Electrophysiological properties of strial pericytes and the effect of aspirin on pericyte K^+ channels

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Abstract. The present study was designed to investigate the electrophysiological properties of strial pericytes and the effect of aspirin on pericyte K^+ channels. Pericytes were identified by determining their morphological characteristics and using pericyte-associated immunofluorescence techniques. The electrophysiological properties of strial pericytes were observed with a whole-cell patch-clamp technique. Alterations in the outward current of cochlear pericytes in the stria vascularis of guinea pigs were examined following the application of K^+ channel retardants. The effects of aspirin on pericyte K^+ channels were also evaluated with the whole-cell patch-clamp technique. The results demonstrated that pericytes were desmin positive, and their nuclei were large and surrounded by a small proportion of the cytoplasm. Cytoplasmic processes gradually declined in size as branches grew parallel to the capillary axis. Thus, capillaries were surrounded by tips. The electrophysiological properties of the cochlear pericytes in the stria vascularis of guinea pigs were also determined. The membrane capacitance of the pericytes was 5.9±0.3 pF, while the membrane resistance and resting potential were 2.2±0.3 GΩ and -30.9±1.2 mV, respectively. The current densities of the pericytes (pA/pF) were 3.2±0.7, 10.6±1.0, 15.7±0.9 and 21.3±1.2 at command voltages of 0, +20, +40, and +60 mV, respectively. The K^+ channels were activated when the pericytes were within the range of -20 mV to +20 mV, particularly at 0 mV. The inhibition rates of the outward current of cochlear pericytes in the stria vascularis of the guinea pigs were determined by administering iberiotoxin (IBTX) and IBTX + 4-aminopyridine. Once the background leakage current was removed, the following inhibition rates were obtained with 3, 10, 30, 300 and 1,000 μmol/l aspirin: 20.8±4.8, 34.1±6.9, 48.2±6.7, 63.6±7.1 and 65.7±8.1%, respectively. The outward current of the cochlear pericytes in the stria vascularis was inhibited by aspirin with a half maximal inhibitory concentration of 24.5±4.5 μmol/l. The membranes of the pericytes in the stria vascularis are characterized by high-conductance calcium-activated K^+ (BK_{Ca}) and voltage-dependent K^+ (K_V) channels. The outward current of the cochlear pericytes in the stria vascularis of guinea pigs was inhibited by aspirin in a concentration-dependent manner. In addition, BK_{Ca} and K_V channels were inhibited by aspirin.

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Introduction

The stria vascularis maintains blood flow stability in the inner ear and also the balanced state of the endocochlear potential (endolymph positive potential), ion transport and endolymph (1,2). Within the microcirculation of the inner ear, the spiral artery of the cochlea has been extensively investigated (3-11). In addition, the morphological characteristics of the pericytes of the stria vascularis have also been widely explored (12,13). However, further research investigating potassium channels (K^+ channels) in the capillary membranes of pericytes within the intrastria space (IS) of the corpus striatum in the stria vascularis is required. These channels may be closely associated with the formation of endocochlear potential, particularly electrophysiological properties.

The high potassium and endocochlear potentials in the inner lymphatic fluid are established by the vascular lines of

the lateral wall of the cochlea (1,2). Following entry into hair cells, K^+ exits across the basolateral membrane via K^+ channels and reaches the lateral cochlear wall either by a perilymphatic route or via the gap-junctional network comprising Deiters' cells and epithelial cells on the basilar membrane (2). K^+ is subsequently transported across the lateral cochlear wall and finally returned back to endolymph (1,2,12,13).

Aspirin (acetylsalicylic acid) is commonly used to treat pain, fever and inflammation (14); however, it is associated with a number of side effects, including tinnitus and hearing loss (15). As such, the toxicity and effects of acetylsalicylic acid on the auditory system have been evaluated previously, though definitive conclusions have yet to be drawn. For instance, aspirin is thought to cause cochlear damage (16,17). Blood flow volume is reduced by sodium salicylate, which influences the metabolism of calcium ions in hair cells, and alters their form and kinetic characteristics (16,17). Sodium salicylate also causes neural damage of the spiral ganglion. Cellular damage to hair cells and spiral ganglion as a result of the ototoxicity of aminosalicylic acid has also been described; however, the effects of aspirin on capillary pericytes in the stria vascularis have yet to be reported. The potential ototoxicity of aminosalicylic acid in the auditory system, in terms of the alterations in the high potassium potential of the endolymph, is also unknown.

In the present study, the function and associated mechanism of the effects of aspirin on the cochlear pericytes of the stria vascularis were observed and recorded using a whole-cell patch-clamp technique. The aim was to evaluate the capillary pericytes in the stria vascularis in order to present experimental evidence regarding the mechanisms associated with maintaining the stability of the internal environment of the stria vascularis and the function of the cochlea, and also to provide a novel theoretical basis for the causes of aspirin toxicity.

Materials and methods

Animals. A total of 100 Dunkin-Hartley guinea pigs (weight, 250-350 g; Animal Testing Center of Xinjiang Medical University, Xinjiang, China) were selected regardless of sex. The use of animals in the present study was approved by the Committee of Animal Experimental Ethics of The First Affiliated Hospital of Medical College, Shihezi University (Shihezi, China) (animal use certificate no. SCXK Xin 2003-0001). Guinea pigs were housed in separate cages in a specific pathogen-free environment at 24±3˚C with a relative humidity of 40-70% under a 12 h light/dark cycle, and were provided with free access to food and water. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the Medical College of Shihezi University and consistent with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (18).

