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Lead (Pb^{2+}) modulation of potassium currents of guinea pig outer hair cells

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Abstract

Outer hair cells (OHC) are mechanosensitive sensory cells of the inner ear cochlea and are involved in modulating the activity of inner hair cells in the transduction of an acoustic stimulus. Potassium (K^+) currents play an important role in the sensory transduction process. K^+ currents were recorded from acutely dissociated OHC obtained from the guinea pig organ of Corti. The whole-cell patch clamp technique was employed. We identified a channel that exhibited outward current of the delayed rectifier type (Kv). Kv channels mediating inward currents carried by potassium ions were also identified and took on the appearance of a previously described inwardly rectifying current. Lead (Pb^{2+}) acetate at concentrations of 0.1, 1.0, 10, and 100 μM was bath applied. Time to activation for outward-going current was not affected by Pb^{2+}. The time course of Pb^{2+} effects was seen as a dose-dependent reduction of K^+ current over time, with very little or no recovery after washout. Pb^{2+} inhibited the outward Kv relative current with values of 0.10, 0.14, 0.18, and 0.30 at 0.1, 1.0, 10, and 100 μM, respectively. Pb^{2+} did not modulate time to activation, peak current, or inactivation of inward I_K. The effects of Pb^{2+} on the potassium currents of OHC are not remarkable and therefore OHC are probably not a major cause of purported peripheral hearing loss observed in Pb^{2+}-exposed animals and humans.
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Keywords: Outer hair cells, OHC; Potassium channels; Outward K^+ current; Inward K^+ current; Lead (Pb^{2+}) acetate; Patch clamp technique

1. Introduction

Lead (Pb^{2+}) is an insidious environmental toxicant that causes irreversible medical health effects, including central auditory processing problems [11,29], cognitive dysfunctioning [5], psychologic and classroom deficits [8,31], changes in auditory evoked potentials [15,22,36], and purported decreased hearing sensitivity [35,41,42,44,49]. A blood lead level (BLL) as low as 0.5 μM (10.34 μg/dl) has been reported to cause decreased hearing sensitivity [44]. It has been shown that the threshold of the maximum voltage of the N_1 peak of the compound action potential (AP) recorded from the auditory nerve in the guinea pig injected with 100 mg/kg of Pb^{2+} was elevated by about 15 dB SPL, and the latency of the N_1 peak of the AP was increased [50]. Contrary to these findings are reports of essentially normal hearing sensitivity in a group of children from the Andes exposed to BLLs of 5.32 μM (110 μg/dl) [7]. Thus, it is not at all clear that Pb^{2+} causes significant peripheral hearing loss from acute or chronic exposure, and if so, the underlying mechanisms at the membrane level responsible for the supposed sensory deficit remain undefined.

In an attempt to better understand basic underlying mechanisms for Pb^{2+} effects at the membrane level, a number of investigations have been conducted on ion channels using several biological preparations [3,4,21,28,30,33,34,37,39,43,48]. K^+ channels have been the focus of several investigations in rat dorsal root ganglia [9], neurons of *Lymnaea stagnalis L* [45], of cloned neurons [24], as well as hippocampal neurons [25,47,52]. K^+ channel kinetics underlie the

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shape, frequency, and duration of ionic conductances in these and a variety of other cells. The effects of Pb^{2+} on K^+ currents of hair cells, however, have not been the focus of any previous investigations. Thus, the basis for acute differential modulation of outer hair cells (OHC) by various concentration of Pb^{2+} is unknown and warrants investigation.

Different types of potassium conductances [delayed rectifier K channels, $I_{K(dr)}$; calcium-activated K channels, $I_{K(Ca)}$; and inward rectifier leak current, $I_{K(n)}$] of OHC of guinea pig have been reported and are purported to play a role in frequency tuning and maintaining the cell resting membrane potential [1,2,16,17,18,26,27]. K^+ channels have been proposed as a drug target in the treatment of myasthenia gravis, multiple sclerosis, Huntington chorea, and Alzheimer disease [40]. Furthermore, the effects of Pb^{2+} on OHC 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 are present near the stereocilia bundle [10]. Thus, this study sought to identify more precisely the effects of lead acetate on guinea pig solitary OHC voltage-sensitive (Kv) potassium channels [i.e., $I_{K(dr)}$ and $I_{K(Ca)}$]. To identify the effects of Pb^{2+} on guinea pig OHC K^+ channels, the whole cell patch clamp technique was employed and the results formed the bases for the present findings.

