the α9-containing nicotinic ACh receptor (α9nAChR): homomeric α9 nicotinic receptor or heteromeric α9α10 nicotinic receptor (Elgoyhen et al., 1994, 2001; Hiel et al., 1996; Vetter et al., 1999; Katz et al., 2000). The activation of α9nAChR in hair cells will initiate the hyperpolarization by activation of an outward K^+ current, identified as a small conductance, Ca^{2+}-activated K^+ current (SK), being virtually suppressed by apamin (Elgoyhen et al., 1994; Glowatzki and Fuchs, 2000; Jagger et al., 2000; Katz et al., 2000; Oliver et al., 2000). In addition, two types of large conductance, Ca^{2+}-activated K^+ currents (BK) were suggested to be present in hair cells: a fast and transient BK current (Jagger and Ashmore, 1999a,b; Armstrong and Roberts, 1998, 2001) and a non-inactivating BK current (Armstrong and Roberts, 1998, 2001), being virtually suppressed by iberiotoxin (IBTX) (Armstrong and Roberts, 1998).

There are different views with the source of the increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). The increase of $[Ca^{2+}]i$ in chick cochlear hair cells (Shigemoto and Ohmori, 1990) and K_{Ca} channels in bullfrog saccular hair cells (Yoshioda et al., 1994) produced by muscarinic agonists were not dependent on extracellular Ca^{2+}. However, the activation of Ca^{2+}-activated K^+ currents (K_{Ca}) produced by ACh in guinea pig OHCs demonstrated a dependence of the influx of Ca^{2+} ions (Housley and Ashmore, 1991; Fuchs and Murrow, 1992a; Kakehata et al., 1993).

Type II vestibular hair cells (VHCs II) are considered to be phylogenetically older than type I hair cells. Until now, there are a limited number of recent literatures available concerning the physiological features of ACh-induced current in mammalian VHCs II. In the present study, we isolated VHCs II from guinea pig by collagenase type IA to investigate the properties of ACh-induced currents by utilizing the conventional whole-cell patch clamp technique. These data indicate that ACh stimulates a sustained and non-inactivating BK current in VHCs II by activation of the influx of Ca^{2+} ions. The BK current is stimulated by an ACh receptor that could not be defined to be the odd-number muscarinic receptor (m1, m3, m5).

2. Materials and methods

2.1. Dissociation of hair cells

Guinea pig (300–350 g) was killed by cervical dislocation and the temporal bones were removed. The vestibular organ (crista or macula) was removed and incubated for 5 min at room temperature (20–24 °C) with 0.2 mg/ml collagenase type IA (Sigma) in a low Ca^{2+} and Mg^{2+}-free balanced salt solution (137 mM NaCl, 5.4 mM KCl, 0.1 mM $CaCl_2$, 0.2 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 10 mM Glucose (pH 7.2)). Washing stopped the enzymatic action with the normal external solution containing 2 mM $CaCl_2$. Then, hair cells were isolated by gentle mechanical dissociation and given half an hour to settle on the bottom of the experimental chamber, which was coated with the self-made rat-collagen (Guo and Kong, 2000).

2.2. Electrical recording

ACh-induced currents were recorded in the conventional whole-cell configuration with the use of a PC-IIB patch clamp amplifier (Hua-Zhong University of Science and Technology, WuHan, PRC). Patch electrodes were fabricated from thick-walled borosilicate glass capillary and fire-polished to fine resistances of 3–5 MΩ when filled with the internal solution as described below. All experiments were performed at room temperature (20–24 °C). Records were low-pass filtered at 5–10 HZ with a four-pole Bessel filter. Usually, the agent was employed to a patched cell for 10 s at a holding potential of −50 mV.

2.3. Solutions and pharmacological agents

All of the drugs were purchased from Sigma (St. Louis, MO, USA), with the exception of IBTX from Bachem (King of Prussia, PA, USA). ACh, apamin, atropine, Cd^{2+}, charybdotoxin (CTX), d-tubocurarine (DTC), IBTX and tetraethylammonium (TEA) were directly dissolved in the external solution. Nifedipine was first dissolved in ethanol; the final concentration of ethanol was $\leqslant 0.1\%$ in the bathing solution. Cd^{2+}, heparin and inositol 1,4,5-trisphosphate (IP3) sometime were added to the internal solution, respectively. After breaking the patch membrane, Cd^{2+}, heparin and IP3 will diffuse into the cell quickly. The efficiency of diffusion through a patch pipette is reported to be high from a reversal potential analysis (Ohoei, 1985). The effective blocking concentration of IBTX on BK currents ranged from 100 nM to 1 μM, due to the loss of activity of the blocking agent with time (also cf. Armstrong and Roberts, 1998).