Reagents and instruments. Collagenase, trypsin, tetraethylammonium (TEA), 4-aminopyridine (4-AP, cat. no. 275875), propidium iodide (PI), Triton X-100, aspirin and iberiotoxin (IBTX, cat. no. 56718) were all obtained from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany). Antibodies against desmin (cat. no. ab32362; Abcam, Cambridge, MA, USA) and α-smooth muscle actin (SMA, cat. no. 6198; Sigma-Aldrich; Merck KGaA) were also used. The remaining reagents were of analytical grade and were locally procured. An Axon 700B amplifier (Axon Instruments; Molecular Devices, LLC, Sunnyvale, CA, USA) and a laser scanning confocal microscope (Zeiss LSM 510; Zeiss AG, Oberkochen, Germany) were also used in the following experiments.

Immunofluorescence identification of pericytes in the stria vascularis. Guinea pigs were randomly divided into the experimental and control groups (n=5/group) in order to identify the pericytes of the stria vascularis. The guinea pigs were intramuscularly injected with 10% chloral hydrate (0.35 ml/100 g body weight, solution of 10 g chloral hydrate and 100 ml of normal saline) and then sacrificed by bloodletting under anesthesia. All protocols were approved by the IACUC at the Medical College of Shihezi University and consistent with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (18). The cochlea was removed and placed in 4% paraformaldehyde at 4˚C for 4 h. The stria vascularis and the spiral ligament were removed from the cochlea with self-made injector-bent needles under a dissecting microscope. The stria vascularis was obtained by separating the stria vascularis and spiral ligaments using microforceps, which were then placed into Eppendorf (EP) tubes containing PBS solution and processed by centrifugation at 111.8 x g for 6 min at room temperature; the supernatant was then discarded. The specimens were rinsed thrice, and the immunostaining blocking liquid (5% PBS) with 0.1% Triton X-100 was added to the stria vascularis. Each sample was incubated for 1 h at room temperature and then rinsed with PBS. Samples in the experimental group were treated with anti-desmin diluents (1:100; Abcam) or anti-α-SMA (1:100; Sigma-Aldrich; Merck KGaA), and the control group was treated with PBS only. The samples were placed in a wet box at 4˚C for 12 h. Rewarming was performed the following day for 1 h at 37˚C. The stria vascularis in the EP tube was rinsed three times in PBS solution and the extra liquid was absorbed with filter paper strips. The samples were treated with secondary antibody (1:100; fluorescein isothiocyanate (FITC)-labeled second antibody, ZF-0311; OriGene Technologies, Inc., Beijing, China), placed in a wet box and further incubated for 1 h at room temperature. Each sample was rinsed for 30 sec with PBS at room temperature prior to staining with PI for 30 sec at room temperature. The stria vascularis was rinsed three times with PBS; during the first two washes, the tubes were centrifuged at 111.8 x g for 6 min at room temperature and the supernatant was discarded. Finally, the stria vascularis and PBS were aspirated with a 1 ml pipette, and then transferred onto microscope slides. The extra PBS was absorbed with filter paper prior to sealing the slide with 85% glycerinum for fluorescence quenching. The fluorescence was observed and recorded with a laser scanning confocal microscope (Zeiss LSM 510; Zeiss AG, Oberkochen, Germany).

Preparation of single pericytes from the cochlear stria vascularis. As aforementioned, guinea pigs in the experimental and control groups were given intramuscular injections of 10%

chloral hydrate (10 g of chloral hydrate in 100 ml of normal saline) and sacrificed by bloodletting under anesthesia. The cochlea was immediately removed and placed in a low-Ca^{2+} buffer solution containing: 142 mM NaCl, 5 mM KCl, 0.05 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Na-HEPES, 6 mM HEPES (pH 7.2) and 7.5 mM glucose (pH was adjusted to 7.4). The stria vascularis with spiral ligaments was removed from the cochlear lateral wall under a dissecting microscope using self-made injector-bent needles. The stria vascularis was obtained by separating the stria vascularis and spiral ligament using microforceps, and was then placed in a mixture containing digestive enzymes including 0.1% collagenase and 0.1% trypsin for 10 min in a water bath at 37˚C. The sample was centrifuged at 111.8 x g at room temperature for 6 min and the supernatant was discarded. A cell suspension was manually cleaned in a Petri dish filled with an aerated normal external solution composed of: 138 mM NaCl, 5 mM KCl, 1.6 mM $CaCl_2$, 1.2 mM $MgCl_2$, 5 mM Na-HEPES, 6 mM HEPES and 7.5 mM glucose (pH 7.4; osmolarity, 300 mOsm/l). The cell suspension was transferred into a clean and dry Petri dish and left to stand for 30 min in the saline solution at room temperature. The liquid in the Petri dish was substituted twice with the saline solution to remove the other cells once the pericytes became adherent. The whole-cell patch-clamp experiments were then performed according to pericyte morphology following 10 min.

