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Mercury (Hg^{2+}) suppression of potassium currents of outer hair cells

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Abstract

The heavy metal mercury (Hg^{2+}) is an insidious environmental pollutant that causes toxic effects on sensory systems. It is well known that the group IIB divalent cation Hg^{2+} is an inhibitor of the group I monovalent potassium (K^+) cation pore-forming channel in several biological preparations. Here, we used the whole cell patch clamp technique on freshly isolated outer hair cells (OHCs) of the guinea pig cochlea to record outward K^+ currents and inward K^+ currents treated with mercuric chloride ($HgCl_2$). $HgCl_2$ affected K^+ currents in a voltage- and dose-dependent manner. The effects of $HgCl_2$ at 1.0–100 μM are more pronounced on onset peak current than on steady-state end current. $HgCl_2$ depolarized also the resting membrane potential. Although the effect of $HgCl_2$ at 1.0 μM was partially washed out over several minutes, the effects at 10 and 100 μM were irreversible to washout. Since K^+ channels of OHCs are targets for $HgCl_2$ ototoxicity, this may lead to auditory transduction problems, including a loss in hearing sensitivity. A better understanding of fundamental mechanisms underlying K^+ channelopathies in OHCs due to $HgCl_2$ poisoning may lead to better preventive or therapeutic agents.

Keywords: Cochlea; Potassium channels; Outer hair cells; Mercuric chloride ($HgCl_2$); K^+ outward currents; K^+ inward currents

1. Introduction

Neurotoxicity of organic (MeHg) or inorganic (Hg^{2+}) mercury has been well documented in both humans [8,13,14,19,29,32,33,35,48] and experimental animals [1,11,28,29,31,40,47]. The developing premature and immature CNS is more sensitive to damage from MeHg than the adult CNS [8,13]. In general, weeks or months are needed to manifest phenotypic conditions due to Hg^{2+} such as hearing loss, ataxia, weakness and visual and sensory changes from acute or chronic Hg^{2+} poisoning [17,32,33]. The auditory deficit extends almost over the entire frequency range of hearing, and in the most severe instances, Hg^{2+} can lead to clinically significant auditory deficits [4,19,35]. The sensory cochlea and vestibular apparatus of the inner ear containing the hair cells and central auditory structures have been a target of Hg^{2+} ototoxicity [4,9,14,16,17,24,26,35,40,43,47].

In various biological preparations, Hg^{2+} has been shown to increase intracellular free Ca^{2+} [20,42]. Nerve and muscle membranes are also affected by mercuric chloride ($HgCl_2$) and MeHg [3,6,7,12,25,34,39], and voltage-activated calcium [10,28,29,37,41,46], sodium [38], chloride [12,23] and potassium (K^+) [11,15,18,38] channels are similarly suppressed. The GABA receptor channel [5,22,36], the Na, K-ATPase pump and excitatory amino acid receptors [31] have also been the target of Hg^{2+} modulation.

While the effects of Hg^{2+} on several biological preparations have been studied [3,32,33], the effects of Hg^{2+} on patch clamp electrophysiology of outer hair cells (OHCs), however, have not been the subject of attention. Thus, the basis for possible differential modulation by different concentrations of Hg^{2+} on OHCs is unknown. There are several well-known K^+ conductances in OHC of guinea pig and they are believed to play an important role in frequency tuning, subthreshold excitability and maintaining the resting membrane potential [30]. The effects of Hg^{2+} on K^+ channels were of primary interest since the stereocilia of OHCs are bathed in endolymph, which has a high concentration of K^+ ions, and pores conducting K^+ conductances

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are present near the stereocilia. Thus, the whole cell patch clamp technique was employed to identify more precisely the effects of $HgCl_2$ on guinea pig solitary OHC voltage-sensitive (Kv) K^+ channels.

2. Materials and methods

2.1. Dissociation of OHCs

Pigmented adult guinea pigs were rapidly decapitated. The bullae were removed and placed in ice-cold minimum essential medium (MEM). A bulla was opened on ice and the sensory epithelium was gently isolated from the spiral organ of Corti. The cochlear coils were incubated in collagenase (0.125–0.25 mg/ml, type I, Sigma–Aldrich, St. Louis, MO) in MEM for 5–15 min at a room temperature of 23–25 °C. Enzyme use was followed by rinsing the cochlear coils three times using MEM. The OHCs were dissociated mechanically from the sensory epithelium by gently pipetting the media 1 × using a constricted glass pipette. A small amount of the cell suspension was added to 1–2 ml of MEM in the recording chamber. The cells were allowed approximately 5 min to attach to the bottom of the chamber. Most cells obtained were from the middle and apical turns of the cochlea, and the cells ranged in length