2. Materials and methods

2.1. Dissociated OHC

Pigmented adult guinea pigs were rapidly decapitated (Animal permit N7c/98, Karolinska Institutet, Stockholm, Sweden). A midline incision was made through the head; the temporal bones were rapidly removed and placed on ice. The bony labyrinth was chipped away to expose the sensory epithelium. The spiral organ of Corti was visualized after removal of the stria vascularis. The sensory epithelium was isolated and incubated in a 150-μl solution using trypsin (0.5 mg/ml, type VI; Sigma, St. Louis, MO) and/or collagenase (0.125–0.5 mg/ml, type I; Sigma) in minimum essential medium (MEM) for 5–15 min at 23–25 °C. Enzyme use was followed by rinsing three times using MEM. The cells were dissociated mechanically from the sensory epithelium by gently pipetting the media using a constricted glass micropipette. A small amount of the cell suspension was added to approximately 0.8 ml of MEM in a recording chamber. The cells were allowed approximately 15 min to attach to the bottom of the chamber as well as recover from the mechanical dissociation. Most cells obtained were from the middle and apical turns of the cochlea, and therefore their cell length ranged from approximately 40 to 90 μm. The mean cross-sectional diameter of the cells was approximately 10 μm, but varied from 9.0 to 13.5 μm in accordance to the decreasing length of the cell. The cells were maintained in good physiological condition by employing a number of standard but significant cell culture practices, such as rapid isolation, dissections on ice, filtration of media and solutions, and maintenance of isotonic tension.

2.2. Electrophysiologic recordings

Ionic currents were recorded using the whole-cell patch clamp technique. Patch pipettes were made from 1.5 mm standard wall borosilicate glass capillary tubes (GC150F-10, Clark Electromedical Instruments, Pangbourne, England). The pipettes were pulled on a two-stage puller (Mecanex, Switzerland) to have a resistance of 8–12 mΩ and a narrow bore of 1.0–3.0 μm. Two recording protocols were employed, both from a holding potential (V_H) of −70 mV. The first protocol consisted of a 300-ms duration pulse that was stepped from V_H to −130 mV a total of five times, separated by an interstimulus interval (ISI) of 60 s. The second protocol was similar to the first protocol except that the pulse was stepped from the V_H to +30 mV a total of five times, with each pulse separated also by an ISI of 60 s. Voltage clamp command pulses were applied through the pipette and a pellet/3.0 M KCl—agar bridge was used as the reference electrode. The pipette potentials were corrected for a liquid junction potential of approximately −7 mV. Membrane current passing through the pipette was recorded by a patch clamp amplifier (EPC 7, List-Medical, Darmstadt, Germany). Data were acquired using software (pClamp 7, Axon Instruments, Burlingame, CA) with a 16-bit A/D converter (Digidata 1200, Axon Instruments). Some fast capacitive transients were subtracted manually if time permitted. Series resistance was compensated for between 70% and 80%. Currents were sampled at 20 kHz (total data throughput) and filtered with a three-pole Bessel filter from DC—5 kHz.

2.3. External and internal solutions

The external solution consisted of (in mM) the following: NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 10, Glucose 10, TTX 0.2 μM, with pH adjusted to 7.4 with 1 N NaOH and osmolality of 300 mOsm/kg. In certain experiments, tetraethylammonium chloride (TEA-Cl) (25 mM), cadmium chloride ($CdCl_2$) (100 μM), or 4-aminopiridine (4-AP) (100 μM) was used to suppress potassium currents sensitive to these compounds in order to verify K^+ currents. The internal solution consisted of (in mM) the following: KCl 140, HEPES 5, EGTA 0.5, $MgCl_2 \cdot 6H_2O$ 2.0, ATP-Na 1.0, GTP-Na_2 0.1, pH adjusted to 7.3 with 1 N KOH, and osmolality of 290 mOsm/kg. All experiments were conducted at a room temperature of 23–25 °C. Individual donor animals contributed no more than one cell per treatment group. The data are therefore based on observations of three to four OHC at 0.1, 1.0, 10, and 100 μM Pb^{2+} that were well clamped and in good physiological condition. Lead acetate [$Pb(C_2H_3O_2)2 \cdot 3H_2O$] was made in double

distilled H_2O as a stock solution of 10 mM and stored at − 20 °C until further use.

