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PATCH CLAMP RECORDINGS OF HAIR CELLS ISOLATED FROM ZEBRAFISH AUDITORY AND VESTIBULAR END ORGANS

M. HADEN, R. EINARSSON AND B. YAZEJIAN*

Natural Science Division, Pepperdine University, Malibu, CA 90263, USA

Abstract—The senses of hearing and balance in vertebrates are transduced by hair cells in the inner ear. Hair cells from a wide variety of organisms have been described electrophysiologically but this is the first report of the application of these techniques to the genetically tractable zebrafish model system. Auditory and vestibular hair cells isolated from zebrafish lagenae and utricles were patch clamped and both inward and outward currents under voltage clamp, and changes in membrane potential under current clamp were recorded. Cells displayed substantial diversity in their morphology, constellation of channel types, and level of excitability. While all cells showed evidence of the presence of fast-inactivating (A-type) K^+ channels, other K^+ channel types, including delayed rectifier, inward rectifier and large conductance Ca^{2+}-activated K^+ (BK) channels were less common. Recorded Ca^{2+} currents were identified pharmacologically as L-type. Non-linear regenerative voltage responses were evoked in more than half of the cells studied. © **2013 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: electrophysiology, K^+ channels, Ca^{2+} channels, lagena, utricle, inner ear.

INTRODUCTION

While the zebrafish is increasingly becoming the preparation of choice for the study of the genetic basis of hearing and balance (Nicolson, 2005), very little is known about the ion channel characteristics of its auditory and vestibular hair cells. A more complete understanding of the properties of these channels is lacking largely because of the absence in the literature of patch clamp recordings made from individual hair cells. One major question to be answered is how the channels that underlie neurotransmitter release from these cells vary across and between inner ear end organs and how they (may) differ from those in other preparations. In a previous report (Einarsson et al., 2012), we demonstrated the methods for isolation of hair cells from all of the inner ear organs of zebrafish, illustrated the relative positions of the end organs in the animal and showed representative responses of the cells under voltage clamp. Here, we present a more extensive analysis of the voltage-gated channels of the hair cells found in two of these end organs: the lagena, a mixed auditory and vestibular organ, and the more anteriorly located utricle that has a purely vestibular function.

EXPERIMENTAL PROCEDURES

All animals used in this study were handled in compliance with the policies and procedures of the Animal Care and Use Committee at Pepperdine University and with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). The number of animals used and their suffering were minimized. Adult, wild-type zebrafish (*Danio rerio*) of either gender were sacrificed by immersion in 0.02% MS-222 (Sigma St. Louis, MO USA; Catalogue No. A5040). Lagenae and utricles from the inner ear were removed and placed in a low Ca^{2+} solution (in mM: 100 NaCl, 3 KCl, 0.05 $CaCl_2$, 0.05 $MgCl_2$, 3 glucose, 30 Na^+–HEPES, pH 7.35) containing 0.05% L-cysteine (Sigma C1276) and 0.2% papain (Sigma P3375). After incubation in this solution for 30 min, the inner ear structures were transferred to a solution of NZR (Normal Zebrafish Ringer's, (in mM): 116 NaCl, 2 KCl, 2 $CaCl_2$, 3 glucose, 5 Na^+–HEPES, pH 7.35) + 0.4% bovine serum albumin (BSA) (Sigma A2153). After at least 30 min in the BSA-containing solution, cells were scraped off of the inner ear structures with a dog hair into a recording chamber containing either NZR (for recording of K^+ currents and for current clamp) or TEA-ZR (in mM: 86 NaCl, 30 TEACl, 2 KCl, 10 $CaCl_2$, 1 3,4-diaminopyridine (Sigma D7148), 5 Na^+–HEPES, pH 7.35) for recording inward currents. Patch pipettes of 1–3 MΩ (with the solutions used) were fabricated from borosilicate glass capillary tubing (Sutter Instruments, Novato, CA USA; Catalogue No. BF 150-86-10) using a multi-stage puller (Sutter P1000). Pipettes were filled with one of two solutions (in mM): for K^+ currents and current clamp, 52 K_2SO_4, 38 KCl, 1 mM K^+–EGTA, 5 K^+–HEPES, pH 7.3; for inward currents, 52 $CsMeSO_4$, 38 CsCl, 1 EGTA, 1 3,4-diaminopyridine, 50 glucose, 5 HEPES, pH 7.3. After addition of 200–300 μg/ml amphotericin B to the pipette solution,