Whole-cell patch-clamp recording. The whole-cell patch-clamp experiment was conducted at room temperature (20-25˚C) with an Axon 700B amplifier. The recorded diameter of the electrode tip was 1 μm, and the electrode impedance was ~7-9 MW. The electrode contained 130 mmol/l K-gluconate, 10 mmol/l NaCl, 2.0 mmol/l $CaCl_2$, 1.2 mmol/l $MgCl_2$, 10 mmol/l HEPES, 5 mmol/l EGTA and 7.5 mmol/l glucose; the pH was adjusted to 7.2, with an osmotic pressure of 290 mOsm/l. Sealing-in was obtained with negative pressure once the micromanipulator came into contact with the cell; the membrane was broken and a whole-cell patch-clamp was formed with electrical stimulation or instant high negative pressure following the compensation of capacitors and electrodes (Fig. 1). The compensation did not cover the membrane capacitance current due to the online monitoring of membrane parameters, as well as the calculation of membrane capacitance (C_{input}) and membrane resistance (R_{input}). C_{input} was acquired with the following formula: $C_{input} = Q/V$, where Q, the electric quantity, was acquired from the membrane capacitance charge-discharge initiated by V, the command current. When observing the differences in the outward current following the application of 1 mmol/l TEA and 1 mmol/l 4-AP, the discontinuous square-wave pulse stimulation proceeded as follows: Patch-clamp potential of -40 mV, readings were recorded from -80 mV step stimulation depolarization to +60 mV; time of 150 msec; sampling interval of 10, 20 and 100 μsec; and the membrane current was processed with 10 kHz (-3 dB) low-frequency filtration. The data were analyzed with pClamp™ v10.2 software (Axon Instruments; Molecular Devices, LLC).

Detection of the type of potassium channel in the pericytes membrane. Step stimulation (from -80 mV to +60 mV, interval was 20 mv) and ramp stimulation (from -80 mV to +60 mV; wave width=150 ms) were applied. Clampfit v10.2 software (Molecular Devices, LLC) was used to obtain the current-voltage (I/V) curve.

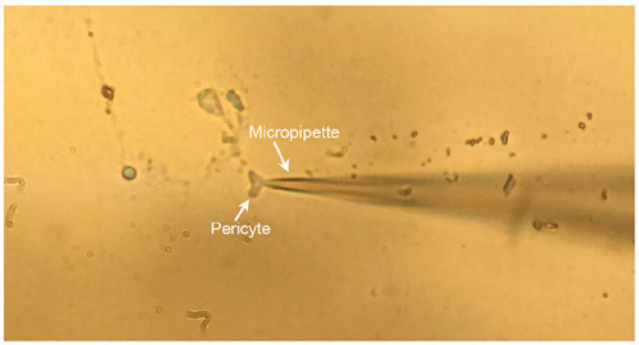

Figure 1. Sealing of a pericyte was observed using an inverted microscope (Scale bar, 50 μm).

The effect of aspirin on the outward current of pericytes. Following the rupture of the membrane formed the whole-cell configuration, 3, 10, 30, 300, 1,000 μmol/l aspirin were successively injected in to each cell. Using Clampfit v10.2 software (Molecular Devices, LLC), the I/V curve of the net current of the diastolic blood vessel was obtained, and the ion mechanism of the outgoing current induced by aspirin was analyzed according to the I/V curve.

Determination of the type of channel that mediates the outward current that is inhibited by aspirin. Following the rupture of the membrane formed the whole-cell configuration; aspirin (300 μmol/l), IBTX (1 nmol/l) + 4-AP (300 μmol/l), Aspirin (300 μmol/l) + IBTX (1 nmol/l) + 4-AP (300 μmol/l) were successively injected in to each cell.

Immunofluorescence identification was conducted once the whole-cell patch-clamp experiments were performed to ensure that the experimental objects were the pericytes of the stria vascularis. Then, the identified pericyte of the experimental and control groups was gently sucked into a glass pipette (tip, ~40 μm), and transferred onto a gelatine-smeared slide. Then, the cells were immobilized in acetone at 4˚C for 15 min, prior to washing thrice with PBS (pH 7.4), for 5 min per wash. The immunostaining blocking liquid (5% PBS) with 0.1% Triton X-100 was applied for 1 h incubation at room temperature. The anti-desmin diluent (1:100, rabbit anti-guinea pig monoclonal antibody; Abcam) was applied prior to overnight incubation at 4˚C. Following 1 h of rewarming at 37˚C, the stria vascularis was rinsed thrice with PBS solution (5 min for each rinse), and excess liquid was absorbed using filter paper. The sample was treated with the secondary antibody (1:100, FITC-labeled second antibody, ZF-0311; OriGene Technologies, Inc.) and incubated in a wet box for 1 h at room temperature, rinsed with PBS and stained with PI for 30 sec at room temperature. The excess fluid at the edge of the sample was absorbed with filter paper following each rinse. The slide was sealed with 85% glycerinum to prevent fluorescence quenching. Fluorescence was observed and recorded with a laser scanning confocal microscope (FITC and PI staining was detected at 488 and 536 nm, respectively; Zeiss LSM 510; Zeiss AG).

