Inhibitory Effects of Heavy Metal Ions on Endolymphatic DC Potential and Cochlear Microphonics in the Guinea Pig

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ABSTRACT. Endocochlear DC potential (EP) and cochlear microphonics (CM) were investigated in the guinea pig under the influence of the following heavy metal ions; manganese, nickel, cobalt, cadmium and lanthanum. The scala tympani and scala vestibuli were perfused with control and test solutions. CM decreased gradually to 50-80%, but EP showed no change after perfusion with a solution containing 1 mM of metal ions. At a concentration of 10 mM, EP decreased from +80 mV to +11-+48 mV and CM decreased to 15-55%. These decreases in EP and CM were irreversible, and perfusion of the area with the standard solution for 20 min had no effect.

Key words: endocochlear DC potential — cochlear microphonics — perilymphatic perfusion — heavy metal ions — Ca channel blocker

Extensive investigations have been carried out on the mechanism of the generation of cochlear endolymphatic DC potential (EP), but its nature is not yet fully understood.[1] It is now generally accepted that a Na^+-K^+ pump located on the basolateral membrane of the marginal cells plays a primary role in the generation of EP. In addition, the active K^+ pump is proposed at the luminal membrane of the marginal cells,[2] although this two pump model hypothesis is opposed to the single pump model hypothesis.[3] Recently, it has been suggested that an active Ca^{2+} pump is located on the same side of the marginal cells as a K^+ pump as well as on Reissner's membrane.[4-7]

If the active transport system in the marginal cells is regulated by intracellular Ca^{2+}, and Ca^{2+} is mediated, at least in part, by voltage dependent Ca^{2+} channels, such as those in excitable or secretory cells, then the application of a Ca^{2+} channel blocker will affect the transport system and produce a resultant change in EP. The previous work[8] in our laboratory indicated that EP was decreased by perilymphatic perfusion of nifedipine, although the concentrations were about 10 times higher than those adopted for experiments on isolated tissues. If there are calcium channels on marginal cells, polyvalent heavy metal ions might affect them. In the present study, EP and cochlear microphonics (CM) were investigated under the influence of these ions, which are competitive Ca^{2+} blockers.[9]

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MATERIALS AND METHODS

The method for recording EP and CM was almost the same as that described by Sato.[8] Briefly, more than 50 guinea pigs (230–400 g) were anesthetized with 30 mg/kg Na-pentobarbital. After the cochlea was exposed, a small hole with a diameter of 50 μm was made in the bony wall of the basal turn with a thin needle, which was revolved by a hand under a binocular microscope. EP was recorded with this hole. Two additional holes which were used as the inlet and outlet for permission, were made on both sides of the first hole along the basal turn. An inlet pipette was inserted into the scala tympani and an outlet hole was scala vestibuli. Special care was taken so that the pipettes fit the holes well. A standard artificial perilymphatic solution containing 130 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 0.1 mM NaH_2PO_4, 10 mM $NaHCO_3$, 5 mM glucose and 10 mM HEPES, adjusted to pH 7.4, was used.

First, a recording microelectrode with the resistance of 3–10 MΩ was inserted into the hole and was advanced by a micromanipulator. Positive potential was detected suddenly when the tip of the electrode reached the scala media. Then, a pipette filled with the standard perilymphatic solution was inserted into the hole of the scala tympani, and the solution was ejected at a flow rate of 10 μl/min. After EP stabilized at a constant level, the perfusion pipette was removed and a cylinder filled with the test solution was inserted into the hole. Then the pipette, this time filled with the test solution was again inserted into the same hole. The test solution was prepared by dissolving La^{3+}, Cd^{2+}, Ni^{2+}, Co^{2+} or Mn^{2+} in the standard perilymphatic solution.

Sound stimuli were applied to the outer ear through a silicon tube. The stimulation sound was a burst of 2,000 Hz with a rise and fall time of 2 msec and a plateau time of 50 msec with a 2 X threshold intensity usually 100 dB. CM and EP were both recorded with the same electrode, with the CM being superimposed over the EP. The peak CM decreased by 5% at most after additional holes, but it did not change further during perfusion with the standard solution. Therefore, any errors in peak CM due to perilymphatic perfusion could be disregarded.

RESULTS

Throughout the following experiments, preparations with an initial EP smaller than +70 mV were discarded. First, the perilymphatic spaces were perfused with the standard solution for several minutes. EP sometimes fluctuated slightly for a few minutes, but it soon became stabilized. Fig. 1 shows that La^{3+} at 1 mM caused a very small decrease in EP. Although the change in EP was not obvious, La^{3+} certainly led to a decrease in CM. All the heavy metal ions examined here attenuated peak CM by 50–80% at a concentration of 1 mM but did not produce a noticeable change in EP. The peak CMs in the solutions with metal ions are shown in Table 1.

The effects of the metal ions on EP and CM were tested at higher concentrations than those adopted for experiments on isolated tissues.

