Temperature Enhances Activation and Inactivation Kinetics of Potassium Currents in Inner Hair Cells Isolated from Guinea-Pig Cochlea

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INTRODUCTION

In the mammalian cochlea, there are two types of hair cells that subserve distinct functions and receive characteristic patterns of innervations. Inner hair cells (IHCs) receive nearly all afferent innervations and are primary acoustic transducers. The three IHC potassium currents are distinguishable by their pharmacology and their activation kinetics [1-3]. The fast activating current, \( I_{K,f} \), is blocked by tetraethylammonium (TEA) but is resistant to 4-aminopyridine (4-AP). \( I_{K,s} \) is activated more slowly on depolarization and is blocked by 4-AP but not by TEA. Another potassium current, \( I_{K,n} \), is already activated at the resting potential of the cell and thus determines the membrane potential and membrane constant. To date, most presently available biophysical studies of IHC potassium currents have been carried out at room temperature. This limits meaningful comparisons between in vivo findings and in vitro experiments such as the whole-cell configuration.

Neurosecretion has been shown to be highly temperature sensitive in various preparations. The kinetics of endocytotic membrane retrieval following transmitter release was described...
as being highly temperature sensitive [4,5]. Similarly in mice IHCs, temperature enhances IHC presynaptic function [6]. The number of readily releasable vesicles available at the active zone is higher at physiological temperatures (35°C-37°C) with no significant change in the rate of sustained release, suggesting that experimental results acquired at room temperature should be scaled up if they are to be related to in vivo findings. Until recently, most patch-clamp recordings in IHCs were performed at room temperature. There are several reports in which experiments were performed in the physiological temperature [1,3], but they did not precisely mentioned about the kinetics of potassium currents. Potassium currents are known to participate in repolarization and discharge behaviors of action potentials in neurons [7,8], so changes in IHC potassium currents at physiological temperatures may cause enhanced IHC presynaptic function.

In the present study, IHCs were isolated from a mature guinea-pig cochlea and potassium currents were recorded at room temperature (around 25°C) and physiological temperatures (35°C-37°C). The effect of temperature on the amplitude of currents and kinetics, i.e., activation and inactivation, were investigated.

MATERIALS AND METHODS

Preparation of isolated IHCs

Adult albino guinea-pigs (200-350 g) with a normal Preyer reflex were killed by rapid cervical dislocation, both bullae were removed, and the cochlea exposed. The cochlea, fused to the bulla, was placed in a Ca²⁺-free external solution (mM: 142 NaCl, 4 KCl, 3 MgCl₂, 2 NaH₂PO₄, 8 Na₂HPO₄, adjusted to pH 7.4 with NaOH). The otic capsule was opened, allowing removal of the organ of Corti attached to the modiolus. The organ of Corti was treated with trypsin (0.5 mg/mL, T-4665, Sigma-Aldrich; replacing 25 mM NaCl in the standard external solution) was applied under pressure (PMI-200, Dagon, Minneapolis, MN, USA) using pipettes with a tip diameter of 2-4 μm positioned around 50 μm from the IHCs. TEA-sensitive currents were reconstructed by subtracting the current recorded in TEA from that recorded in standard saline.

Temperature control

Temperature was adjusted by heating the microscope stage with a dual channel temperature controller (DTC-200, Dagon). Temperature was measured by a miniature thermistor close to the IHC. The influence of temperatures on potassium current properties was first investigated on two different populations of IHCs (at room and physiological temperature), however, to allow direct comparison between room and physiological temperature, the currents of successive changes in temperature (35°C-37°C and 25°C-26°C) in individual cells were recorded. Since the cells were easily moved by convection of heated solution, fixed cells onto the chamber floor were selected. Most experiments were performed in higher to lower temperature to avoid moving the cells by convection by heating.

The temperature dependence of potassium currents was described by its temperature coefficient (Q₁₀), which was calculated from the van’t Hoff equation: \(Q_{10} = (k_2 / k_1)^{(T_2-T_1)/10}\), where \(k_1\) and \(k_2\) are the values of potassium currents measured at lower (T₁) and higher (T₂) temperatures, respectively.

