Inhibition of potassium currents in outer hair cells and Deiters’ cells from guinea pig cochlea by linopirdine

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Abstract: To study the functional expression of KCNQ gene in outer hair cells (OHCs) and Deiters’ cells, the effects of linopirdine on the whole cell K⁺ current were investigated by using the whole cell variant of patch clamp technique in the present study. The outward tetraethylammonium (TEA)-sensitive K⁺ current and the inward K⁺ current (I_{Kn}) in OHCs were recorded and measured before and after the administration of linopirdine. Simultaneously, the whole cell currents in Deiters’ cells were also observed in normal solution and in the presence of linopirdine. After the application of 100 µmol/L linopirdine to OHCs, the peak K⁺ current was reversibly blocked and the late K⁺ current was partly reduced. In addition, the decay time constant of the TEA-sensitive K⁺ current was prolonged in the presence of 100 µmol/L linopirdine. The inward current in OHCs was totally inhibited after the superfusion of 100 µmol/L and 200 µmol/L linopirdine respectively. The outward rectifier K⁺ current (I_k) was the dominant K⁺ current in the whole cell currents in Deiters’ cells. In the presence of 200 µmol/L linopirdine, the I_k current was not significantly affected. Our findings demonstrate that the KCNQ heteromeric or homomeric potassium channel is possibly the molecular basis for the peak outward K⁺ current and that the inward I_{Kn} current is mediated by KCNQ potassium channel. KCNQ potassium channel in OHCs can not only permit the K⁺ efflux but also limit the depolarization. In the present study, no expression of KCNQ potassium channel is found in Deiters’ cells.

Key words: KCNQ; potassium channels; outer hair cells; Deiters cells; patch clamp technique; linopirdine
Five members of KCNQ gene family, named KCNQ1-5 respectively have been described so far, of which four members have been identified in inner ear [1,2]. However, only the functional expressions of KCNQ1 and KCNQ4 gene have been demonstrated in cochlea [3,9]. KCNQ1 subunits, together with the smaller subunit minK protein (KCNE1), form a KCNQ1/minK channel in the marginal cells of the stria vascularis and in the vestibular dark cells [53]. KCNQ4 potassium channel is expressed in hair cells and spiral ganglionsof the mammalian cochlea. Mutations in KCNQ4 gene give rise to a form of nonsyndromic hereditary deafness (DFNA2) [1,4,5]. Recent studies indicated the involvement of KCNQ potassium channel in cochlear function and the possible role of KCNQ potassium channel in the K+ recycling in cochlea, which is very important for potassium homeostasis in endolymph [6]. So the expression characteristics and function properties of the KCNQ gene in inner ear are the crucial point to understand the role of these genes in the pathophysiology of progressive hearing loss. It is no doubt that the hair cells in cochlea play the pivotal role in sound transduction [7]. Moreover, recent findings have prompted that Deiters’ cells in cochlea may maintain the homeostasis of the organ of Corti and contribute to the electrical and micromechanical environment of hair cells rather than a simple support for outer hair cells (OHCs) [8,9]. The cochlea OHCs and Deiters’ cells express a wide variety of K+ channels, with the outward tetraethylammonium (TEA)-sensitive K+ currents and the inward K+ currents (I_K) in OHCs and the dominant delayed outward rectifier K+ currents (I_K) in Deiters’ cells, which plays an fundamental physiological function in setting the resting membrane potential and shaping the voltage responses of cells, as well as in the K+ recycling in cochlea [7,10,12]. The outward K+ currents in OHCs had been suggested to mediated by two types of voltage-dependent potassium channels, one is a Ca2+-activated K+ channel with a high conductance, the other is a K+ channel had a low conductance and a small outward rectification [13], whereas, the outward K+ currents in Deiter’s cells was regarded as the Kv1.5 subtype [12]. Although the electrophysiological properties of these K+ channels have been specially characterized, much less is known about their molecular structure.