It may be possible that the diffusion of the metal ions to the sensory cells or spiral segment from the scala tympni is slower than that in free solution. To determine the effects of metal ions more rapidly, they were tested at higher

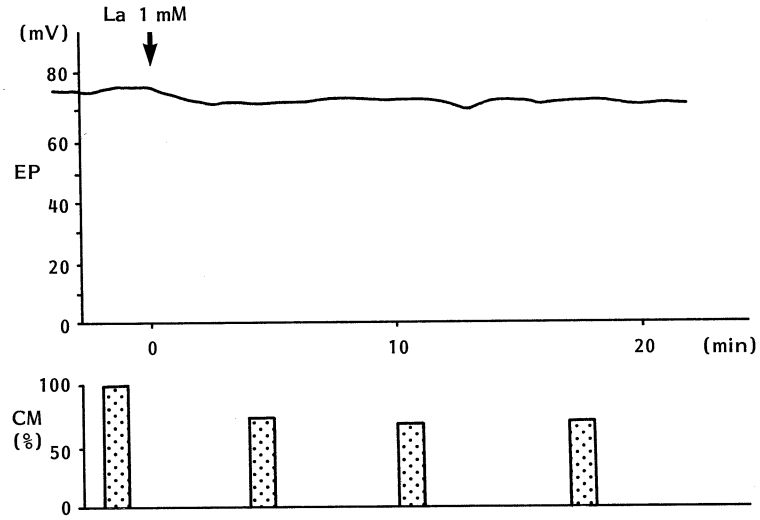

Fig. 1. EP and CM during perilymphatic perfusion with a standard solution containing 1 mM lanthanum. Peak CM amplitudes are indicated by % of the control on the ordinate. The time after the start of perfusion with the La^{3+} solution is scaled on the abscissa.

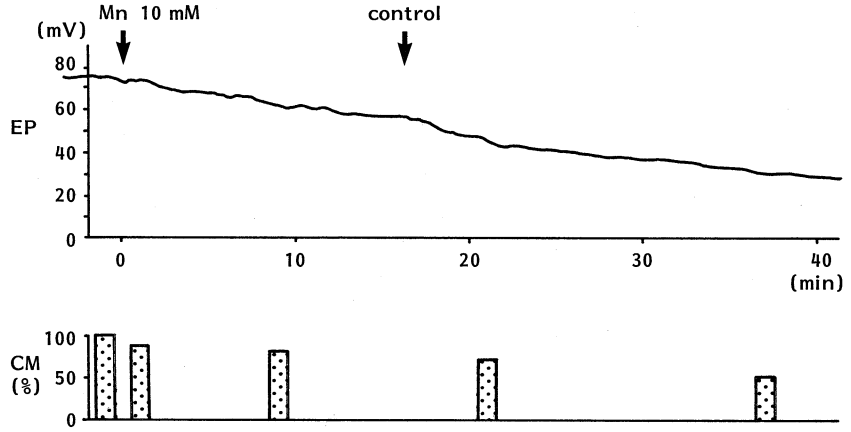

Fig. 2. EP and CM during perilymphatic perfusion with 10 mM manganase. At the time indicated by the first arrow.

TABLE 1. Decrease in CM (%) after 20 min perfusion with 1 mM of metal ions.

Ni^{2+}	Co^{2+}	Mn^{2+}	Cd^{2+}	La^{3+}
63	68	60	50	55
75	70	65	53	60
76	77	80	60	74
71.3±7.2	71.6±4.7	68.3±10.4	54.3±5.1	63.0±9.8

(mean±1SD)

concentrations than those adopted for experiments on isolated tissues. Fig. 2 shows one of the results of consecutive perilymphatic perfusion with 10 mM Mn^{2+} and Fig. 3 shows that with 10 mM Cd^{2+}. EP started to decrease after a latent period of 3 min and it attained a stationary value of 0–+20 mV within 20–60 min. These actions were slow and not completely reversible. The decrease in CM was nearly parallel with the change in EP. These changes are summarized in Table 2.

The effect of 1 mM and 10 mM of metal ions on CM was in the order of $Cd \geqq La^{3+} > Ni^{2+} > Co^{2+} \geqq Mn^{2+}$, while the effect on EP was in the order of

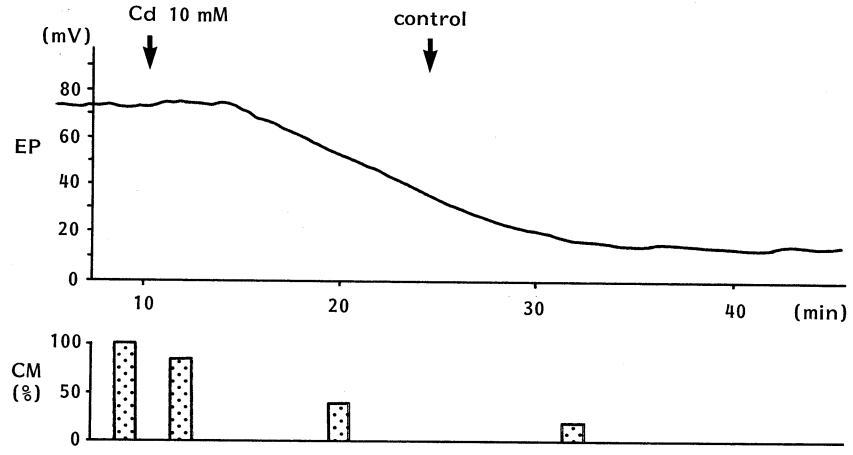

Fig. 3. EP and CM during perilymphatic perfusion with 10 mM cadmium. The decrease in EP was faster than that in the case of Mn^{2+}.