3. Results

All cells from which K^+ currents were obtained displayed a basally located nucleus without appreciable blebs, a prominent cuticular plate, were phase bright, exhibited no Brownian motion and possessed a readily identifiable stereocilia bundle. Ohmic leakage current (I_L) of from approximately 50 to 400 pA that shifted the baseline from zero was corrected offline. I_L was present for the much longer hair cells (>70 μm) than the shorter cells (< 40 μm) and as the recording time of the experiments progressed. The maximum time that cells remained completely healthy for recordings was approximately 20–40 min. A compromised cell condition was readily detectable in the form of a more positive resting potential (ranged from − 25 to − 55 mV). All recordings were terminated when V_{rest} declined to ≤ 25 mV, when prominent Brownian motion occurred, a visible swollen membrane occurred, a grossly deformed morphology was present, or the giga-ohm seal was lost.

3.1. Effects of 0.1, 1.0, 10, and 100 μm Pb^{2+}

The effects of 0.1 μM Pb^{2+} (Fig. 1A), 1.0 μM Pb^{2+} (Fig. 1B), 10 μM Pb^{2+} (Fig. 1C), and 100 μM Pb^{2+} (Fig. 1D) on Kv outward currents and a lack of effects on K^+ inward currents are presented. The rapid current spikes at the onset and offset of the voltage pulses at each concentration (except 100 μM Pb^{2+}) are indicative of a mixture of the electromotor activity of the OHC and membrane capacitive transients [1]. In each instance, the outward going current displayed kinetics of the delayed rectifier type (Kv) by its slow onset and its failure to inactivate

