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Effects of gentamicin and pH on $[Ca^{2+}]_i$ in apical and basal outer hair cells from guinea pigs

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Abstract

Aminoglycosides are widely used antibiotics and frequently produce acute ototoxicity. In this study we attempted to comparatively investigate the effects of gentamicin on Ca^{2+} influx of apical and basal outer hair cells (OHCs) isolated from guinea-pig cochlea. Since the solution of gentamicin sulfate salt is acidic (pH 3.1–3.3), we also explored the effect of external acidification on Ca^{2+} influx. By means of fura-2 microspectrofluorimetry, we measured the intracellular calcium concentration ($[Ca^{2+}]_i$) of OHCs bathed in Hanks' balanced salt solution (pH 7.40) during either a resting state or high K^+-induced depolarization. Our results show that at the resting state, the baseline $[Ca^{2+}]_i$ in apical OHCs (94 ± 2.0 nM) was slightly lower than that in basal OHCs (101.1 ± 2.4 nM). By contrast, the increase in $[Ca^{2+}]_i$ evoked by high K^+ depolarization in apical OHCs was about two-fold greater than that in basal OHCs. Nifedipine (30 μM) abolished the increased $[Ca^{2+}]_i$ in both types of OHCs, suggesting that Ca^{2+} influx was mainly through L-type Ca^{2+} channels of OHCs. While gentamicin and extracellular acidification (pH 7.14) can separately attenuate this increase in $[Ca^{2+}]_i$ in both types of OHCs, their suppressive effects are additive in basal OHCs, but not in apical OHCs. The implications of these findings are that: (1) apical and basal OHCs behave differently in response to depolarization-increased $[Ca^{2+}]_i$, and (2) basal OHCs are more vulnerable to the impairment of Ca^{2+} entry during depolarization by a combination of gentamicin and extracellular acidification, which is correlated with the clinical observation that ototoxicity of aminoglycosides at the basal coil of OHCs is more severe than that at the apical coils. Moreover, the possibility that extracellular acidification may enhance the acute ototoxic effects of aminoglycosides should be considered especially in topical applications.

Key words: Outer hair cell; Gentamicin; Intracellular calcium concentration; Fura-2; Extracellular pH

1. Introduction

Cochlear outer hair cells (OHCs) are known to be the primary targets of ototoxic effects of aminoglycoside antibiotics (Huizing and de Groot, 1987; Govaerts et al., 1990). Previous studies demonstrated that administration of aminoglycosides inhibits Ca^{2+} influx in response to membrane depolarization evoked either by high $[K^+]_o$ (Dulon et al., 1989) or by an electrical current (Nakagawa et al., 1992) in OHCs isolated from guinea pigs, suggesting that this inhibition may be due to antagonistic effects of these compounds on Ca^{2+} channels. This notion has also been confirmed by other investigators (Kroese and van den Bercken, 1982; Takada and Schacht, 1982; Haws et al., 1996). Since intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is important in regulating many cellular functions (Flock et al., 1986; Zenner, 1986; Schacht and Zenner, 1987; Dulon and Schacht, 1992), Ca^{2+} antagonism may provide a possi-

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Abbreviations: OHCs, outer hair cells; $[Ca^{2+}]_i$, intracellular calcium concentration; HBSS, Hanks' balanced salt solution; HB/40, HBSS alone with a pH value of 7.40; NF/40, nifedipine dissolved in HBSS with a pH value of 7.40; GT/14, gentamicin dissolved in HBSS with a pH value of 7.14; GT/40, gentamicin dissolved in HBSS with an adjusted pH value of 7.40; HB/14, HBSS with a pH value of 7.14

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ble mechanism of the acute ototoxic effects of aminoglycosides on OHCs (Corrado et al., 1989). Pathological studies have pointed out that the ototoxicity of aminoglycosides starts at the basal coil of OHCs and then gradually progresses to the apical coil (Tange et al., 1982; Huizing and de Groot, 1987). This observation suggests that OHCs derived from these two different coils of cochlea have different vulnerabilities to ototoxic actions of aminoglycosides. However, whether aminoglycosides may exert different blocking effects on the Ca^{2+} influx in apical and basal OHCs remains to be investigated.