*Corresponding author. Present address: Department of Physiology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA. Tel: +1-310-825-7870.
E-mail address: yaz@ucla.edu (B. Yazejian).

Abbreviations: BSA, bovine serum albumin; EGTA, ethylene glycol bis(β-aminoethylether)-*N*,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NZR, Normal Zebrafish Ringer's.

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perforated-patch clamp recordings (Horn and Marty, 1988; Rae et al., 1991) were made using a MultiClamp 700B patch-clamp amplifier and a Digidata 1440A digitzer controlled by pCLAMP 10 software (Molecular Devices, Sunnyvale, CA USA). Series resistances were measured (before compensating 85–95%) and averaged 25.7 ± 12.3 MΩ (sd, range 6–67 MΩ). K^+ currents are presented as the average of three presentations of each waveform, and inward currents as the average of ten presentations. Current clamp traces are of single presentations. Cell capacitance averaged 2.44 ± 0.75 pF (sd, range 1.0–4.3 pF). Experiments were performed at room temperature (22–25 °C).

RESULTS

Voltage and current clamp recordings

Voltage and current clamp recordings were made from hair cells acutely isolated from two inner ear organs, the lagena and utricle, of adult wild-type zebrafish. There was substantial diversity—both among cells from the same structure as well as between the two structures—in the morphology of cells isolated and in their complements of voltage-gated channels and extent of excitability. Figs. 1 and 2 illustrate this diversity for the lagena and utricle respectively. In Fig. 1, electrophysiological properties of three representative cells from the lagena (shown in a′, b′ and c′) are illustrated. The top panel (A, B and C) shows the currents evoked with a K^+ based internal solution in response to voltage steps between −130 mV and +110 mV delivered from a holding potential of −70 mV. The outward currents generated in A had at least three components: a slowly-activating delayed-rectifier type K^+ current; a rapidly activating and inactivating A-type current reminiscent of that first described in molluscan neurons (Connor and Stevens, 1971) and a Ca^{2+}-activated K^+ current. The telltale N-shaped region seen in the normalized peak current vs. voltage curve for this set of traces (see Fig. 1D) indicates the presence of a K_{Ca} current (Meech and Standen, 1975). Although a similar combination of current types is apparent in the recording of Fig. 1C, the magnitude and density of the