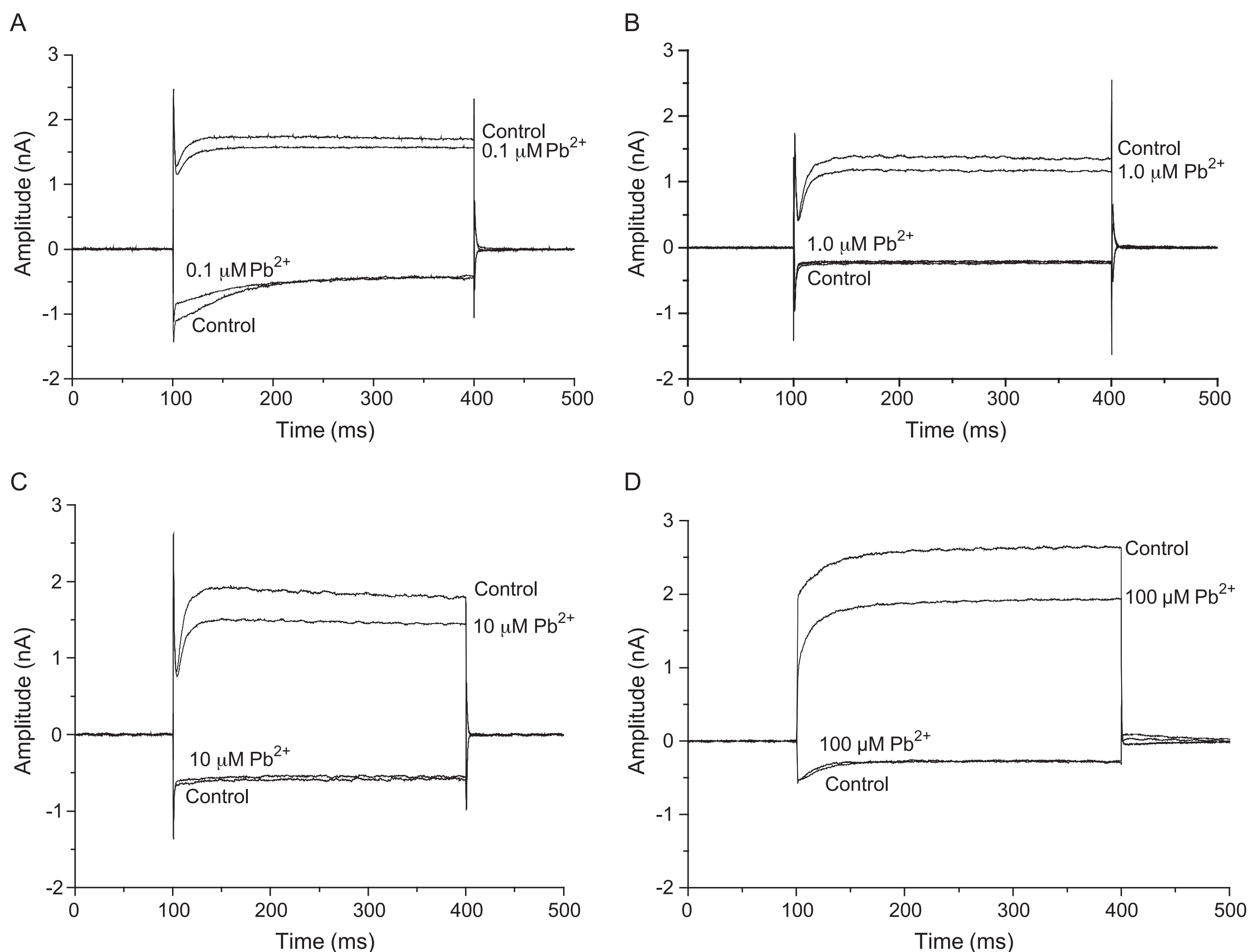

Fig. 1. Potassium currents (Kv) and block by Pb^{2+} elicited by hyperpolarization and depolarizing voltage steps in guinea pig OHC. Voltage steps of 300 ms duration were used at a holding potential of − 70 mV, with voltage stepped to − 130 mV (inward traces) and + 30 (outward traces) mV. (A) Pb^{2+} acetate bath applied at 0.1 μM for 5.0 min. (B) Pb^{2+} acetate bath applied at 1.0 μM for 5.0 min. (C) Pb^{2+} acetate bath applied at 10 μM for 5.0 min. (D) Pb^{2+} acetate bath applied at 100 μM for 5.0 min.

over the period of the 300-ms pulse. The control currents could be partially suppressed with the organic cations of 25 mM TEA or 100 μM 4-AP applied to the bath (four cells, data not shown). The application of 100 μM $CdCl_2$ further suppressed the outward currents suggesting a calcium component (e.g., $I_{K(Ca)}$) as part of these currents (data not shown).

The application of Pb^{2+} applied to the bath was investigated on three or four OHC for each concentration. The cell motility/capacitive spikes, present at the three lowest concentrations, were largely unaffected by the application of Pb^{2+} (Fig. 1). The ratio of the difference between control recordings and the application of Pb^{2+} for the outward current for the individual cells of Fig. 1 was 0.10 at 0.1 μM, 0.14 at 1.0 μM, 0.18 at 10 μM, and 0.30 at 100 μM. Thus, the outward current was inhibited while the inward current due to Pb^{2+} treatment was unaffected. That is, Pb^{2+} only slightly decreased the peak amplitude of the inward current, and the steady state end current amplitude of the inward K^+ current was not inhibited at any of the concentrations. The inward I_L was unaffected by the external application of Pb^{2+}. Pb^{2+} did not shift the reversal potential for any of the Pb^{2+} concentrations tested (data not shown). Given that Pb^{2+} did not affect the inward currents, the time course of these currents will not be displayed.

3.2. Time-dependent effects of Pb^{2+}

The time-dependent effect by which bath-applied 1.0 μM Pb^{2+} inhibited OHC Kv was investigated in three OHC. Fig. 2 shows the time inhibition of the mean outward peak currents including ± 1.0 S.E.M. Control recordings were obtained in which outward currents were recorded to five presentations of the positive pulses at 1-min intervals from a

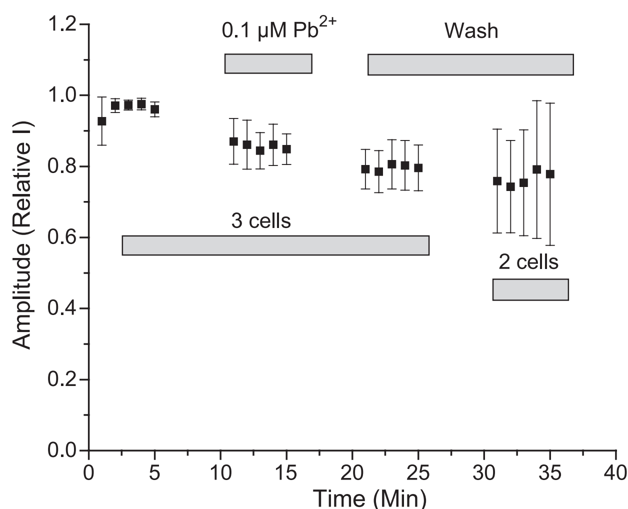

Fig. 2. Time course of 0.1 μM Pb^{2+} to outward Kv peak current of three OHC. The first five data points are control recordings in normal external solution; the second five data points were obtained in the presence of 0.1 μM Pb^{2+}. The final 10 data points were obtained during wash with normal external solution.