Another problem is that the solution of aminoglycoside sulfate salt has a low pH value (Winkler and Trese, 1992), which is claimed to be capable of exerting acute ototoxic effects (Ikeda and Morizono, 1989). Actually, local application of aminoglycosides has been shown to cause acidification of the tissue fluid of the ears (Ikeda and Morizono, 1989), eyes (Winkler and Trese, 1992), and synovial joints (Lloyd et al., 1988). In OHCs isolated from guinea pigs, external acidification alone may interfere with voltage-sensitive Ca^{2+} channels and decrease the Ca^{2+} influx in response to membrane depolarization evoked by high $[K^+]_o$ (K^+ depolarization) (Ikeda et al., 1991). Therefore, it is possible that the aminoglycoside-induced inhibition of Ca^{2+} influx of OHCs may be complicated by external acidification when these compounds are administered. If so, in addition to Ca^{2+} antagonism of aminoglycosides per se, external acidification resulting from administration of these compounds might also contribute to overall acute ototoxic mechanisms. Nevertheless, experimental evidence to support or repudiate this possibility remains to be established; especially differences in Ca^{2+} influx between basal and apical OHCs in response to external acidification need to be clarified.

The objectives of this study were to investigate and compare (1) the blocking effects of gentamicin on Ca^{2+} influx during a resting condition and in response to K^+ depolarization in apical and basal OHCs isolated from guinea pigs, and (2) the influences of external acidification on these blocking effects in these two types of OHCs.

2. Materials and methods

The use and care of animals reported in this study was approved by National Taiwan University's Animal Care and Use Committee.

2.1. Preparation of isolated OHCs

OHCs were isolated from guinea pig cochleas as described previously (Zajic and Schacht, 1987). In brief, guinea pigs weighing 200–300 g with Preyer's reflexes were decapitated after adequate anesthesia with intraperitoneal injection of pentobarbital (Nembutal, Abbott Laboratories, USA) and their temporal bones were removed from the skull. After the tympanic bulla was opened, the bony capsules of the cochlea were picked away under a stereomicroscope (SMZ-2T, Nikon, Japan) to expose the spiral ligament. The lateral walls of the cochlea were peeled away from the basilar membrane. The organ of Corti in the first and the third coils was dissected and bathed in Hanks' balanced salt solution (HBSS; Gibco-BRL, Life Technologies, USA) containing type IV collagenase (1.0 mg/ml; Sigma, USA). After 10 min of collagenase treatment at 37°C, strips of the organ of Corti were transferred gently with a 50-μl syringe to collagenase-free HBSS. OHCs were mechanically dissociated by gentle trituration. The cells collected from the first and third coils were regarded as apical and basal OHCs, respectively.