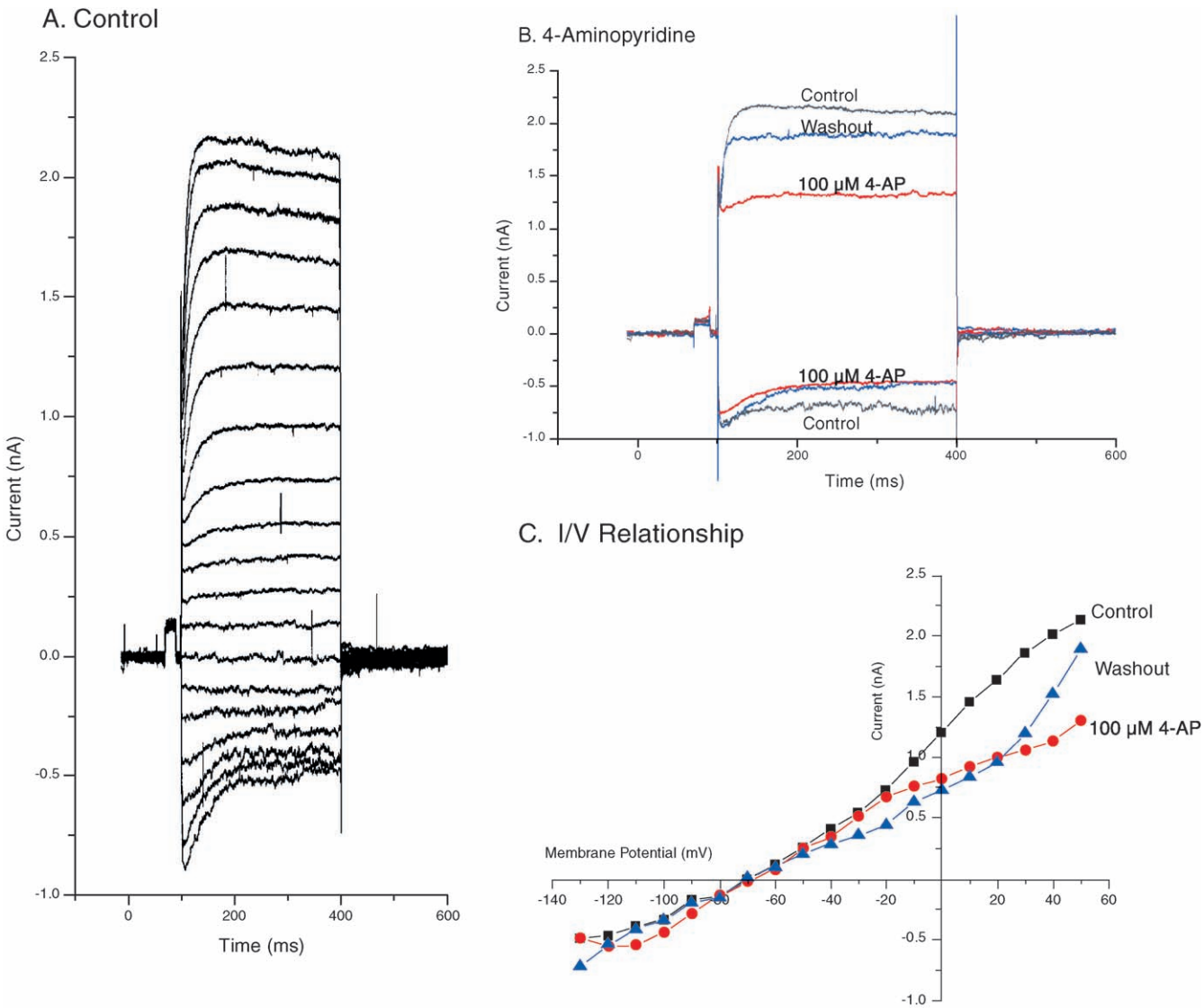

Fig. 1. Voltage-activated K^+ currents (Kv) from a guinea pig OHC. (A) Whole cell membrane currents elicited from a holding potential of −70 mV to potentials stepped from −130 to +50 mV in 10 mV steps. A small leak current of 42 pA at the holding potential is plotted as zero current. The pulse duration was 300 ms. (B) Same cell and same protocol as in (A). For clarity, only two traces, −130 and +50 mV, are shown for each of the conditions. Membrane currents were recorded before (Control, black), during 100 μM 4-AP (red) application and during washout (blue). The typical blocking effect of 4-AP on the outward peak current is demonstrated. A 5 min wash returned the currents close to the control level. (C) Steady-state *I*/*V* plots of the currents of (A) and (B) revealed primarily that the outward currents above −20 mV were decreased by 4-AP in a voltage-dependent manner. A decrease of the currents by 4-AP is indicative of Kv.

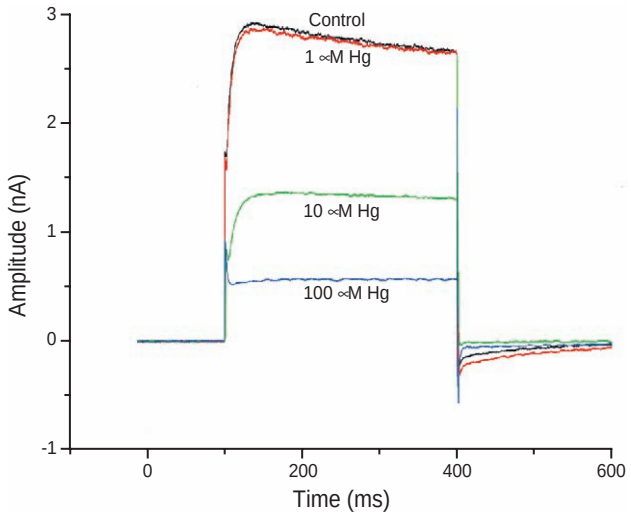

Fig. 2. Effects of $HgCl_2$ on outward K^+ currents ($I_{K(dr)}$ and/or $I_{K(Ca)}$) of a guinea pig OHC. The top trace (black) is a control recording without $HgCl_2$ in the bath. The next three traces of decreasing magnitude are outward K^+ currents recorded in the presence of 1 μM (red), 10 μM (green) or 100 μM (blue) $HgCl_2$.

from ~60 to 80 μm. The second cochlea was kept on ice and processed in a similar manner. All animal procedures were performed in accordance with Swedish Government regulations for the care and use of animals (approval N7c/98).

2.2. Electrophysiologic recordings

Voltage-sensitive K^+ currents were recorded using the whole cell patch clamp technique. Patch pipettes made of 1.5 mm standard wall borosilicate glass capillary tubes (GC150F-10, Clark Electromedical Instruments, Pangbourne, England) were pulled on a two-stage puller (BB-CH, Mecanex, Switzerland) to have a resistance of 8–15 MΩ filled with the internal solution. Voltage clamp command pulses were applied through the pipette and a pellet/3.0 M KCl agar bridge was used as a reference electrode. The pipette potentials were maintained by initially correcting for the liquid junction potential of ~5–6 mV. A patch clamp amplifier (EPC 7, List-Medical, Darmstadt, Germany) recorded membrane currents passing through the pipette. Data were acquired using pClamp 7 (Axon Instruments, Union City, CA) with a 16-bit A/D converter (Axon Instruments). P/4 leak subtraction was used for some of the data and capacitive currents were subtracted manually. Currents were sampled at 10 kHz and filtered with a three-pole Bessell filter from DC −5 kHz.