TABLE 2. A 50% decrease in EP (time) and the final decrease in CM (%) with 10 mM of metal ions.

	Ni^{2+}	Co^{2+}	Mn^{2+}	Cd^{2+}	La^{3+}
EP	26	35	24	14	11
	30	40	31	15	15
	48	45	32	20	20
	34.6±11.7	40.0±5.0	29.0±4.4	16.3±3.2	15.3±4.5
CM	15	20	39	20	30
	20	40	50	25	35
	38	55	55	40	40
	24.3±12.1	38.3±17.6	48.0±8.2	28.3±10.4	35.0±5.0

(mean±1 SD)

$La^{3+} > Cd^{2+} > Mn^{2+} > Co^{2+} > Ni^{2+}$. The order could not be determined exactly, but La^{3+} and Cd^{2+} seemed to exhibit stronger actions than Ni^{2+}, Co^{2+} and Mn^{2+}.

DISCUSSION

The present study showed that perfusion of the scala tympani through vestibuli with an artificial perilymphatic solution containing polyvalent metal ions decreased both CM and EP. At a concentration of 1 mM, however, only change in CM could be clearly detected. Marcus[10] reported that perfusion through the scala tympani with 0.5 and 5 mM Ba^{2+} caused elevation of the EP level and a decrease in peak CM. As for a decrease in the peak CM, he noted that Ba^{2+} injected into scala tympani diffused into the scala media across the basilar membrane and reduced the K^+ permeability of the hair cells. The effect of metal ions on CM may be similarly explained, since the metal ions used here are known to not only block inward Ca^{2+} current but also to reduce Na^+- and K^+- permeability. The initial step of depolarization of the hair cells is the entry of Ca^{2+} during cilia movement, followed by K^+ entry through a Ca^{2+}-activated K^+ channel. If K^+ permeability is reduced by metal ions, depolarization in response to the same cilia movement and concomitant peak CM must be decreased.

Sterkers *et al.*[1] indicated that the endolymph originates in the perilymph. The possible pathways from the scala tympani to the scala media are considered to be diffusion across the basilar membrane under Corti's organ, diffusion through the spiral ligament and stria vascularis, and diffusion through the spiral ligament, scala vestibuli and across Reissner's membrane. The very slow time course of the change in CM suggests a long pathway for diffusion from the scala tympani to the scala media.

The decrease in EP during perfusion with 10 mM of metal ions is also explained by their stabilization on the luminal membrane of the marginal cells. According to the single pump hypothesis, the basolateral membrane is not permeable to K^+ and is provided with Na^+-K^+ pump, but the luminal membrane is highly permeable to K^+.[1,11] EP should decrease, if high K^+ permeability is reduced by metal ions. The slow time course of the change in EP suggests that the diffusion pathway should be via the stria vascularis. Marcus *et al.*[11] observed an increase in the EP when the scala tympani was perfused with 0.5–5 mM Ba^{2+}, probably because of the reduction of K^+ permeability in the basolateral membrane of the marginal cells. We hypothesized that the luminal membrane is stabilized, not the basolateral mambrane. The difference in the action of metal ions and that of Ba^{2+} may be ascribed to the difference in the degree of membrane stabilization, that Ba^{2+} is stronger than our metal ions. Actually, if the concentration of metal ions is raised, the EP is elevated transiently.

The decrease in EP might also be due to an inhibition of the pump activities in the stria vascularis or Reissner's membrane by the heavy metal ions. Ahlstrom *et al.*[12] observed the presence of adenylate cyclase in the cochlea, and recently, Doi *et al.*[13,14] reported that an increase in EP was due to stimulation of the Na^+-K^+ pump rather than to a decrease in the negative component of EP. It is considered that cAMP induced phosphorylase activity requires Ca^{2+}, which are stored in intracellular sites, and that intracellularly stored Ca^{2+} is associated with Ca^{2+} entering from the extracellular space.[15] From this standpoint, we assume that Ca^{2+} enter to the marginal cells through voltage dependent channels and that the channels are blocked by heavy metal

ions, as widely noticed in other excitable or secretory cells. Blocking of Ca^{2+} entry leads to exhaustion of the stored Ca^{2+} and consequently, a reduction in cAMP dependent phosphorylation and Na^{+}-K^{+} pump activity, leading to a fall in EP due to a decrease in the positive component of EP.

In the present study, the concentration of the metal ions required to obtain the changes in EP or CM were about 10 times higher than those adopted in experiments on isolated cells. Although the order of the action could not be definitely determined, it was considered to be $La^{3+} > Cd^{2+} > Co^{2+} > Mn^{2+} > Ni^{2+}$. Probably, perilymphatically injected metal ions would be bound to surrounding tissues and free metal ions to demonstrate their effects on the hair cells or the marginal cells may be much less than the injected ones.

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