2.2. $[Ca^{2+}]_i$ measurements

$[Ca^{2+}]_i$ was measured by a previously described method using microspectrofluorimetry and using fura-2 as the Ca^{2+}-sensitive indicator (Grynkiewicz et al., 1985; Nilles et al., 1994; Yao et al., 1999). In brief, isolated OHCs bathed in HBSS were loaded with 2 μM fura-2 AM (acetoxymethyl ester form, Molecular Probes, USA) for 30 min at room temperature. After washing with HBSS, OHCs were transferred to a 150-μl chamber (9 mm in diameter) mounted on the stage of an inverted microscope (Axiovert 135-TV, Zeiss, Germany). Excitation of fura-2 was at 340 and 380 nm with emitted light monitored at 510 nm. Cell-derived fluorescent images were visualized using a 40×, numerical aperture 1.3 oil immersion objective (Fluar, Zeiss) and were captured with an OlymPix cooled CCD camera (Life Science Resources, UK). The digitized images were calculated for ratio images using the Merlin image processing package (Life Science Resources). Calcium concentration was calculated according to the formula listed as follows (Grynkiewicz et al., 1985): $[Ca^{2+}]_i = K_d[(R-R_{min})/(R_{max}-R)](S_{f2}/S_{b2})$, where R is the measured ratio, K_d is the dissociation constant of fura-2 (200 nM), and S_{f2} and S_{b2} are the emitted fluorescent intensity (510 nm) excited by 380 nm at R_{min} and R_{max}, respectively. The values of R_{max} and R_{min} were obtained from the average value of the calibration procedure using ionomycin (10 μM) and EGTA (5 mM). OHCs with the following criteria were selected for subsequent experiments: (1) cells appeared structurally intact and had no signs of swelling, wrinkled membranes, granular cytoplasm, or high-positioned nuclei; (2) cells had a calcium signal above background as judged by the photomultiplier output; and (3) cells had a stable and rea-

sonable baseline $[Ca^{2+}]_i$ sustained for at least 5 min. Cells not fitting any of these criteria were discarded.

2.3. Testing solutions and agents

HBSS consists of 137 mM NaCl, 5.4 mM KCl, 1.25 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 0.4 mM KH_2PO_4, 0.33 mM Na_2HPO_4, 5.5 mM D-glucose, and 5 mM sodium *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate. HBSS was initially adjusted to pH 7.40 ± 0.01 with NaOH and to 300 ± 2 mOsm with NaCl. All procedures were carried out at room temperature. In this study, adding 1 mM gentamicin (Atlanta, B20292, California, USA) lowered the pH of the HBSS to a value of 7.14. Five types of testing solution were then prepared for experimental interventions: (1) HBSS alone with a pH value of 7.40 (HB/40), (2) nifedipine (a Ca^{2+} channel blocker; 30 μM, RBI, Natick, MA, USA) dissolved in HBSS with a pH value of 7.40 (NF/40), (3) 1 mM gentamicin dissolved in HBSS with a pH value of 7.14 (GT/14), (4) 1 mM gentamicin dissolved in HBSS with an adjusted pH value of 7.40 (GT/40), and (5) HBSS with a pH value of 7.14 (HB/14). Test solutions with high $[K^+]$ were prepared by replacing NaCl with equivalent KCl. Nifedipine was first dissolved in dimethyl sulfoxide (DMSO) and then diluted with HBSS to the desired concentration. The final concentration of DMSO was estimated to be less than 0.1% (nontoxic to OHCs). All solutions were freshly prepared.

2.4. Experimental procedures

In total, 52 apical OHCs and 46 basal OHCs were successfully studied. All OHCs were initially bathed in 150 μl of HBSS with a pH value of 7.40 during the control condition. $[Ca^{2+}]_i$ of each OHC was monitored at least 5 min before and 5 min after the original solution was replaced by any of the test solutions mentioned above. Subsequently, 50 μl of the test solution was drawn out of the chamber and replaced with 50 μl of the same type of test solution containing high $[K^+]_o$. The final $[K^+]_o$ of the test solution inside the chamber after mixing was estimated to be 50 mM. After this procedure, $[Ca^{2+}]_i$ of each OHC was monitored for at least another 6 min. It took approximately 1 min to complete the alternation of the bath solution.

2.5. Statistical analysis

Comparisons of $[Ca^{2+}]_i$ between apical and basal OHCs were made by unpaired *t*-test. Comparisons of $[Ca^{2+}]_i$ before and after exposure to any testing solution were evaluated by paired *t*-test. Comparisons of increases in $[Ca^{2+}]_i$ induced by K^+ depolarization among the five test groups were made by a one-factor analysis of variance followed by Fisher's least significant difference procedure when appropriate. $P < 0.05$ was considered significant. All data are presented as the mean ± S.E.M.