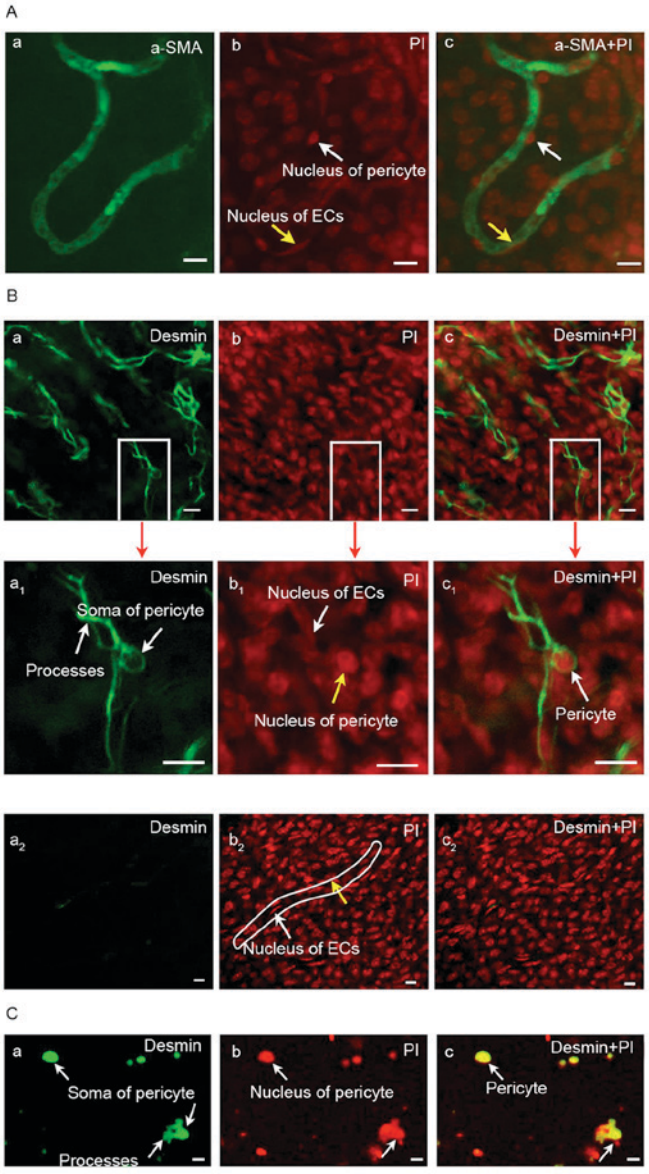

Figure 2. Pericytes can be identified using the marker protein, desmin. (A-a) Image pericytes are negative for α-SMA expression (nonspecific staining of blood vessels); (b) nuclei of stria vascularis cells are stained with PI (red fluorescence); (c) merge of images a and b. (B-a) Image, desmin expression in pericytes (green fluorescence, FITC-labeled second antibody); (b) nuclei of stria vascularis cells are stained with PI (red fluorescence); (c) merge of images a and b. Images (a_1-c_1) are enlargements of the indicated areas in images (a-c), respectively; (a_2-c_2) are the negative control group. The white line in image b_2 represents the vessel shape. (C-a) Image desmin expression of pericytes from the stria vascularis following whole-cell patch-clamp experiments (green fluorescence, FITC-labeled second antibody); (b) nuclei of single cells marked with PI (red fluorescence); (c) merge of images a and b. Scale bar, 10 μm. α-SMA, α-smooth muscle actin; FITC, fluorescein isothiocyanate; PI, propidium iodide.

Data analysis. Current intensity was measured as the difference between the initial current and the maximum activating current during activation. Current density was determined as the ratio between current density and membrane capacitance (pA/pF). Data were analyzed using KaleidaGraph v3.6 (Synergy Software, Inc., Reading, PA, USA), FreeHand MX11, Adobe Photoshop CS5 (both from Adobe Systems, Inc., San Jose, CA, USA), and simplified and localized CAD software (Autodesk, Inc., San Rafael, CA, USA).

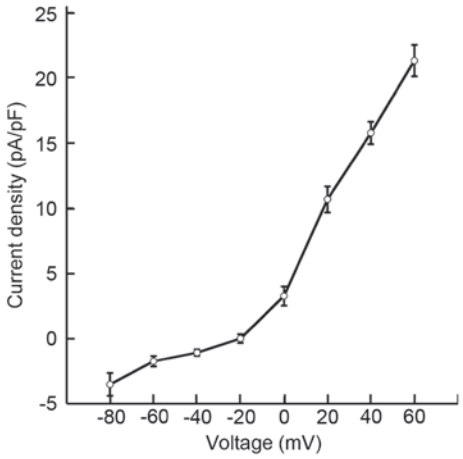

Figure 3. Voltage dependence of the current density in cochlear pericytes. The vertical coordinate is the current density, and the horizontal coordinate is the voltage. Current density was determined as the ratio between current density and membrane capacitance (pA/pF).

Statistical analysis. The results are expressed as the mean ± standard error of the mean (n=6). Statistical analysis was performed using SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL, USA). A homogeneity test for variance was performed followed by one-way analysis of variance, and two-group comparisons were conducted using the least significant difference post hoc test. $P<0.05$ was considered to indicate a statistically significant difference. KaleidaGraph v3.6 (Synergy Software, Inc.) and Adobe Photoshop CS5 (Adobe Systems Europe, Ltd., Maidenhead, UK) were used for graphics processing.

Results

Identification of pericytes with the marker protein desmin. As shown in Fig. 2A, the expression of α-SMA in guinea pig cochlear pericytes from the stria vascularis was negative; however, nonspecific staining of blood vessels was observed. The expression of desmin was positive based on the results of microscopy; enlarged pericytes and large nuclei were observed. Pericytes were detected in the negative control (Fig. 2B and C). The nuclei of pericytes were large and surrounded by less cytoplasm; the cytoplasmic processes gradually became smaller as branches grew parallel with the capillary axis, thereby surrounding the capillaries with its tips (Fig. 2B). This result demonstrated that the identification of pericytes with marker protein desmin may be feasible. Identification experiments were also conducted following the whole-cell patch-clamp experiment to ensure that the experimental objects were the pericyte.