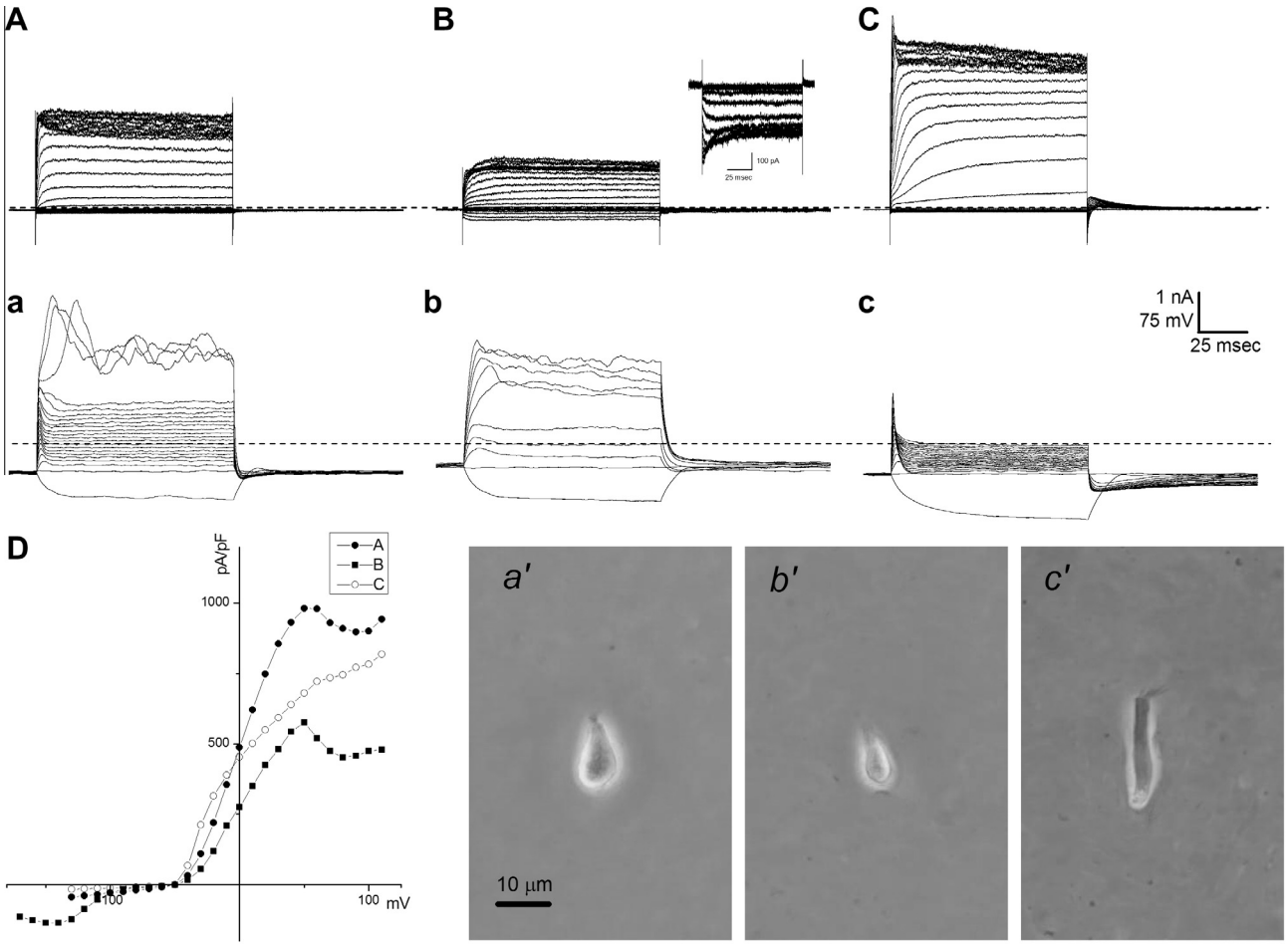

Fig. 1. Patch clamp recordings from three zebrafish hair cells isolated from the lagena. (a′, b′, c′) Photomicrographs of cells used in recordings A, a; B, b; and C, c respectively. (A, B, C) Voltage clamp recordings of currents evoked in response to membrane potential steps of 100-ms duration (without leakage subtraction) delivered in 10 mV increments from −130 mV to +110 mV from a holding potential of −70 mV. Dashed line indicates zero current level. (Inset) Current responses to 10 mV incremental voltage steps to between −170 mV and −80 mV from a holding potential of −70 mV. (a) Current clamp recording of membrane potential changes evoked in response to 100-ms long current injections of between −100 pA and 2 nA delivered in increments of 100 pA. (b) Same as (a), except current steps were from −100 pA to +800 pA. (c) Same paradigm as in (a). Dashed line indicates 0 mV. (D) Plots of currents normalized to cell capacitance vs. voltage taken from recordings of A, B and C.