3. Results

In the control resting state, OHCs initially bathed in HB/40 retained their unique morphological features in vitro. Fig. 1 shows fluorescent images on the video monitor of a typical apical OHC (panel A) and a basal OHC (panel B). As shown, these cells appear structurally normal with nuclei near their bases. Apical OHCs (71.51 ± 1.73 μm) were much longer than basal OHCs (42.46 ± 2.06 μm) (Fig. 1), similar to features described previously (Jagger and Ashmore, 1999). Apical OHCs had a baseline $[Ca^{2+}]_i$ of 94.11 ± 2.06 nM (range 74.42–133.63 nM; $n = 52$), which was slightly lower than that of basal OHCs (101.12 ± 2.42 nM; range 75.05–143.77 nM; $n = 46$; $P = 0.029$). Both apical and basal OHCs were randomly assigned to any of the five groups exposed to different test solutions.

The baseline $[Ca^{2+}]_i$ among the five test groups of either apical or basal OHCs studied did not vary significantly (Fig. 2). The original bath solution of these OHCs was then replaced by one of the five types of test solutions: HB/40, NF/40, GT/14, GT/40, or HB/14. Statistical analysis revealed that exposure to the NF/40, GT/14, or GT/40 solutions caused a significant reduction in $[Ca^{2+}]_i$ in both apical and basal OHCs,

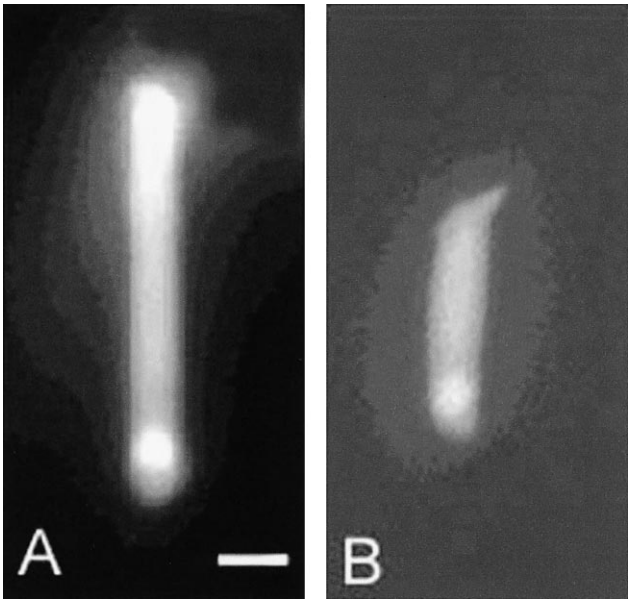

Fig. 1. Typical OHC isolated from the apical turn (A) and the basal turn (B) as described in Section 2. These cells appeared structurally normal with nuclei near their bases. Scale bar = 10 μm.