2.3. External and internal solutions

The external solution (MEM) consisted of (in mM) $CaCl_2{\cdot}2H_2O$ 1.26, KCl 5.37, KH_2PO_4 0.44, $MgCl_2{\cdot}6H_2O$ 0.49, $MgSO_4{\cdot}7H_2O$ 0.40, NaCl 128, $NaHCO_3$ 4.2 and Na_2HPO_4 0.31, buffered with HEPES to pH 7.4. The osmolality of the external solution was approximately 305 mOsm. The calculated equilibrium potential for K^+ (E_K) was −81 mV. In some of the experiments, tetraethylammonium chloride (TEA-Cl, 25 mM), cadmium chloride ($CdCl_2$, 100 μM) or 4-aminopiridine (4-AP, 100 μM) was used to suppress certain K^+ currents sensitive to these compounds. The internal solution consisted of (in mM) KCl 140, HEPES 5.0, EGTA 0.5, $MgCl_2{\cdot}6H_2O$ 2.0, ATP-Na 1.0 and GTP-Na_2 0.1, pH adjusted to 7.3 with 1 N KOH and osmolality of 290 mOsm/kg. Experiments were conducted at a room temperature of 21–23 °C.

2.4. Voltage protocols

Voltage protocol 1: Cells were held (V_H) at a membrane potential of −70 mV, and a 300 ms pulse was stepped to potentials ranging from −130 to +50 mV in 10 mV steps. Each test potential was applied once every 20 ms and stepped back to V_H. Voltage protocol 2: A 300 ms pulse at a V_H of −70 mV was stepped to −130 mV five times and to +50 mV five times, with each pulse separated by a 1.0 min interval.

3. Results

3.1. Confirmation of OHC K^+ currents

All cells used for recording K^+ currents were healthy, exhibited a basally located nucleus and apically located stereocilia, and the cell cytoplasm did not exhibit any Brownian motion. The compounds 4-AP, $CdCl_2$ and TEA were applied to the stable OHC in order to verify the

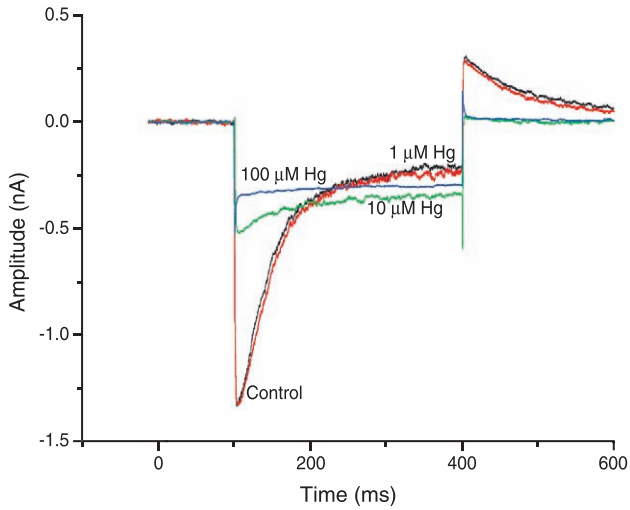

Fig. 3. Effects of $HgCl_2$ on inward K^+ currents ($I_{K(n)}$) of a guinea pig OHC. The Control (black) was recorded without $HgCl_2$ in the bath. The next three traces are inward $I_{K(n)}$ recorded in the presence of 1.0 μM (red), 10 μM (green) or 100 μM (blue) $HgCl_2$.

recording of various K^+ currents. As depicted in Fig. 1A, depolarizing voltage pulses applied from a holding potential of −70 mV and stepped to potentials ranging from −130 to +50 mV elicited large, pronounced slowly developing outward rectified currents that slightly relaxed over the period of the 300 ms pulse. Hyperpolarizing steps exhibited relatively small currents with relaxation at approximately 100 ms of the pulse period to a lower residual noninactivating standing inward current. The compound 4-AP reversibly blocked approximately 40% of the outward currents (Fig. 1B), with less inhibition of approximately 10% of inward going peak onset current and 20% of the steady-state current. The current/voltage relationship (I/V) revealed that only the currents more positive than −40 mV was sensitive to 4-AP (Fig. 1C). The inhibition was returned to near the control values especially at the more positive currents. $CdCl_2$ or TEA suppressed also portions of the outward currents and inward currents (data not shown). Based on these latter observations, we surmised that the outward currents were composed of $I_{K(dr)}$ and $I_{K(Ca)}$, identical to previous K^+ currents recorded from guinea pig OHCs [30]. Similarly, the inwardly going currents were identical to $I_{K(n)}$ [30].