Electrophysiological properties of guinea pig cochlear pericytes. The electrophysiological properties of single pericytes were recorded using the whole-cell patch-clamp recording technique. The membrane capacitance was 5.9±0.3 pF, the membrane resistance was 2.18±0.3 GW and the membrane resting potential was -30.9±1.2 mV. Fig. 3 reveals the current densities of single pericytes. At command voltages of 0, +20, +40 and +60 mV, the pericyte current densities were 3.2±0.7, 10.6±0.9, 15.7±0.8 and 21.2±1.2 pA/pF, respectively. These results

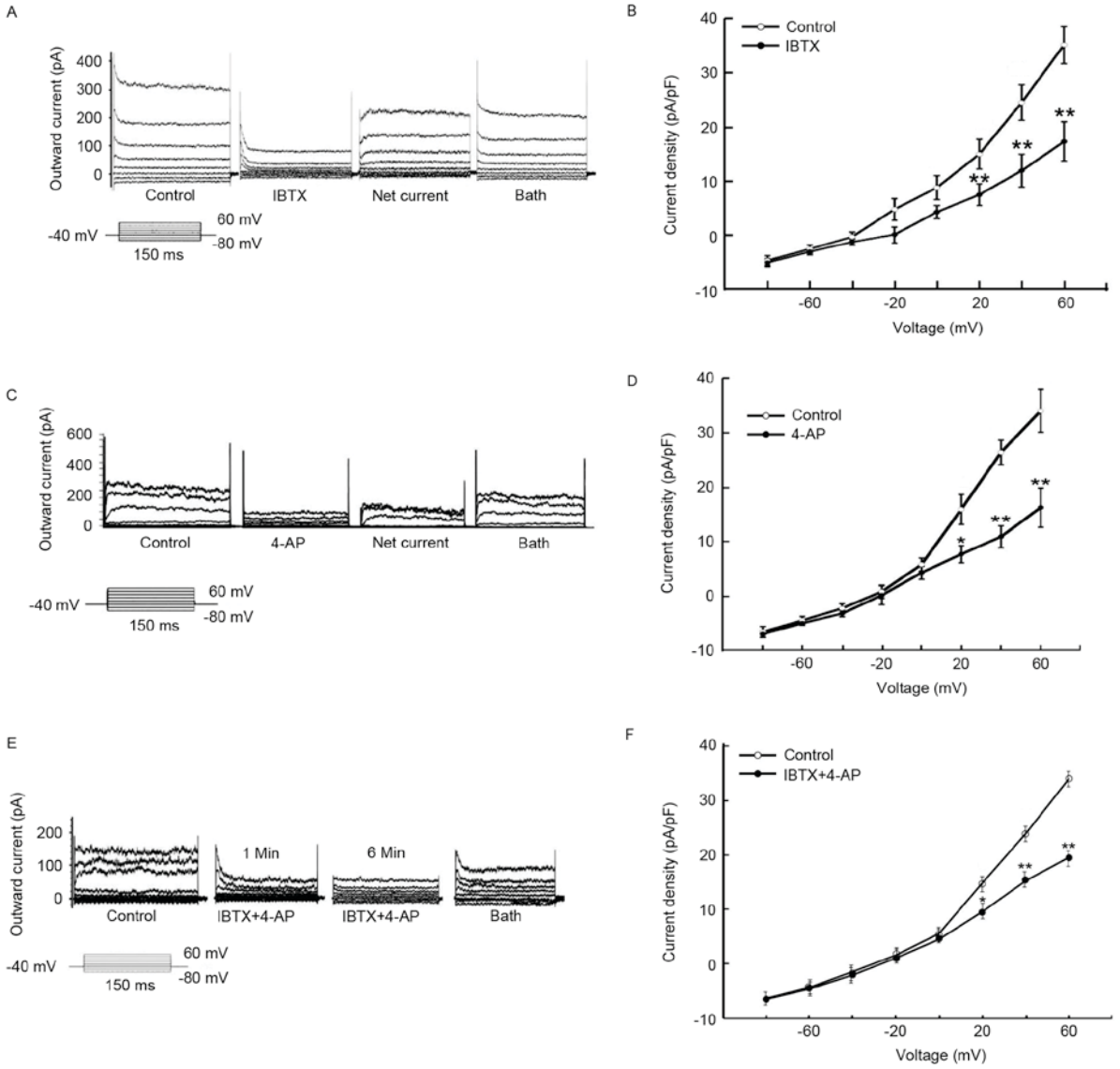

Figure 4. IBTX, 4-AP and IBTX + 4-AP inhibit the outward current of cochlear pericytes from the stria vascularis in guinea pigs. (A) Step stimulation of the whole-cell current indicating that the pericyte outward current was partially inhibited by the BK_{Ca} channel retardant IBTX (1 nmol/l). (B) Pericyte current density prior to and following the application of 1 nmol/l IBTX. (C) Step stimulation of the whole-cell current indicating that the pericyte outward current was partially inhibited by the K_V channel retardant 4-AP (1 mmol/l). (D) Pericyte current density prior to and following the application of 1 mmol/l 4-AP. (E) Step stimulation of the whole-cell current indicating that the pericyte outward current was markedly inhibited by 1 nmol/l IBTX + 1 mmol/l 4-AP. (F) Pericyte current density prior to and following the application of 1 nmol/l IBTX + 1 mmol/l 4-AP. Data are presented as the mean ± standard error of the mean of 6 independent experiments. *P<0.05 and **P<0.01 vs. control. IBTX, iberiotoxin; 4-AP, 4-aminopyridine; BK_{Ca}, high-conductance calcium-activated K^+ channels; K_V, voltage-dependent K^+ channels.

indicated that the current density of cochlear pericytes may be voltage dependent.

Membranes of pericytes in the stria vascularis contain high-conductance calcium-activated K^+ (BK_{Ca}) and voltage-dependent K^+ (K_V) channels. With the step stimulation of -80 mV to +60 mV, the single pericyte current demonstrated marked outwardly rectifying characteristics (Fig. 4A, C and E). The outward current of the pericytes treated with the K^+ channel retardant became sensitive to the specific retardant IBTX (1 nmol/l) in BK_{Ca} channels (Fig. 4A and B). The inhibiting current exhibits a typical graph of voltage-dependent K_V, as displayed in Fig. 4A. At 1 mmol/l 4-AP (specific retardant of K_V channels), the outward current of the pericytes was partially inhibited compared with in the control (Fig. 4C and D). At 1 mmol/l 4-AP and 1 nmol/l IBTX, the outward current of pericytes was also inhibited (Fig. 4E and F). These results demonstrated that the outward current of pericytes may be initiated by activating BK_{Ca} and K_V channels.