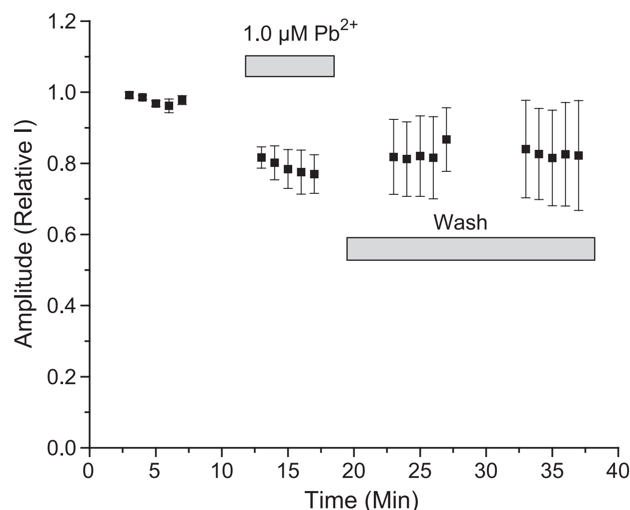

Fig. 3. Time course of 1.0 μM Pb^{2+} to outward Kv peak current of three OHC. The first five data points are control recordings with normal external solution; the second five data points were obtained in the presence of 1.0 μM Pb^{2+}. The final 10 data points were obtained during wash with normal external solution.

V_H of − 70 mV stepped to + 30 mV. This was followed by using the same stimulus protocol in the presence of 0.1 μM Pb^{2+}. Two 5-min washes were employed in an attempt to reverse the effects of Pb^{2+} application. The second wash followed the first one by 6.0 min, in which only two cells were tested, as one cell did not survive the period of the second wash. The inhibition was not reversible with the two consecutive periods of washout with normal external solution. The average fraction of current inhibition for the peak onset current was 0.10 for 0.1 μM Pb^{2+}. The increased variability of the recordings as time increased is reflected by the increased size of the standard error bars.

Fig. 3 shows the mean effects with ± 1.0 S.E.M. of the application of 1.0 μM Pb^{2+} on the outward K^+ currents. The effect of 1.0 μM Pb^{2+} on the peak outward currents over time was 0.14. From the first to the fifth pulse during treatment with Pb^{2+}, the current continued to decrease. Two consecutive washes with normal external solution did not cause appreciable recovery of the amplitude of the current when compared to the control current. Since the outward current contains a mixture of $I_{K(Ca)}$, the Pb^{2+} may be blocking Ca^{2+} entry through the voltage-sensitive calcium channels. Upon the application of Pb^{2+}, the variability of the current recordings increased as reflected in the increased size of the error bars. This was particularly noteworthy for the two 5.0-min washes.

The inhibition effects of adding 10 μM Pb^{2+} to the bath were investigated in four OHC (see Fig. 4). Outward going Kv exhibited an average ratio reduction of 0.18. An initial 5.0-min wash failed to return the currents to the preexposure levels. There was partial recovery of the currents to the second 5.0-min wash. Inactivation kinetics could not be calculated since the duration of the pulses of 300 ms failed to yield any appreciable inactivation.

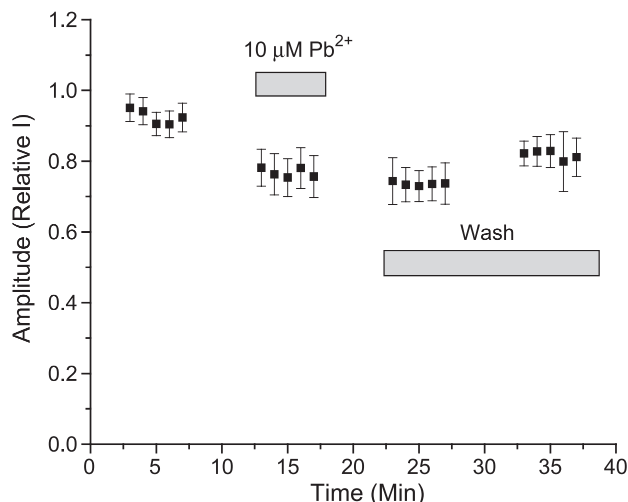

Fig. 4. Time course of 10 μM Pb^{2+} to outward Kv peak current of four OHC. The first five data points are control recordings with normal external solution; the second five data points were obtained in the presence of 10 μM Pb^{2+}. The final 10 data points were obtained during wash with normal external solution.