To explore the functional expression characteristics of KCNQ gene in OHCs and its adjacent supporting Deiters’ cells and the possible molecular basis of the K+ currents in OHCs and Deiters’ cells, the effects of linopirdine, a potent inhibitor in blocking homologues or heterologus KCNQ potassium channel [11], on the whole cell K+ currents in OHCs and Deiters’ cells isolated from guinea pig cochlea were to be observed using the whole cell patch clamp technique in the present study.

1 MATERIAL AND METHODS

1.1 Cells preparation. OHCs and Deiters’ cells were isolated with a standard enzymatic-mechanical procedure as previously described [12,14]. Briefly, adult pigmented guinea pigs (200~300 g) were killed by rapid decapitation, and the bulla was separated and placed in normal external (artificial external lymph) solution. The bone wall of the cochlea was removed and then the basilar membrane was dissected. The organ of Corti was placed in standard external solution containing collagenase [1] (0.5 mg/ml) for 12~15 min at room temperature (20~25ºC). Cells were then isolated and stored in a chamber at room temperature for 30 min. OHCs were identified by its characteristic cylinder shape and stereocilium, and Deiters’ cells, by their comma shape and a characteristic phalangeal process. Several morphological criteria for cells’ viability were used in the experiment: no visible movement of cellular organelles; absence of slight granulation; and the appearance of normal turgor [12,14]. Isolated cells were used in all experiments.

1.2 Recording solutions and chemicals. The standard external solution contained (in mmol/L): 150 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 Glu, and 10 HEPES. The osmolarity of all external solution was adjusted with D-glucose to 300 ± 2 mOsm/kg H2O using a freezing point osmometer. All the external solutions were bubbled continuously with gas containing 95% O2 and 5% CO2 and then the pH was adjusted to 7.4 with 1 mol/L NaOH just before each experiment.

Our standard patch pipette solution for recording K+ currents contained (in mmol/L): 140 KCl, 0.5 CaCl2, 5 MgCl2, 5 Na2ATP, 5 EGTA, and 10 HEPES. The osmolarity of the internal solution was adjusted with D-glucose to 290 ± 2 mOsm/kg H2O and the pH was adjusted to 7.2 with 1 mol/L KOH.

Linopirdine was predissolved in dimethyl sulfoxide (DMSO) and the final concentration of DMSO in the external solution did not exceed 0.1%. TEA was dissolved directly in bath solution. Solutions and drugs were applied to isolated cells via a series of flow pipes positioned close to the chamber and removed by aspiration with a peristaltic pump. The following substances (Sigma Company, USA) were used in the experiment: linopirdine, Na2ATP, HEPES, EGTA, tetraethylammonium (TEA), collagenase IV.
1.3 Electrophysiology and data analysis. The recording chamber containing isolated cells was placed on the stage of an inverted microscope (Zeiss, Axovert-100, Germany) and macroscopic currents were recorded using the conventional whole-cell configuration of the patch-clamp technique. Voltage clamp experiments were employed using an Axon 200A amplifier (Axon Instruments, CA, USA), which connected with a computer equipped with ISO2 software (Germany). Patch pipettes were pulled from quartz glass (Liuhe Laboratory Apparatus Factory, Nanjing, China) using a vertical two-stage puller (Model 700C, David Kopf Instruments, Germany) and had the initial access resistances of 3–5 MΩ, with tip size ranged from 1–1.5 µm. For whole-cell measurements, pipettes were filled with standard internal solution. After the whole-cell configuration had been established, the leakage and slow capacitive currents were not subtracted from currents under voltage clamp, and all voltages have been corrected for liquid junction potential\[15\]. Stimuli and voltage input signal were generated by software ISO2. Voltage-clamp recordings were low-pass filtered at 2 kHz and the recorded signals were stored at hard disk of computer after collection for off-line analysis. Eight outer hair cells and five Deiters’ cells were patched and analyzed.