Inhibition of the outward current by aspirin is concentration-dependent in guinea pig cochlear pericytes from the stria vascularis. The inhibition of the outward current by aspirin in the cochlear pericytes of the stria vascularis was marked and concentration dependent (Fig. 5). Aspirin exerted weak effects on the membrane current within the voltage range of -80-0 mV in the pericyte *I/V* curve; the drug primarily inhibited the activated current within the voltage range of 0-60 mV (Fig. 5B).

With the perfusion of aspirin at different concentrations in the experiment, the effect of aspirin was concentration

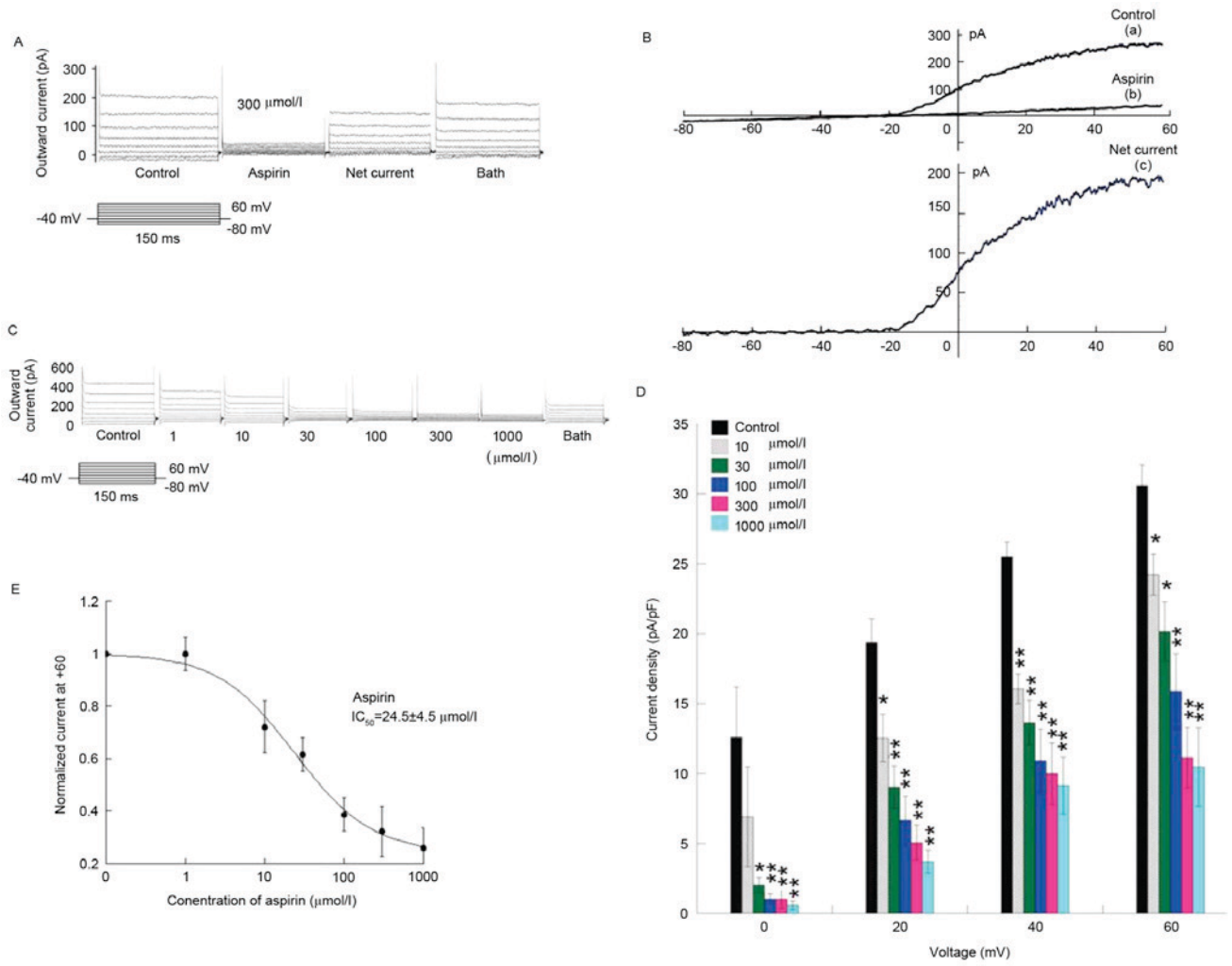

Figure 5. Inhibition of the outward current in cochlear pericytes from the stria vascularis of guinea pigs caused by aspirin. (A) Effects on the pericyte outward current with 300 μmol/l aspirin. (B) Current/voltage curve of the action of 300 μmol/l aspirin in pericytes, where the following is applied: (a) Prior to 300 μmol/l aspirin inhibition; (b) following inhibition; (c) net current inhibited by 300 μmol/l aspirin. (C) Whole-cell current initiated by step stimulation showing the effects aspirin exerts on pericytes at different concentrations. (D) Bar graph displaying the inhibition of the pericyte outward current prior to and following the application of aspirin at different concentrations. (E) Dose-effect curve of aspirin; a half maximal inhibitory concentration of 24.5±4.5 μmol/l was observed. Data are presented as the mean ± standard error of the mean of 6 independent experiments. *P<0.05 and **P<0.01 vs. control.

dependent for the pericyte outward current of stria vascularis capillaries. Following removal of the background leakage current, treatment with 3, 10, 30, 300 and 1,000 μmol/l aspirin yielded the following inhibition ratios: 20.8±4.8, 34.1±6.9, 48.2±6.7, 63.6±7.1 and 65.7±8.1%, respectively (Fig. 5C and D). Under conditions with background leakage current, the half maximal inhibitory concentration (IC_{50}) was 24.5±4.5 μmol/l in terms of the inhibition of the outward current of cochlea stria vascularis pericyte with aspirin (Fig. 5E). These results revealed that inhibition of the outward current of cochlea stria vascularis pericyte with aspirin is concentration dependent.