Fig. 5 displays the effects of bath application of 100 μM Pb^{2+} to three cells. The control recordings reflect very little variability of the five different recordings that established the baseline. When 100 μM Pb^{2+} was applied to the bath, there was an average ratio reduction of 0.30. The variability of the recordings, however, increased with the application of Pb^{2+}. A 5.0-min wash did not return the current amplitude to the preexposure levels, and the variability of the currents increased as reflected in the increased size of the standard error bars.

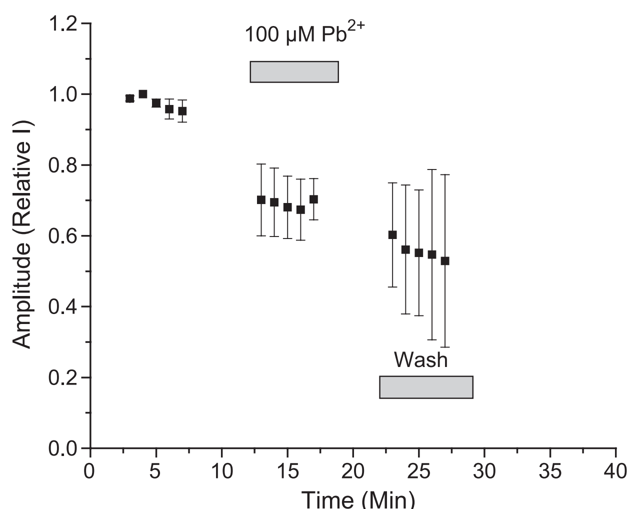

Fig. 5. Time course of 100 μM Pb^{2+} to outward Kv peak current of three OHC. The first five data points are control recordings with normal external solution; the second five data points were obtained in the presence of 100 μM Pb^{2+}. The final 10 data points were obtained during wash with normal external solution.

Table 1
Distribution of statistical values for Pb^{2+} at 0.1, 1.0, 10, and 100 μM

Pb^{2+} μ	$Xc - X_{Pb^{2+}}$	t	df	S.E.	P
0.1	0.10	8.53	4	0.027	0.0010 *
1.0	0.14	11.88	4	0.031	0.0002 *
10.0	0.18	16.13	4	0.023	< 0.0001 *
100	0.30	30.13	4	0.021	< 0.0001 *

Pb^{2+} μ is the concentration of lead, $Xc - X_{Pb^{2+}}$ is control minus the lead treatment, t is the value of the t test, df is degrees of freedom, S.E. is the standard deviation, and P is the two-tailed probability. * Statistical significance beyond the 0.01 level.

3.3. Maximum effects of Pb^{2+} on outward Kv

The maximum inhibition for the amount of Pb^{2+} used in the present investigation was calculated. The current amplitude was expressed as the percentage of the control current (mean ± S.E., $n = 3–4$ cells). The average maximum inhibition for the outward Kv was 30% at 100 μM Pb^{2+}. Given that the maximum current inhibition was less than 50%, an IC_{50} was not computed.

3.4. Statistical analyses

A t test for correlated samples was used to probe for the significance of the difference between the amplitude of the current of the control data and the amplitude of the current of the Pb-treated data at each of the Pb^{2+} concentrations. Table 1 summarizes these data. The Pb-treated inhibitions of the K^+ current were significantly different from the control current at the 0.01 levels for the four concentrations of Pb^{2+} used.

4. Discussion

We have confirmed [1,2] the existence of a distinct Kv channel in guinea pig OHC that generate current of the outward type—the delayed rectifier current with a mixture of calcium-activated components. Channels mediating inward currents carried by potassium channels were also identified and took on the appearance of a fast partial inactivating type and a slow steady state non-inactivating type of the $I_{K(n)}$ type [2,13,16,17,18,23,26,27,32]. Outward going Kv were partially blocked by 25 mM TEA, 100 μM 4-AP, and by 100 μM $CdCl_2$ (data not shown), indicating that the currents obtained were K^+ currents and that parts of the current were Ca activated. These several compounds had no effect on the inward $I_{K(n)}$. Thus, it would appear that the inward $I_{K(n)}$ does not contain a mixture of the Ca-activated component as seen in the outward current.