Data analysis and fitting was performed with the Microsoft Excel 2002 (Microsoft Company, USA) and Origin5.0 (Microcal software, Northampton, USA). Figures were produced by the software of ISO2 and Sigma Plot 2000 (SPSS Inc, Chicago, USA). Results are presented as means±SD. Data were analyzed using Student’s t test. Significance level was taken as P<0.05.

2 RESULTS

2.1 Linopirdine reduced the outward potassium currents in OHCs

The typical outward current in a dissociated OHC (approximately 50 µm in length) in response to a +30 mV depolarizing test pulse from the holding potential of –60 mV was demonstrated in Fig.1a. The macroscopic current trace was composed of two components, one was the peak component and the other was the steady-state component (late current). The outward current was then recorded again in the same OHCs after the superfusion of 100 µmol/L linopirdine (Fig.1c) and 30 mmol/L TEA (Fig.1e) respectively. As shown in Fig.1e, the outward current could be totally blocked by TEA, a potent blocker of various K⁺ channels\[10\], indicating that the outward current was one type of K⁺ current, namely the TEA-sensitive K⁺ currents\[10\]. Linopirdine (100 µmol/L) not only reversibly reduced the late current but also reversibly blocked the peak component, implying the presence of linopirdine-sensitive component in the outward K⁺ current. However, no more decrease of the outward K⁺ current magnitude was found even at a surprising high concentration of linopirdine at 200 µmol/L. Figure 1b shows the block effect of 30 mmol/L TEA on the outward current. Fig. 1. Outward currents elicited by a 30 mV depolarizing test pulse in an isolated OHC (about 50 µm in length). a indicates the outward current in normal solution. b is the current trace after wash-out. The outward current (a) consists of the peak component and the late component. c and d demonstrate the current traces in the presence of 100 µmol/L and 200 µmol/L linopirdine respectively. Note the disappearance of peak component and the reduction of late current. e shows the block effect of 30 mmol/L TEA on the outward current.

A series of test pulses (holding potential –60 mV, from +20 mV to –80 mV, step 10 mV, duration 150 ms) were applied to obtain the whole cell K⁺ currents in OHCs (data not shown). The late currents’ magnitude was measured at the end of each test pulse. As shown in Fig. 3, the outward late K⁺ currents in OHCs after treating with linopirdine
still demonstrated strong outward rectification, suggesting the activation kinetics of the outward late \(K^+\) currents could not be affected by linopirdine. Although the late current amplitude measured at +20 mV test pulse decreased from \((3.13 \pm 0.54)\) nA to \((2.65 \pm 0.41)\) nA \((n=8, r=1.583, P>0.1)\), no statistical difference was found.

2.2 Linopirdine blocked the inward potassium currents in OHCs

The conventional inward potassium current in OHCs (approximately 30 \(\mu\)m in length) could be elicited by a hyperpolarizing voltage step (duration 150 ms) from the holding potential of \(-60\) mV, as shown in Fig.4a. The inward current was regarded as the \(I_{\text{Kn}}\) current, which can be activated by hyperpolarized negative test pulse\(^{10, 11}\). In the presence of 100 \(\mu\)mol/L linopirdine, the inward \(I_{\text{Kn}}\) current was substantially blocked, as shown in Fig.4b. However, at a high concentration of 200 \(\mu\)mol/L linopirdine, no more reduction of the inward \(I_{\text{Kn}}\) current was found.
2.3 Linopirdine had no effect on K⁺ currents in Deiters’ cells

Figure 5 showed the typical whole-cell currents recorded in an isolated Deiters’ cell before and during the application of linopirdine. No obvious inward current was elicited by the −120 mV hyperpolarizing test pulse. The outward current was the potassium current as indicated by the sensitivity to 30 mmol/L TEA (Fig. 5d). Moreover, the outward K⁺ current showed strong outward rectification.
tion since depolarizing steps elicited large current, while hyperpolarizing steps just yielded very small current. Linopirdine had no effect on the outward K⁺ current at the concentration of 100 µmol/L, as shown in Fig. 5b. Whereas linopirdine, at the concentration of 200 µmol/L, could slightly reduce the outward K⁺ current (Fig. 5c). Although, the current amplitude decreased from (5.86 ± 1.67) nA to (5.72 ± 1.41) nA (n=5) measured at +30 mV depolarization voltage potential, no statistical difference was detected by student’s paired t test (t=0.04, P>0.1).