BK_{Ca} and K_v channels are inhibited by aspirin. To understand the type of mediation on the outward current produced by aspirin, the following treatments were perfused at different time intervals in the experiment: 300 μmol/l aspirin, 1 nmol/l IBTX, or 300 μmol/l aspirin + 1 nmol/l IBTX (Fig. 6A and B); 300 μmol/l aspirin, 1 mmol/l 4-AP, or 300 μmol/l aspirin + 1 mmol/l 4-AP (Fig. 6C and D); 300 μmol/l aspirin, 1 nmol/l IBTX + 1 mmol/l 4-AP, or 300 μmol/l aspirin + 1 nmol/l IBTX + 1 mmol/l 4-AP (Fig. 6E and F). Following flushing of the extracellular fluid, the current returned to almost the original levels; however, the current reduced again following treatment with 1 nmol/l IBTX. After 5 min, aspirin (300 μmol/l) + IBTX (1 nmol/l) were injected in the same cell and the current reduced further. The current recovered after flushing. These results indicated that the outward current may be inhibited by aspirin, and mediated by the BK_{Ca} and K_V channels.

Discussion

It is generally believed that pericytes and smooth muscle cells serve similar roles in adjusting blood flow volume and vascular permeability (19-21). Nicosia and Villaschi (19) observed that pericytes in mice can be differentiated from the intima of the mesentery artery and the vascular smooth muscle cell subgroup below the intima during vasculogenesis *in vitro*. Another previous study observed myogenic phenotypes when pericytes were cultured *in vitro* (20). Pericytes can be distinguished from