Animal care

The experimental design was reviewed and approved (Accession No. A23-020-0) by the Animal Care and Use Committee, Kyushu University. All procedures were conducted in accordance with the Guidelines for Animal Care and Use Committee, Kyushu University.
RESULTS

Potassium currents at physiological temperature

Currents in response to hyperpolarizing and depolarizing voltage steps from the holding potential of -60 mV were recorded in IHCs. Typical current records at room temperature and physiological temperature (36°C) were shown in Fig. 1. IHCs had outwardly rectifying currents ($I_{K,f}$) in response to depolarizing voltage pulses, with only a slight inward current ($I_{K,n}$) when hyperpolarized. Fig. 1B demonstrated the inward components by enlarging the scale. The amplitude of steady outward currents was not changed with temperature. The outward current at a membrane potential of 110 mV was $9.2 \pm 3.8$ nA ($n=20$) at room temperature and $8.5 \pm 2.6$ nA ($n=11$) at 36°C (Fig. 2A). The amplitude of peak inward currents was not changed with temperature (Fig. 1B). The inward current at a membrane potential of -130 mV was $0.63 \pm 0.37$ nA ($n=22$) at room temperature and $0.68 \pm 0.34$ nA ($n=11$) at 36°C (Fig. 2B). At room temperature, the currents immediately activated but successive rising phases were observed, however, at 36°C, the currents immediately reached the maximum size, suggesting very fast activation (Fig. 1A).

Temperature-dependent activation kinetics

The initial activation phase of outward currents at a membrane potential of 60 mV at 35.8°C and 25.8°C were normalized by the maximum steady currents, and both traces were superimposed in Fig. 3. The rate of activation at 25.8°C was slower than that recorded at physiological temperature (35.8°C). Activation
kinetics is dependent on membrane potentials, becoming faster at more negative potentials. In Fig. 4, half-times ($t_{1/2}$) for activation were measured at room temperature and 36°C, and plotted against various membrane potentials. This approximation is justified since there is a direct relationship between the time constant ($\tau$) and $t_{1/2}$, i.e., $-t_{1/2} = \ln(1- (0.5)^{1/x}) \tau$, where $x=4$ for activation from Hodgkin-Huxley ($n^4$) modeling of potassium currents [11]. The activation rate was faster at 36°C than at room temperature. Statistical analysis (ANOVA) demonstrated significant differences between 36°C and room temperature at membrane potentials of -10, 10, 20, 30, and 40 mV. The normalized value of half-times in activation at 36°C relative to that at room temperature (averaged from -20 to 60 mV) was 1.94, suggesting that $Q_{10}$ was 1.83 assuming a room temperature of 25°C.

**Temperature-dependent inactivation kinetics**

TEA-sensitive potassium currents in IHCs possess the kinetics of fast inactivation [12]. The inactivation phase could be fitted by a single exponential curve and the time constant of inactivation is dependent on membrane potentials, becoming slower at more negative potentials. Fig. 5 showed the inactivation time constants against various test potentials (from -20 to 60 mV) at room temperature and 36°C. The inactivation rate was much faster at 36°C than at room temperature, showing significant differences at all membrane potentials. The normalized value of time constant at 36°C relative to that at room temperature (averaged from -20 to 60 mV) was 3.58, suggesting that $Q_{10}$ was 3.19. The degree of inactivation could be defined by calculating the ratio of the steady-state ($I_{ss}$) to the peak ($I_p$) current ($I_{ss}/I_p$). Fig. 6 showed $I_{ss}/I_p$ against various test potentials at room temperature and 36°C. The degree of inactivation was less voltage dependent and no differences are identified between 36°C and room temperature.

**DISCUSSION**

The amplitude of potassium currents in IHCs showed no temperature dependence either in outward-going $I_{K,f}$, or inward-going $I_{K,n}$, however, activation rates became faster at 36°C than at room temperature (Fig. 4). Inactivation, that was characteristically possessed in TEA-sensitive $I_{K,f}$, was much faster at 36°C than at room temperature (Fig. 5). $Q_{10}$ of activation and inactivation was 1.83 and 3.19, respectively.