Cell electromotility has been observed previously to hyperpolarizing and depolarizing pulses [1] and was observed in the present investigation. The electromotility of the cells occurs to the changing electric field [1,6,9] when the cells are stimulated. The motile responses of the cells

were unaffected by Pb^{2+} at any of the concentrations. However, a few cells did not exhibit the motility-dependent variation in cell shortening and lengthening as attributed to the kinetics of K^+ channels [16,26,27] prior to treatment. Quinine, another ototoxic agent with properties similar to heavy metals, applied in vitro on OHC at a concentration of 5.0 mM, substantially decreased active forces of cell elongation and shortening [19]. These results are indicative that Pb^{2+} perhaps has different actions on cell membrane properties and motility mechanisms than that of quinine.

The results suggest that OHC outward components can be slightly modulated by various concentrations of Pb^{2+}. The decrease of outward current ranged from 10% to 30% at 0.1–100 μM. Previous reports have reported that chronic exposure to Pb^{2+}, however, substantially increased calcium currents in both invertebrate and mammalian cells [4]. The inward current was not reduced by Pb^{2+}. This may indicate that separate perhaps independent channels are generating the inward currents when compared to the outward currents. The blocked currents were not totally time-dependent reversible after washing. These results are in agreement with several mammalian studies in which Pb^{2+} action proved irreversible to varying degrees in several biological preparations [33,34,48], but not in agreement with other results of various K^+ currents [9, 24,25,45,47,52].

Kv was recorded in all OHC tested, and it is not at all uncommon that slowly inactivating or noninactivating conductances can be found in the same cell [40]. Kv is more slowly activated, and unless a very long pulse is used in the range of several seconds, it will not inactivate. It is interesting to speculate that Pb^{2+} may be acting on OHC similarly to the effects of the anesthetic agent ether $[(CH_3CH_2)_2O]$ of the *Shaker* mutant in which Kv is reduced, and thus membrane currents become electrically unstable [9,25,52].

Another important consideration of the present data is that our isolation technique is biased toward OHC located from the apical and middle part of the cochlea or the mid- to low-frequency regions of the cochlea. Typically, this is a region that has been for the most part insensitive to most ototoxic agents. To what extent our selection of cells that were biased more toward the apical end of the cochlea can explain the small effects observed here is unknown and will have to await a more extensive in vitro electrophysiological survey of OHC from different regions of the sensory epithelium. Another factor important for consideration is that the observations of the present study were obtained at room temperature. Thus, the small effects of the Pb^{2+} may be explained based on the fact that room temperature is not, of course, the normal physiological condition of the cochlea. These data are consistent, however, with the effects of Pb^{2+} on $I_{K(Ca)}$ since the outward currents contain a mixture of K^+ channel conductances that include the calcium component [37].

While OHC are not directly coupled to inner hair cells, but since they are purported to modulate inner hair cell activity via mechanical properties, it is suggested that the present data may account for the underlying results found for the slight voltage threshold modifications of the compound AP in the guinea pig by Pb^{2+} [50]. That is, a modification of K^+ conductances by Pb^{2+} may interfere with the resting membrane potential, the repolarization phase, and after hyperpolarization of the IHC receptor potentials, perhaps causing an abnormal release of neurotransmitter substance(s) and a subsequent modification of AP transduction in afferent auditory nerve fibers [51]. These results may suggest also that at higher doses, Pb^{2+} molecules may get stuck in the channel responsible for the outward currents and cannot be expelled at least within a reasonable time or expulsion may be voltage dependent, which was not tested under these conditions. The fact that the effects of Pb^{2+} are not completely reversible by washing perhaps is indicative that palliative chelation therapy that prevents or reverses the binding of metallic cations to cellular ligands may not hold excellent promise to prevent or reverse peripheral or central effects of the auditory system due to the effects of Pb^{2+} [20]. The association of BLL to a number of neurodevelopmental conditions [12,14,38,46] should not be minimized, however, in spite of the relatively benign effects of Pb^{2+} observed in vitro on hair cells in the present investigation, albeit hair cells have an epithelial origin and may therefore react differently than nerve cells. However, based on the present observations, we cannot reconcile differences between acute effects of lead on ion channel function as measured here and potential effects of chronic developmental exposure, which occur in the human population as a cause of hearing loss [5,7,29,31].

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