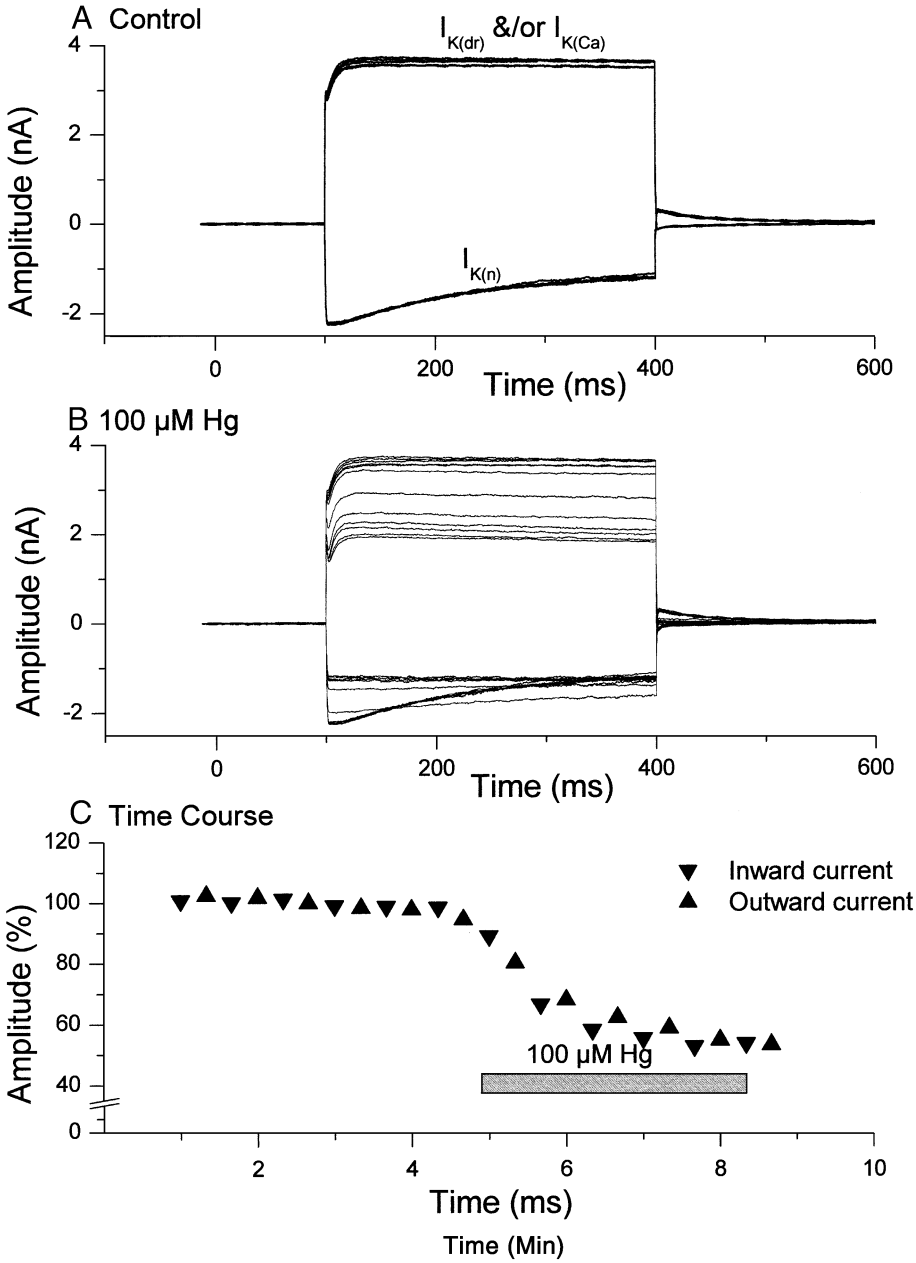

Fig. 4. Time course effects of 100 μM Hg on whole cell current recordings of a guinea pig OHC. (A) Outward K^+ currents ($I_{K(dr)}$ and/or $I_{K(Ca)}$) and inward K^+ currents ($I_{K(n)}$) were activated by protocol 2 (see Section 2) (A and B). (B) A 100 μM $HgCl_2$ bath was applied to the cell and it inhibited $I_{K(dr)}$ and/or $I_{K(Ca)}$ and $I_{K(n)}$ (B and C). $HgCl_2$ primarily reduced the peak $I_{K(n)}$ rather than the steady-state end current. (C) The time course of outward K^+ current inhibition and inward K^+ current inhibition by 100 μM $HgCl_2$.

3.2. Effects of $HgCl_2$ on outward and inward K^+ currents

Fig. 2 depicts the results of outward currents ($I_{K(dr)}$ and/or $I_{K(Ca)}$) in a typical OHC to the application of 1, 10 or 100 μM $HgCl_2$ over the period of the 300 ms pulse. Superfusion of the cell with 1 μM $HgCl_2$ caused only a slight reduction in the onset and steady-state current. There was approximately a 50% reduction of the outward K^+ current in 10 μM $HgCl_2$. The onset peak is slightly more affected than the steady-state current. With the application of 100 μM $HgCl_2$, there was a further decrease of approximately 64% when compared to the control condition. Under this condition, the onset current was practically eliminated. Likewise, there was a corresponding reduction of the tail currents due to the application of various concentrations of $HgCl_2$.

Fig. 3 shows typical results of the inward going currents ($I_{K(n)}$) following the application of 1, 10 or 100 $HgCl_2$ over the stimulus period of 300 ms. For the 1 μM $HgCl_2$ application, the onset peak current was largely unaffected. There was a slight decrease in the inactivation current over the period of the 300 ms pulse, with incomplete inactivation. Application of 10 μM $HgCl_2$ caused approximately a 73% decrease in the peak inward K^+ current, and inactivation was markedly affected (63% decrease). $HgCl_2$ at 100 μM caused an 80% decrease in the onset peak current but a slightly less (22%) effect on the steady-state end current.

3.3. Time course inhibition by 100 μM $HgCl_2$

Fig. 4A exhibits inward currents ($I_{K(n)}$) and outward current ($I_{K(dr)}$ and/or $I_{K(Ca)}$) as produced by potential steps to −130 mV five times and +50 mV five times from a V_H of −70 mV at 1 min intervals. The current traces for the control condition were stable over the period of 300 ms. The inhibition by $HgCl_2$ of the inward currents and outward current was concentration dependent, with no selectivity for either current. $HgCl_2$ at 100 μM rapidly reduced Kv ($I_{K(dr)}$ and/or $I_{K(Ca)}$) as well as $I_{K(n)}$ peak current by 39.73 ± 6.35% and 44.27 ± 7.67%, respectively ($n = 3$ cells) (Fig. 4B).