A temperature dependency of potassium currents has been described in several cells. In guinea pig ventricular cells, the conductance of inward rectifier potassium currents decreased by lowering the temperature with a $Q_{10}$ of 1.28 (whole cell recordings), or 1.41 (single channel recordings) between 20°C and
30°C [13]. The channel open probability was scarcely affected by temperature, however, the time constant of open-time histogram progressively increased as the temperature was lowered and the configuration of closed-time distribution was markedly modified by changing the temperature. These findings strongly suggested the kinetics of potassium channels were dependent on temperature. In rat skeletal muscle (omohyoid muscle), potassium currents were measured from 1°C to 37°C using the three-microelectrode voltage-clamp technique [14]. The effect of temperature on the activation time constant ($\tau_a$) was much greater in the cold than in the warm: $\tau_a$ had a $Q_{10}$ of nearly 6 at temperatures below 10°C, but a $Q_{10}$ of only ~2 over the range of 30°C-38°C. Voltage-gated potassium channels in human lymphocytes, which are similar to $I_{k,1}$ in IHCs for the sensitivity of TEA, were studied at temperatures from 5 to 42°C [15]. Heating the cell increased the current amplitude at any given membrane potentials between -30 and 30 mV. As for conductance, the channel activation rate was increased by warming the cell and the inactivation rate also progressively increased with increasing temperature. However, in contrast with conductance and activation rates, inactivation rates were most sensitive to temperature changes above room temperature (there was no inactivation below 20°C).

In IHCs, a temperature enhancement of presynaptic function was reported [6]. Readily releasable pool exocytosis (within 20 ms) was increased at physiological temperature with a $Q_{10}$ of 2.1. In spite of the temperature enhancement of fast exocytosis, sustained exocytosis (between 20 and 100 ms) was not significantly different between room and body temperature. Voltage-gated L-type Ca$^{2+}$ channels trigger the release of synaptic vesicles at IHC active zones [16]. An increase in temperature increased the L-type Ca$^{2+}$ current amplitude and accelerated activation kinetics [6]. $Q_{10}$ of amplitude is 1.36 and 1.90 in adult IHCs and immature IHCs, respectively [17]. They assumed that since open channel probability of Ca$^{2+}$ channels become less temperature dependent with maturation, $Q_{10}$ is smaller in adult IHCs than in immature IHCs. Lowering the temperature from ~37°C to room temperature slowed the activation time constant of Ca$^{2+}$ currents by about half [6], suggesting that $Q_{10}$ is around 1.8.

Outward potassium currents in IHCs, such as $I_{k,1}$, are expressed during development of auditory responsiveness [18], and allow the cells to perform high frequency transduction by greatly shortening the membrane time constant [1]. Accelerating potassium current activation by temperature can lead to the faster response of IHCs’ exocytosis mentioned above. Fast potassium currents were about 5 times slower at room temperature than at body temperature and showed a clear delay at onset [1]. $Q_{10}$ for the kinetics of the fast current was estimated as 3.0 on the basis of tail current relaxations. In the present study, $Q_{10}$ was larger for inactivation (3.19) than for activation (1.83). Inactivation of potassium currents are considered to be conferred by many treatments and cytosolic domains, such as intracellular Ca$^{2+}$ [19], cyclic adenosine monophosphate [20], papain [21], trypsin [22], and ‘ball’ peptide [21]. Intracellular agents are predominantly entropy driven, so temperature dependency for the inactivation process may be greater than for the activation process.

**CONFLICT OF INTEREST**

No potential conflict of interest relevant to this article was reported.

**ACKNOWLEDGMENTS**

This work was supported by a Grant-in-Aid for Scientific Research 21592160 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and the Health Labour Sciences Research Grant FAHK230048, FAHK210014 from the Ministry of Health, Labour, and Welfare of Japan.

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