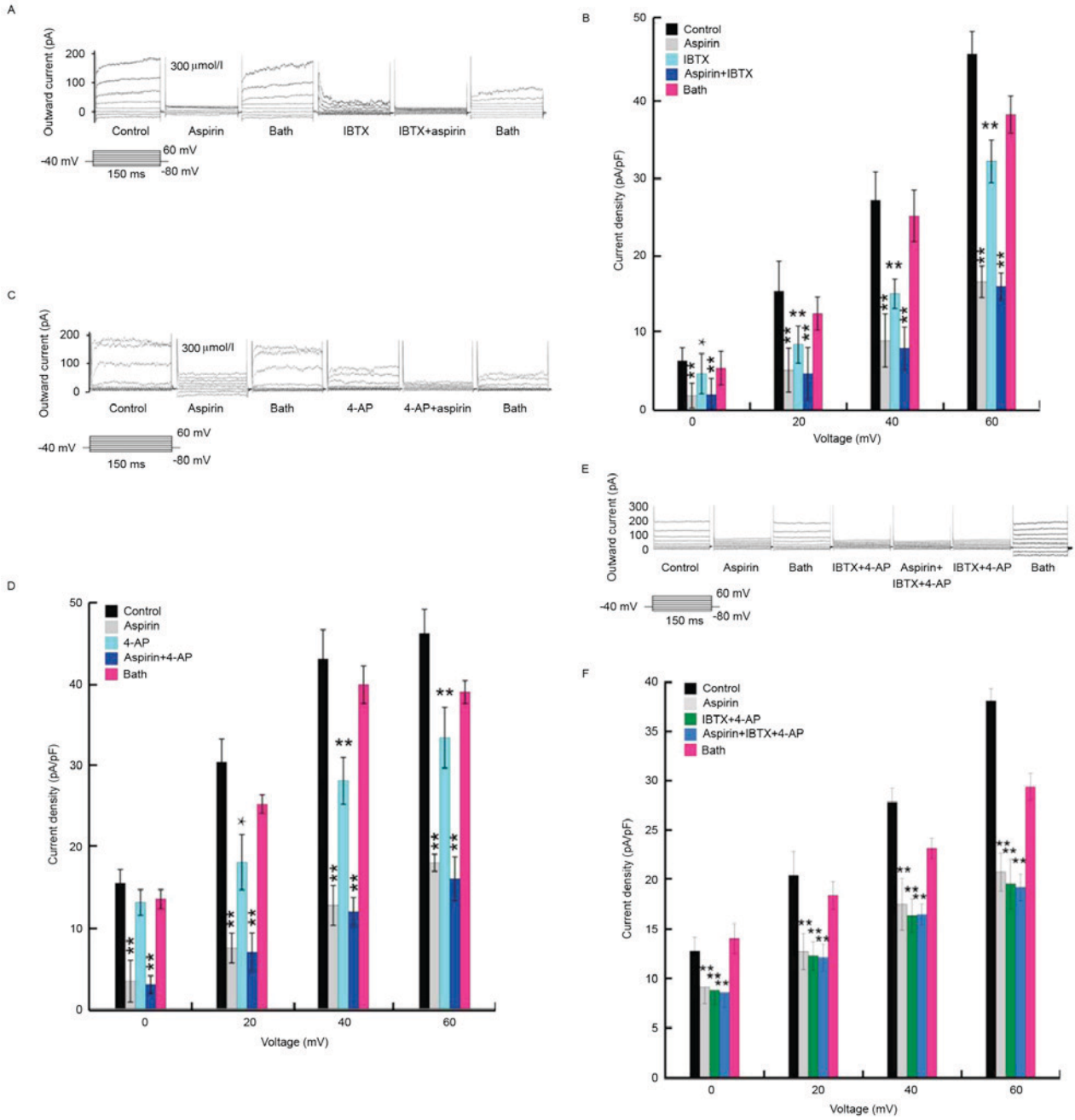

Figure 6. BK_{Ca} and K_v channels can be inhibited by aspirin. (A) Whole-cell current initiated by step stimulation showing the influence of 300 μmol/l aspirin, 1 nmol/l IBTX, and 300 μmol/l aspirin + 1 nmol/l IBTX, on the outward current. (B) Pericyte current density following the application of 300 μmol/l aspirin, 1 nmol/l IBTX, and 1 nmol/l IBTX + 300 μmol/l aspirin. (C) Whole-cell current initiated by step stimulation showing the influence of 300 μmol/l aspirin, 1 mmol/l 4-AP, and 300 μmol/l aspirin + 1 mmol/l 4-AP on the outward current. (D) Pericyte current density following the application of 300 μmol/l aspirin, 1 mmol/l 4-AP, and 300 μmol/l aspirin + 1 mmol/l 4-AP. (E) Whole-cell current initiated by step stimulation showing the influence of IBTX + 1 mmol/l 4-AP, 300 μmol/l aspirin, and IBTX + 1 mmol/l 4-AP + 300 μmol/l aspirin on the outward current. (F) Pericyte current density following the application of IBTX + 1 mmol/l 4-AP, 300 μmol/l aspirin and IBTX + 1 mmol/l 4-AP + 300 μmol/l aspirin. Data are presented as the mean ± standard error of the mean of 6 independent experiments. *P<0.05 and **P<0.01 vs. control. IBTX, iberiotoxin; 4-AP, 4-aminopyridine; BK_{Ca}, high-conductance calcium-activated K^+ channels; K_V, voltage-dependent K^+ channels.

smooth muscle cells under certain conditions (20). Comparisons between the results of the present study and those of a previous study (21), in regard to membrane resistance, membrane conductance and current density of the single smooth muscle cells of the cochlear spiral artery, showed no significant differences between the pericytes and smooth muscle cells in terms of their electrophysiological properties. Therefore, pericytes and smooth muscle cells may have similar electrophysiological properties.

Aspirin is a widely used medicine, and its main side effects include tinnitus and hearing loss. Considerable research has been conducted on the toxicity and side effects of acetylsalicylic acid to the auditory system (21,22). A previous study in mice demonstrated that sodium salicylate inhibits the K_V channels of cochlear spiral ganglion cells (SGNs) in a concentration-dependent manner, with an IC_{50} of ~1.15 mmol/l (22). Sodium salicylate inhibits the outward rectification of the potassium current in

cochlear SGNs and the tail current, as the inactivation of the tail current is accelerated; these effects lead to the abnormal electrophysiological activities of cochlear SGNs, thereby damaging the auditory system (22). Sodium salicylate inhibits the voltage-gated calcium channel current in a concentration-dependent manner. The IC_{50} value of sodium salicylate to hippocampal neurons in the voltage-gated Ca^{2+} channel current was 1.64 mmol/l (21). Sodium salicylate has also been observed to inhibit the K^+ channels of temple cortex neurons in a concentration-dependent manner (0.1-10 mmol/l) in mice (22); the IC_{50} value of sodium salicylate inhibition on the K^+ channels was 2.13 mmol/l (22). This experiment indicates that aspirin at 1-1,000 μmol/l can inhibit pericyte BK_{Ca} and K_V channels depending on its concentration; the greater the concentration, the greater the inhibition (22). At a concentration of 300 and 1,000 M, the inhibitory effect of the outward current on the cochlear pericytes was not marked in the present; however, the inhibition rate of aspirin increased with the increase in concentration. This observation demonstrated the effects of various blood concentrations on hearing. According to the results of the present, the IC_{50} value of aspirin for inhibiting the outward current of cochlear pericytes of the stria vascularis was 24.5±4.5 μmol/l.

Pericytes serve an important role in adjusting blood flow volume and vascular permeability (12). The results of the present study indicated that the ototoxicity of aspirin may depend on two aspects. Firstly, pericytes lose their timely repolarization once the outward current of pericytes is inhibited by aspirin, which affects the blood flow volume of the inner ear as the capillary network contracts in the stria vascularis. Secondly, the aspirin-induced inhibition of the outward current of pericytes affects the K^+ concentration and potential in the stria vascularis. Therefore, two conditions are required to activate the Na^+/K^+-ATP enzyme of border cells: i) An adequate ATP supply; and ii) a suitable K^+ concentration and potential in the stria vascularis. However, aspirin influences these conditions. Therefore, this drug may affect the normal auditory system by terminating K^+ transport from the stria vascularis to border cells via the Na^+/K^+-ATP enzyme, or by preventing K^+ from entering the endolymph via K^+ channels in the membranes of border cells. The results of the present study enhanced the current understanding of the electrophysiological properties of pericytes, and the toxic and general side effects of aspirin on the auditory system. Applying the technologies of immunohistochemistry, western blotting and RT-qPCR technology, detection of aspirin for the pericytes of the stria vascularis, the effects of potassium ion channel expression may provide a theoretical basis for the prevention of aspirin ototoxicity.

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