To obtain the whole cell K⁺ currents, a series of voltage potentials from +20 mV to −120 mV, with the holding potential of −60 mV (test step 10 mV and duration 150 ms), were applied to Deiters’ cells (data not shown). The late current’s magnitude was measured and then the current-voltage relation (I-V curve) was plotted in Fig. 6a. The effect of linopirdine (200 µmol/L) on the whole cell K⁺ currents compared with that of TEA were both plotted as I-V curve in Figs. 6b and 6c respectively. Similar to the normal outward K⁺ current, the whole cell K⁺ current in Deiters’ cell after treating with linopirdine still displayed strong outward rectification (Fig. 6b). In summary, all these findings demonstrated that KCNQ potassium channel had no contribution to the outward rectifier K⁺ current in Deiters’ cells.

3 DISCUSSION

Mutations in two genes (KCNQ1 and KCNQ4) encoding potassium channel subunits of the KCNQ family have been found to result in hereditary deafness[1,5]. The expression characteristics and function properties of the KCNQ gene in the inner ear have been investigated by various methods including the immunofluorescence, RT-PCR, in situ hybridization, patch clamp and the gene knockout animal. Expression of KCNQ4 gene has been thought to be restricted to cochlear OHCs, vestibular hair cells, and central auditory neurons[1,4,5]. Recent reports focus on expression of KCNQ4 in IHCs on the basis of in situ hybridization and RT-PCR analysis[3,5]. Other members of the KCNQ family have not been detected unequivocally in the organ of Corti, but a weak RT-PCR signal for KCNQ3 has been reported for cochlear cells[5]. However, no expression of KCNQ gene in Deiters’ cells has been found so far. Considering the lower expression level of the other KCNQ gene and a less dense localization of KCNQ potassium channel within the cells plasma membrane, the functional expression of KCNQ gene in the organ of Corti was studied by observing the block effect of linopirdine on the whole cell K⁺ currents in OHCs and in Deiters’ cells.

Linopirdine had previously been characterized as a potent inhibitor of K⁺ channel belonging to the KCNQ potassium channel at a low concentration of 15 µmol/L[10]. The high concentration at 100 µmol/L of linopirdine could significantly reduce the IKn current in hippocampal neurons and no drug toxicity was found at this concentration[17]. So the high concentration (100 µmol/L) was chosen as the basic concentration in the present study to observe the blocking effect on the whole cell K⁺ currents in OHCs and Deiters’ cells.

Investigations of the K⁺ currents on cochlear OHCs reported that these sensory hair cells were endowed with two voltage-dependent K⁺ currents, one was the outward TEA-sensitive K⁺ current and the other was the inward IKn current. In the present study, we recorded the outward currents in OHCs with the zero-current potential (−61±3.3 mV, n=8) which was close to the theoretical K⁺ equilibrium potential from Nernst equation: EK=(RT/F)×ln([K+]o/[K]i). Moreover, the application of 30 mmol/L external TEA could totally block the outward current. All these kinetic and pharmacological features suggested that the outward current recorded in our experiment was the TEA-sensitive K⁺ current. The inward current elicited by hyperpolarized potential in this work was very similar to the K⁺ current activated at nearly rest potential (IKn) which has been detail described[10,11]. Considering the differential expression of K⁺ current in OHCs[18], the shorter OHCs (about 30 µm) were chosen to record the IKn currents while the longer OHCs (about 50 µm) were used when recording the outward K⁺ currents.