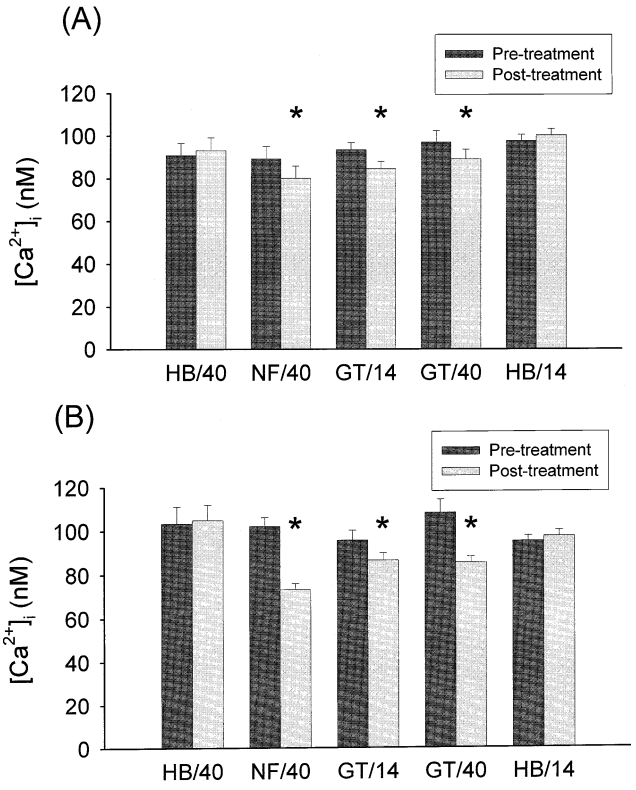

Fig. 2. $[Ca^{2+}]_i$ measured in apical (A) and basal (B) OHCs during the baseline period and after being bathed in various types of testing solutions: HB/40, NF/40, GT/14, GT/40, and HB/14. *Significantly different from baseline (pretreatment) values. Data are presented as the mean ± S.E.M.

whereas exposure to the HB/40 or HB/14 solutions failed to do so (Fig. 2).

These OHCs were subsequently subjected to the challenge of K^+ depolarization. Fig. 3 shows typical tracings of increases in $[Ca^{2+}]_i$ induced by K^+ depolarization in OHCs bathed in the HB/40 solution. As shown, K^+ depolarization induced a marked elevation of $[Ca^{2+}]_i$ of apical OHCs, whereas it caused a more rapid but smaller increase in $[Ca^{2+}]_i$ of basal OHCs. After reaching its peak, $[Ca^{2+}]_i$ in the apical OHC declined gradually toward its baseline, while $[Ca^{2+}]_i$ in the basal OHC declined more rapidly (Fig. 3). Statistical analysis revealed that the peak increase in $[Ca^{2+}]_i$ in response to K^+ depolarization in apical OHCs ($\Delta = 41.6 \pm 3.1$ nM above the pre-depolarization level; $n = 9$) was significantly greater than that in basal OHCs ($\Delta = 20.9 \pm 3.9$ nM; $n = 9$).

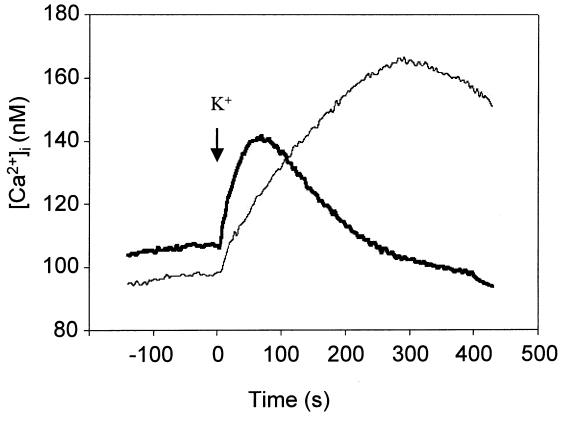

Fig. 3. Typical tracings of increases in $[Ca^{2+}]_i$ in response to depolarization evoked by high $[K^+]_o$ (50 mM) in apical (thin line) and basal (thick line) OHCs bathed in HB/40. $[Ca^{2+}]_i$ was measured with a fura-2 fluorescence ratio imaging technique.

In apical OHCs (Fig. 4A), the increase in $[Ca^{2+}]_i$ in response to K^+ depolarization was totally abolished by the NF/40 solution, but only partially inhibited by the GT/14, GT/40, or HB/14 solutions. Consequently, the peak increase in $[Ca^{2+}]_i$ of apical OHCs bathed in the GT/14, GT/40, or HB/14 solutions was significantly smaller than that in HB/40. These were also significantly greater than that in the NF/40 solution, yet they did not vary significantly among these three groups (Fig. 4A). Similarly, in basal OHCs (Fig. 4B), the in-