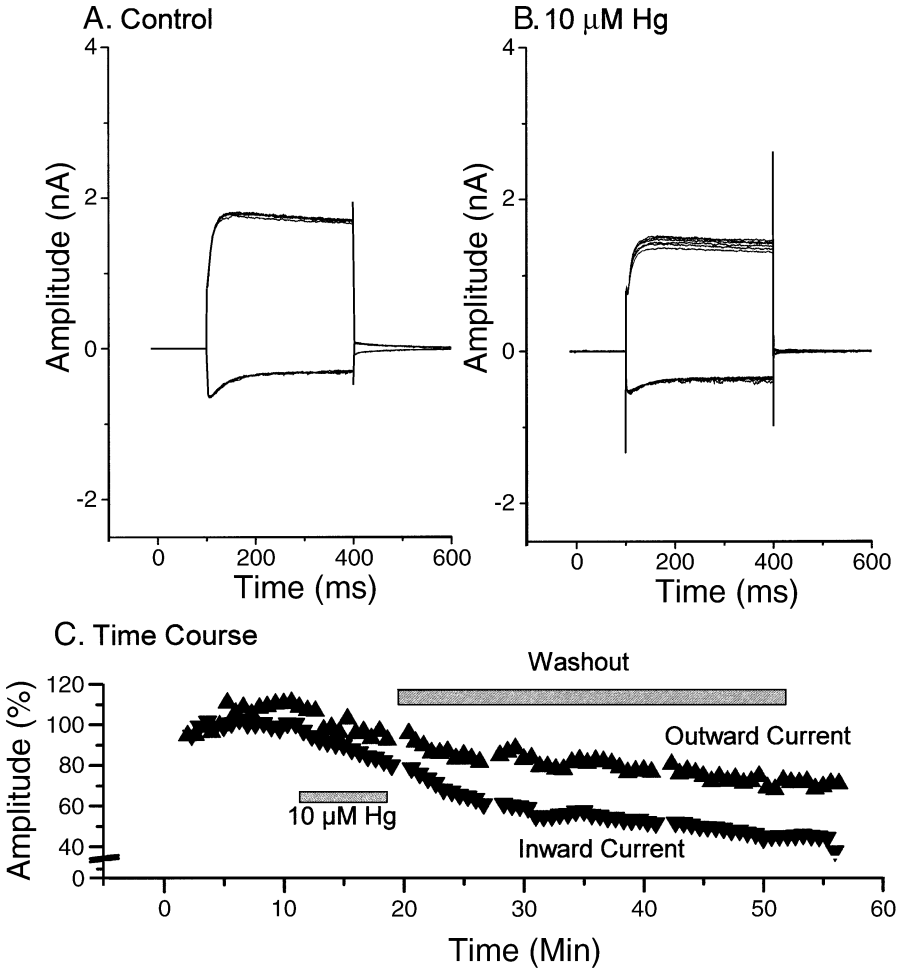

Fig. 5. Time course effects of 10 μM Hg^{2+} on whole cell current recordings of guinea pig OHC. (A) Outward K^+ currents ($I_{K(dr)}$ and $I_{K(Ca)}$) and inward K^+ currents [$I_{K(n)}$] were activated by protocol 2 (see Section 2). (B) A 10 μM $HgCl_2$ bath was applied to the cell. $I_{K(dr)}$ and $I_{K(Ca)}$ and/or $I_{K(n)}$ were partially blocked by 10 μM $HgCl_2$. (C) Time course of outward current inhibition of $I_{K(dr)}$ and/or $I_{K(Ca)}$ by 10 μM $HgCl_2$ and the time course of inward current inhibition of $I_{K(n)}$ by 10 μM $HgCl_2$. A 65 min wash did not return the $HgCl_2$-inhibited current to the control level.

Unlike the control condition, the reduction of the currents over the 300 ms period is quite apparent with the application of $HgCl_2$. The peak current (at the beginning of the pulse) of the inward $I_{K(n)}$ was reduced by $HgCl_2$ considerably more than the steady-state end current (Fig. 4B). The time course of inhibition of $I_{K(n)}$ and/or $I_{K(dr)}$ by 100 μM $HgCl_2$ is illustrated in Fig. 4C. The inhibition of both outward current and inward current is rapid and became stable over the course of the $HgCl_2$ application. OHCs in 100 μM $HgCl_2$ tended to collapse in a short time period, reducing from the control recording time of 38.3 ± 4.18 min (mean ± 1.0 S.E., $n = 3$ cells) to 16.67 ± 2.18 min (mean ± 1.0 S.E., $n = 3$ cells) (41%). The percent reduction for both inward and outward currents in this instance was approximately 41%. A washout condition in two other cells (data not shown) failed to return the currents to their pretreatment condition.

3.4. Time course of inhibition by 10 μM $HgCl_2$

Fig. 5A depicts outward and inward currents in the control condition. The outward current developed over a slow time course and relaxed slightly over the period of the 300 ms pulse. The inward current developed more rapidly and inactivated over the 300 ms pulse. Fig. 5B shows that 10 μM $HgCl_2$ decreased the outward peak and inward peak currents by 22.61 ± 3.74% and 11.96 ± 3.75% in three OHCs, respectively. Thus, the peak inward current was more affected by 10 μM $HgCl_2$ than the inward steady-state current. Compared to Fig. 4, 10 μM $HgCl_2$ gradually reduced the current in a slower time course of 2.8% and 1.35% per minute for the outward and inward currents, respectively (Fig. 5C). The inward currents were substantially reduced more than the outward current (Fig. 5C). A 36 min washout changed the rate of inhibition to 0.79% for the