In the present study, we found that linopirdine not only erased the peak component of the outward K⁺ current in OHCs but also diminished the late currents amplitude. However, the reduction effect of linopirdine on the late current was not statistically significant. Exclude for the drug toxicity, the block effect of linopirdine on the outward K⁺ currents in OHCs indicated that there is connection between the KCNQ potassium channel and the peak component of the outward K⁺ currents. Moreover, we also demonstrated that 100 µmol/L linopirdine could totally inhibit the inward IKn currents in all tested OHCs. Since linopirdine was a selective blocker of KCNQ channel, the inhibition of IKn currents by linopirdine was possibly realized by the inhibition of KCNQ channel. This inhibition suggested the KCNQ potassium channel may be the mediator for IKn currents, however, the molecular structure for IKn currents remains to be testified by molecular-biol-
ogy technique. The time and location of $I_{Kn}$ current was firstly detected in the inner ear were consistent with those of KCNQ potassium channel also strongly testified our conclusion[19]. Although the recently cloned KCNQ4 potassium channel was not very sensitive to linopirdine when the homomeric channel was expressed, but a heteromeric channel formed by a combination of KCNQ3/4 was more sensitive to linopirdine[1]. Considering the sensitivity of $K^+$ current to linopirdine, it was strongly suggested that KCNQ potassium channel can be the mediator for inward $I_{Kn}$ currents and the peak outward $K^+$ currents in OHCs. Alternatively, OHCs may express a new type of KCNQ potassium channel or heteromers comprising a KCNQ family member with another class of $K^+$ channels. In summary, the molecular species underlying the OHCs linopirdine-sensitive current remains to be identified, but a KCNQ potassium component is likely to be involved.

A voltage-dependent outward rectifier current ($I_o$) in Deiters’ cells was recorded in the present study. The zero-current potential ($-60 \pm 4.6 \text{ mV}$, n=5) of the current indicated that the current was mainly carried by potassium ion. The electrophysiological characteristics and pharmacological properties hinted that the voltage-dependent outward rectifier current was the major contributor to the outward current in Deiters’ cells[13]. The subtype of $K_v1.5$ channel belonging to the voltage-gated Shaker potassium channel family may be the molecular basis for $I_o$ currents[12]. In this work, we found a slightly decrease of $I_o$ current by linopirdine at a high concentration (200 µmol/L), however, no significant difference was testified. A reasonable explanation for this reduction might be the linopirdine’s toxicity at high concentration. Coupled with the localization of KCNQ potassium channel in cochlea and the insensitivity of the $I_o$ current to linopirdine, it was strongly suggested that KCNQ potassium channel has no contribution to the outward currents in Deiters’ cells.

In the inner ear, the stereocilia of hair cells are bathed in endolymph that has an unusually high concentration of potassium (150 mmol/L K+) with a positive endocochlear potential ($+85 \text{ mV}$). The latter provides a very large driving force for $K^+$ entry into the hair cells. This steep electrochemical gradient dictates the apical $K^+$ channels located at the top of the hair cell stereocillum allows $K^+$ influx and the followed depolarization. The basolateral channels, which are not subjected to the unusual electrochemical gradient, may be responsible for $K^+$ efflux across the basolateral membrane of the hair cells. Our finding demonstrated that the KCNQ potassium channel in OHCs not only mediate the $K^+$ efflux by the outward TEA-sensitive currents but also limit the more depolarization by $I_{Kn}$ currents, which maintained the resting membrane potential in OHCs. Recently, KCNQ-type potassium channel was found to set the resting potential in inner hair cells in mouse cochlea[2]. The effluxed $K^+$ ions from OHCs were taken up by the adjacent supporting Deiters’ cells and then flowed through the gap junction system[20]. The finding in the present study also indicated that the KCNQ potassium channel had no contribution to the $K^+$ efflux in Deiters’ cells.

In conclusion, we found that the KCNQ potassium heteromeric or homomeric channels may be the molecular basis for the peak outward $K^+$ currents in OHCs and testified that KCNQ potassium channel can be the mediator for inward $I_{Kn}$ current. However, we did not find the expression of KCNQ potassium channel in supporting Deiters’ cells in the present study.

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