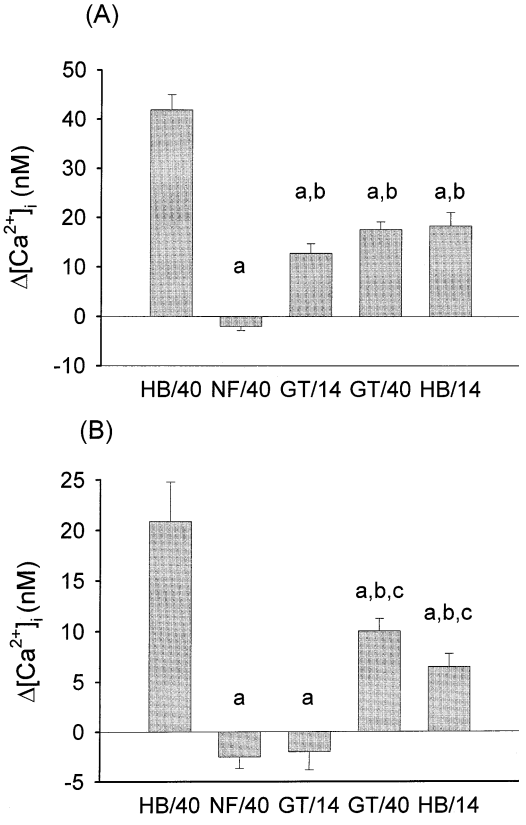

Fig. 4. Changes in $[Ca^{2+}]_i$ in response to depolarization evoked by $[K^+]_o$ (50 mM) in apical (A) and basal (B) OHCs bathed in different solutions. Testing solutions are described in the legend to Fig. 2. [a]Significantly different from the value in the HB/40 group; [b]significantly different from the value in the NF/40 group; [c]significantly different from the value in the GT/14 group. Data are presented as the mean ± S.E.M.

crease in $[Ca^{2+}]_i$ in response to K^+ depolarization was totally abolished when cells were bathed in the NF/40 solution and was attenuated when cells were bathed in the GT/40 or HB/14 solutions. However, in contrast to the situation in apical OHCs, the increase in $[Ca^{2+}]_i$ was completely prevented when cells were bathed in GT/14 (Fig. 4B). Consequently, the peak increase in $[Ca^{2+}]_i$ of basal OHCs bathed in the GT/40 or HB/14 solution was significantly smaller than that in HB/40. These were also significantly greater than that in the NF/40 or GT/14 solutions, yet they did not vary significantly between these two groups (Fig. 4B).

4. Discussion

In this study, we demonstrate that the increase in $[Ca^{2+}]_i$ evoked by K^+ depolarization in apical OHCs is approximately two-fold greater than that in basal OHCs, despite the fact that the baseline $[Ca^{2+}]_i$ in apical OHCs is slightly smaller than that in basal OHCs. This increase in $[Ca^{2+}]_i$ in these two types of OHCs is apparently due to Ca^{2+} entry because it was totally abolished by nifedipine, a voltage-dependent L-type Ca^{2+} channel blocker. This is the first evidence showing that, with respect to Ca^{2+} entry leading to an elevation of $[Ca^{2+}]_i$, apical and basal OHCs respond differently to membrane depolarization evoked by the same stimulus. It is conceivable that factors such as electrophysiological properties of the cell membrane and Ca^{2+} channels may contribute to this difference between these two types of OHCs. For example, although K^+ concentrations in the solutions bathing apical and basal OHCs are the same, it may depolarize the membrane of these two types of OHCs differently. Furthermore, while L-type Ca^{2+} channels have been shown to exist in hair cells (Hudspeth, 1986; Nakagawa et al., 1992; Chen et al., 1995), their features and densities may not be the same in apical and basal OHCs. It is well established that OHCs are not a homogeneous group of cells and may have differences in their characteristics including morphometric features, electrophysiological properties, ionic currents, and physiological functions (Ashmore, 1987; Liu et al., 1997; Nenov et al., 1997; Jagger and Ashmore, 1999). Furthermore, it is also known that $[Ca^{2+}]_i$ is important in OHC motility (Flock et al., 1986; Schacht and Zenner, 1987) and that the shortening of apical OHCs in response to membrane depolarization is much greater than the shortening of basal OHCs (Ashmore, 1987). However, what dissimilarities of characteristics are responsible for the difference in the Ca^{2+} entry during K^+ depolarization between apical and basal OHCs, and whether this difference is related to the distinct physiological functions of these cells remain unclear at present.