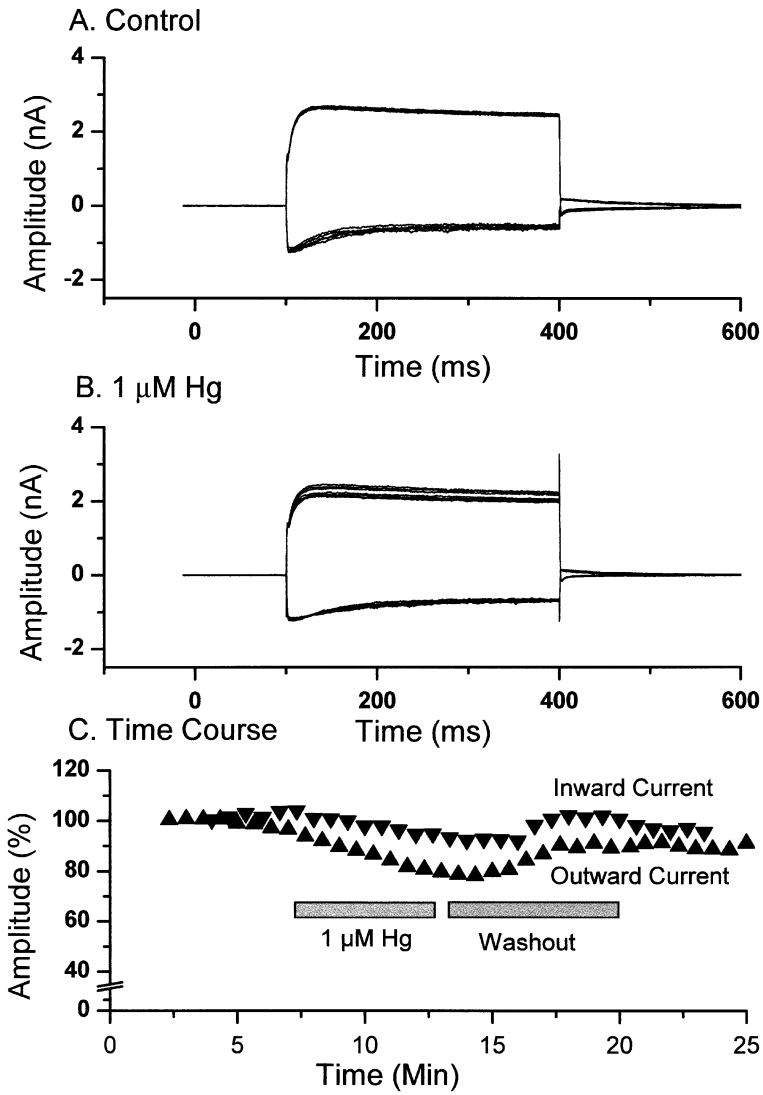

Fig. 6. Time course effects of 1.0 μM Hg on whole cell current recordings of guinea pig OHC. (A) Outward K^+ currents [$I_{K(dr)}$ and/or $I_{K(Ca)}$] and inward K^+ currents [$I_{K(n)}$] were activated by protocol 2 (see Section 2). (B) A 1.0 μM Hg bath was applied to the cell with minimal effect on both $I_{K(dr)}$ and/or $I_{K(Ca)}$ and $I_{K(n)}$. (C) Time course of outward current inhibition of $I_{K(n)}$ and I_K by 1.0 μM Hg.

outward current and 0.36% for the inward current. This is an indication that washout can delay the deterioration of the OHC due to $HgCl_2$ exposure, but the 36 min washout failed to return to its pretreatment level. The time of cell viability in 10 μM was reduced from 38.3 ± 4.18 min for control to 20 ± 4.4 min (mean ± 1.0 S.E., $n = 4$ cells). There was an average of 36% reduction of the outward current and a 52% reduction of the inward current.

3.5. Time course of inhibition by 1.0 μM $HgCl_2$

A control condition is shown in Fig. 6A in which inward and outward currents were elicited using protocol 2 in a single cell. The OHC currents were elicited by 300 ms test pulses from a holding potential of − 70 mV to potentials of − 130 mV five times and to + 50 mV five times, with each pulse separated by a 1.0 min interval. Over the period of the 300 ms pulse, both inward and outward peak currents were slightly decreased by 1.0 μM $HgCl_2$, approximately 5% for the inward currents and approximately 15% for the outward currents (Fig. 6B). Fig. 6C depicts the time course of the effects of 1.0 μM $HgCl_2$ on the outward and inward peak currents. The block was fully reversible in a few minutes for the peak inward current, but not for the peak outward current, and the latter recovered within 10% of the presu-perfusion condition. Thus, although $HgCl_2$ at high concentrations reduced both inward peak and outward peak currents, at a low concentration, $HgCl_2$ primarily reduced the outward currents. In 1.0 μM $HgCl_2$, the recording time was reduced from 32.3 ± 3.18 min for the control condition to 30.6 ± 1.5 min (mean ± 1.0 S.E., $n = 5$ cells).

3.6. Dose–response relations for $HgCl_2$ block

The dose–response relations for $HgCl_2$ block are depicted in Fig. 7 for K^+ outward peak and inward peak currents. The current amplitudes in $HgCl_2$ are expressed as percentages of control (mean ± S.E., $n = 3–4$ cells). The