We further demonstrate that bath application of a gentamicin solution largely attenuates the increase in $[Ca^{2+}]_i$ during K^+ depolarization in both apical and basal OHCs. This result suggests that gentamicin per se without the low pH factor can impair Ca^{2+} entry in OHCs and confirms the observation reported by Dulon et al. (1989) who also studied the blocking effect of gentamicin on OHCs. Several electrophysiological studies have revealed that membrane Ca^{2+} currents in isolated OHCs (Nakagawa et al., 1992), GH3 pituitary cells (Suarez-Kurtz and Reuben, 1987), frog motor nerve endings (Redman and Silinsky, 1994), and skeletal muscle fibers (Haws et al., 1996) are inhibited by aminoglycosides. It appears that there is an antagonistic relationship between gentamicin and Ca^{2+}. This notion is consistent with observations that the aminoglycoside-induced loss of cochlear microphonics (Takada and Schacht, 1982) and impairment of the mechano-electric transduction process of sensory hair cells (Kroese and van den Bercken, 1982) can be reversed by Ca^{2+}.

On the other hand, bath application of an acidic solution also largely attenuated the increase in $[Ca^{2+}]_i$ during K^+ depolarization in both apical and basal OHCs. This result suggests that extracellular acidification alone can impair Ca^{2+} entry in OHCs and confirms the observations obtained from the study of effects of low pH on OHCs (Ikeda et al., 1991) or on other types of cells (Tombaugh and Somjen, 1996; Zhou and Jones, 1996; Sitges and Rodriguez, 1998). Several investigators (Ikeda et al., 1991; Zhou and Jones, 1996) have proposed that the inactivation of Ca^{2+} channels by extracellular acidification is due to a neutralization of the external surface charge by an increase in extracellular $[H^+]$. Whatever the mechanisms, gentamicin and extracellular acidification seem to act differently on the Ca^{2+} channel. As shown in this study, the baseline $[Ca^{2+}]_i$ of apical or basal OHCs was significantly reduced by bath application of gentamicin with either the pH 7.14 or pH 7.40 solutions, yet was not affected by bath application with the pH 7.14 solution alone. The alternative is that external acidification to pH 7.14 is insufficient to affect the baseline $[Ca^{2+}]_i$ in these OHCs. However, this concentration is in agreement with the finding that external acidification to as low as pH 6.4 still does not affect their baseline $[Ca^{2+}]_i$ (Ikeda et al., 1991). On the other hand, the decrease in the baseline $[Ca^{2+}]_i$ induced by gentamicin was due to the antagonistic effects of gentamicin on spontaneous Ca^{2+} influx in unstimulated OHCs (Dulon et al., 1989). Nifedipine produced a similar inhibition, suggesting that a dynamic activation of the L-type Ca^{2+} channel plays a role in regulating the baseline $[Ca^{2+}]_i$.

Since gentamicin and extracellular acidification can separately attenuate the increase in $[Ca^{2+}]_i$ during K^+ depolarization in OHCs, it is reasonable to speculate as