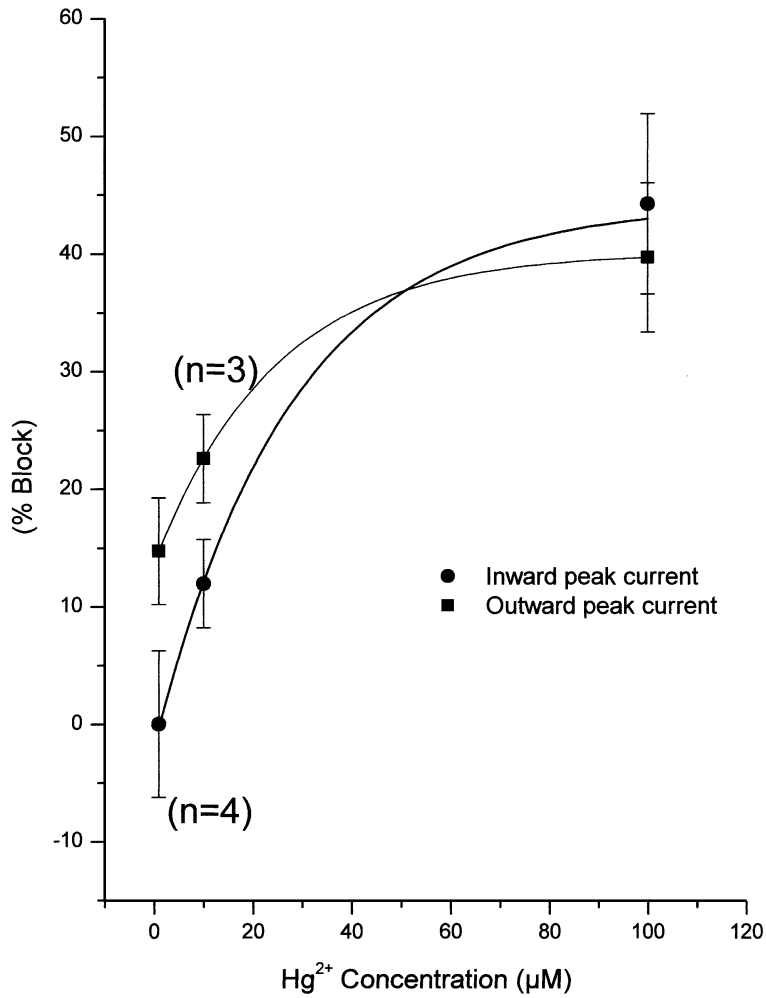

Fig. 7. Dose–response curves for $HgCl_2$ on outward and inward peak K^+ currents in guinea pig OHCs. The $HgCl_2$-blocked current amplitude at the peak of the outward going currents ($I_{K(dr)}$) and the peak of the inward going K^+ currents ($I_{K(n)}$ and/or $I_{K(Ca)}$) are expressed as percent of control (mean ± S.E., $n = 3–4$ cells). The solid lines are functions of best fit by the Boltzmann distribution [$h(\infty) = 1/(1 + \exp((V - Vo)/d)]$, where Vo is the membrane potential, at which $h(\infty) = 0.5$ and d is the slope factor. The IC_{50} for the outward current was 21.5 μM; the IC_{50} for the inward current was 27.2 μM.

solid lines represent the best fit for the Boltzmann distribution $[h(\infty) = 1/(1 + \exp((V - Vo)/d)]$, where Vo is the membrane potential at which $h(\infty) = 0.5$ and d is the slope factor. The average maximum block for the outward component was 39%; the average maximum block for the inward component was 42.5%. The IC_{50} for the outward current was 21.5 ± 1.53 μM (mean ± 1.0 S.E.); the IC_{50} for the inward current was 27.2 ± 0.88 μM (mean ± 1.0 S.E.).

3.7. Effects of $HgCl_2$ on resting membrane potential

Fig. 8 reveals the average resting membrane potential as a function of recording time for control cells ($n = 2$) and cells exposed to $HgCl_2$ at 1.0 μM ($n = 4$), 10 μM ($n = 4$) and 100 μM ($n = 5$). This group of cells ($n = 15$) was not subjected to the two voltage protocols. The control condition was essentially stable over the 20 min period, with a depolarization of the resting membrane of only −5 mV over the last 5 min period. In 1.0 μM $HgCl_2$, the resting membrane was depolarized over the 20 min period to a final value of −13 mV. In 10 μM $HgCl_2$, the membrane was first hyperpolarized and then depolarized to −27 mV. The treatment with 100 μM $HgCl_2$ resulted in a precipitous depolarization to −7 mV over a 15 min recording period. We cannot explain why there is initial hyperpolarization before depolarization with 10 μM $HgCl_2$ or why there is a greater depolarization for 1.0 μM $HgCl_2$ than for 10 μM $HgCl_2$.

4. Discussion

The dominant species of $HgCl_2$ complexes are $HgCl_4^{2-}$ in physiological chloride concentration and Hg^{2+} in extreme low chloride concentration. Hg^{2+} is far less potent than $HgCl_4^{2-}$, indicating that $HgCl_4^{2-}$ plays a major role in $HgCl_2$ neurotoxicity [22]. Another factor worthy of consideration is extracellular pH, well known to modulate the activity of ligand-gated channels [22]. An informative table summarizing Cl^- concentration as a function of pH has been published (Ref. [22], p. 509). These two factors, chloride concentration and pH, are two important factors worthy of consideration to determine the correct species of different solutions. However, since the concentrations of $HgCl_2$ used in the present study were in the micromolar range, and pH of the extracellular solution was maintained

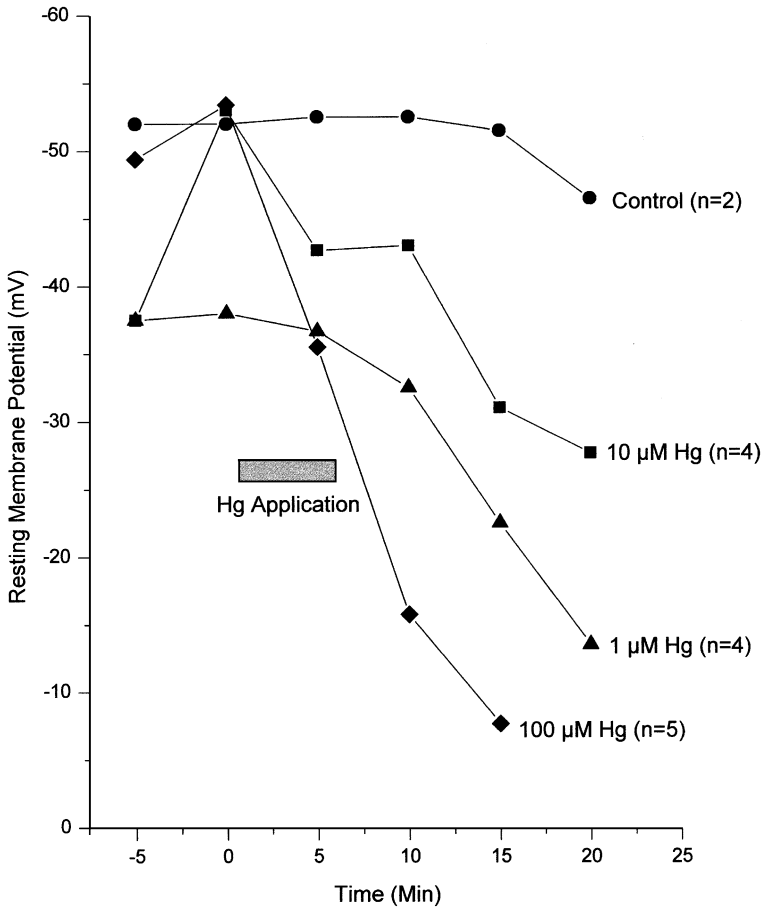

Fig. 8. Effects of $HgCl_2$ on the resting membrane potential. Functions are depicted for Control ($n = 2$ cells, ●), 100 μM Hg ($n = 5$ cells, ◆), 10 μM Hg ($n = 4$ cells, ■) and 1.0 μM Hg ($n = 4$ cells, ▲).

at 7.4, the levels of $HgCl_2$ were not expected to be changed to additional chemical complexes that would have compromised the interpretations of these data [22].