to whether a combination of these two factors may have additive suppressive effects on OHCs. Indeed, in this study, addition of gentamicin (1 mM) to the bath solution lowered its pH value from 7.40 to 7.14 and additively suppressed $[Ca^{2+}]_i$ elevation in basal OHCs but not in apical OHCs. Addition of aminoglycosides has been shown to induce a concentration-dependent decrease in the pH of solutions (Brown et al., 1990). These results suggest that gentamicin and extracellular acidification have additive inhibitory effects on Ca^{2+} entry during K^+ depolarization only in basal OHCs, but not in the apical OHCs. The mechanisms by which such a difference between these two types of OHCs is produced are obscure. Again, factors such as electrophysiological properties of Ca^{2+} channels may contribute to this difference between these two types of OHCs. For example, although it has not been proven in OHCs, different subtypes of L-type Ca^{2+} membrane channels have been found to exist in cells from other tissues (Garrido et al., 1990; Guo et al., 1995; Watanabe et al., 1998). Hence, it is plausible that gentamicin and extracellular acidification mainly act on the same subtype of L-type Ca^{2+} membrane channels in apical OHCs, while they act on different subtypes in basal OHCs. Consequently, the blocking effect on Ca^{2+} channels induced by a combination of these two factors would be comparable to that induced by either of these two factors in apical OHCs, but would be much greater than that induced by either of them in basal OHCs. Accordingly, a previous study on the heart supports this notion: Name et al. (1989) showed that bath application of a solution either containing an aminoglycoside or with a low pH value depressed the Ca^{2+}-dependent cardiac contractile force, and that the cardiac depressant action of the former was aggravated by the latter.

Apical and basal OHCs bathed in a GT/40 solution displayed $[Ca^{2+}]_i$ responses to K^+ depolarization reaching 48% and 42%, respectively, of their corresponding control responses. In view of these reductions, it appears that the blocking effect of gentamicin per se in apical OHCs is quite similar to that in basal OHCs. However, owing to the fact that the inhibitory effects were additive, basal OHCs became more vulnerable to the suppression of Ca^{2+} entry during K^+ depolarization by a combination of gentamicin and extracellular acidification, as compared to the response of apical OHCs. Several pathological studies (Tange et al., 1982; Huizing and de Groot, 1987) have reported that basal OHCs are more vulnerable to injurious insults by aminoglycosides. Among them, a study (Richardson and Russell, 1991) using mouse cochlear cultures revealed that neomycin rapidly induces morphological damage to OHCs and that basal coil cultures are more sensitive to the insult of neomycin than are those in apical coil cultures. Additionally, the neomycin-induced morphological damage to OHCs can be blocked by excess Ca^{2+}, suggesting that the acute ototoxicity of aminoglycosides is also linked to the antagonistic effects of these compounds on Ca^{2+}. Since $[Ca^{2+}]_i$ is important in regulating and maintaining many cell functions, perhaps our findings may partly explain why there is a difference in the vulnerability to the acute ototoxicity of aminoglycosides.

Application of aminoglycosides with the complication of extracellular acidification is frequently found in clinical practice involving the use of various ototopical medications (Ikeda and Morizono, 1989). Solutions of these antibiotics commonly have a low pH value (Winkler and Trese, 1992). It has been demonstrated that a small amount of neomycin, locally applied to the round window, can gain access to the inner ear (Harada et al., 1986). It has also been shown that local application of an acetic acid solution to the round window membrane results in a lowering of both the endolymph and perilymph pH and a reduction in the endocochlear potential (Ikeda and Morizono, 1989). Previous studies revealed structural damage of the organ of Corti following application of otic drops of aminoglycosides to the middle ear (Harada et al., 1986; Spandow et al., 1988). Based on these findings, we propose that the acute ototoxicity of these ototopical aminoglycoside solutions is due to a synergistic effect of these compounds and their acidic solutions.

In summary, our data show that, although apical OHCs have a smaller baseline $[Ca^{2+}]_i$ than do basal OHCs, the former display a greater increase in $[Ca^{2+}]_i$ in response to K^+ depolarization than do the latter. Furthermore, while either gentamicin or extracellular acidification can attenuate the increase in $[Ca^{2+}]_i$ in response to K^+ depolarization in both types of OHCs, basal OHCs are more vulnerable to the suppressive effects of a combination of gentamicin and extracellular acidification on this increase in $[Ca^{2+}]_i$. This finding provides evidence for the clinical observation that basal OHCs are more susceptible to the acute ototoxic effect of acidic gentamicin solutions than are apical OHCs.

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