The effects of $HgCl_2$ at the lower concentration of 1.0 was substantially reversed by superperfusion with normal solution, unlike at the 10 and 100 μM concentrations where the effect was irreversible. This is perhaps an indication that the action of $HgCl_2$ on OHCs was not due to a nonspecific effect but would perhaps suggest that the block was caused by sulfhydryl (-SH) oxidation, the important functional group of thiols [3]. Usually, mercurials are effective only when added to the cytoplasmic side of the membrane [25,27]. We observed an effect, however, when $HgCl_2$ was applied to the external face of the hair cell membrane. Reversible block of a calcium-activated nonselective cation channel was observed in brown fat cells by the sulfhydryl reagent $HgCl_2$ as well as by thimerosal [25]. On the other hand, thimerosal completely reversed the effect of $HgCl_2$ in the whole cell recording mode [27]. This is an indication that thimerosal-induced current can be rendered reversible and are not due to irreversible membrane permeability changes, and this may explain the data for the lower concentrations of $HgCl_2$ used in the present investigation. The immediate inhibitory effect of external 1,4-dithiothreitol [21,27] was attributed to an action on the external face of the cell [44], which leads us to arrive at a similar conclusion. It should be noted also that thiols are oxidized to disulfides by oxidizing agents such as $HgCl_2$. Since proteins (hence, K^+ ion channels) contain numerous disulfide bonds, it is likely that at the higher concentrations used in the present investigation, $HgCl_2$ disrupted these links in an irreversible manner; thus, a reduction or a reversal of oxidation was not possible. It therefore follows that OHC homeostasis was perhaps disrupted by the sulfhydryl reducing agent -$HgCl_2$.

We measured the normal membrane potential (V_r) in 10 cells and found the values to range from -35 to -66 mV (mean $= -50.5$ mV, ± 2.96 mV). $HgCl_2$ shifted the V_r to a lower value of 84% at 100 μM and 40% at 1.0 μM. This may indicate that our higher concentrations of $HgCl_2$ were perhaps fundamentally interfering with the normal electrochemical gradient of K^+ ions across the cytoskeletal membrane of the OHCs.

Given that when the V_r is shifted too low for certain excitable cells, it renders the cell incapable of depolarization, evident in the present investigation for certain of our data. In this case, it is likely that $HgCl_2$ in addition to altering the concentration gradient was interfering also with regulation of cell volume [23]. We observed extreme cell swelling under the microscope in the present investigation, especially at the higher concentration of 100 μM. It is likely also that $HgCl_2$ interferes with the electrogenic Na^+, K^+-ATPase pump as well as interferes with the chord conductance as reflected in the transmembrane electrical potential differences of K^+ and Na^+ ions [2]. Alternatively, these data could be explained using the constant field model [2] as a guide to a disruption in normal OHC homeostasis.

The concentrations of $HgCl_2$ used in the present investigation, viz., 1.0–100 μM, decreased the time of viability of our OHCs when compared to that of control cells. This was much more evident at the high concentration of 100 μM than that for the lower dose of 1.0 μM. Most of the in vivo as well as in vitro studies have demonstrated similar abnormal status of biological preparations other than OHCs when exposed to higher levels of mercury concentrations [21,27]. Interestingly, in comparison to clinical data, concentrations of $HgCl_2$ at these levels are not for the most part manifested in large deficits in hearing thresholds shifts [14], although central auditory processing was largely affected by the mercury [35], but unfortunately, the high dose of dimethylmercury exposure in this case proved fatal to the patient.

While the high concentration of $HgCl_2$ used in this study caused outward peak and inward peak currents to decrease, the effect was prolonged and worked gradually as a function of time. One should note that long time washout only partially restored the K^+ currents to their pretreatment values and would suggest that blocking the deterioration of OHCs due to $HgCl_2$ poisoning does not occur immediately. This, in part, accords well with the clinical data from the Iraqi study [8] and the Minamata Bay study [45] of a long-term latent period in which patients exposed to MeHg did not immediately show any phenotypic changes in electrophysiologic measures. The data on the neuromuscular junction, dorsal root ganglion cells, and abdominal ganglia cell, however, exhibited a reversal in blocked activity [5–7].

The present data suggest that $HgCl_2$ is a membrane-permeable compound that navigates the permeation K^+ pore or may cause an alteration in ion–ion interactions within the K^+ pore [18]. One should consider also that the long-term recovery, or lack of, at higher concentrations, could suggest that the $HgCl_2$ works in an "indirect-gated receptor mode" [2]. Given that Hg^{2+} in the micromolar range will gradually decrease the population spike amplitude in membranes [7], one cannot perhaps dismiss the notion that $HgCl_2$ alters OHC membrane depolarization and thus increases the threshold required for cell firing, which may lead to a deficit in hearing sensitivity [4]. However, the central effects of $HgCl_2$ inhibition are perhaps more important than the peripheral effects [14,35].

As can be seen, there are some inexplicable and confusing aspects of these data; that is, lower concentrations of $HgCl_2$ at times caused more of an effect than higher concentrations of $HgCl_2$ in certain instances. Furthermore, there was a differential effect on outward current versus inward current and across various concentrations. This may be related to the small number of cells that were observed under various treatment conditions as well as an inability to hold the cells for extended periods of time for a more complete assessment of their biophysical properties. The most compelling factor is that V_{rest} compromised with various concentrations of $HgCl_2$ did not lead to the most ideal conditions for membrane recordings. Future studies

will be undertaken to attempt to address many of these vexing problems.

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