Voltage-dependent K Channels in Supporting Cells Isolated from Guinea Pig Cochlea

Kanae OKUMOTO, Yozo ORITA and Takeshi AKISADA

Department of Otorhinolaryngology, Kawasaki Medical School, Kurashiki 701-0192, Japan

Accepted for publication on March 14, 1998

ABSTRACT. The cochlea hair cells of the mammalian inner ear are held mechanically by a surrounding network of various supporting cells in the organ of Corti located on the basilar membrane. Recently, it has been suggested that these supporting cells have both mechanical and functional connections with sensory hair cells. In this study, Deiters' cells (DCs) and Hensen's cells (HEs) were freshly isolated from the guineapig cochlea, and voltage-dependent outward currents (VDOCs) in both DCs and HEs were measured with conventional whole-cell patch recording techniques. The results obtained were as follows.

1) The whole-cell currents in both cells were VDOCs, which were evoked at about -20 mV with depolarizing pulses, and were suppressed completely by intracellular Cs^+. The current-voltage curve in DCs had a plateau phase at high depolarizing voltages. However, in a Ca-free extracellular solution, this plateau phase disappeared and the current-voltage curve showed a straight line with depolarizing voltages.
2) In an extracellular solution containing 10mM TEA, the VDOCs in both DCs and HEs were suppressed by about 80% of the control K currents. Therefore, the main currents in both cells were rectifying K currents.
3) An intracellular solution containing 10mM 4-AP suppressed voltage-dependent outward K (VDO-K) currents in both cells. The slow components of these VDO-K currents in HEs were suppressed by more than 90% of the control currents, whereas those in DCs were suppressed by about 40% of the control currents with depolarizing voltages. But these 4-AP-resistant components were disappeared completely with an extracellular solution containing 10mM TEA.

From these results, it was concluded that VDO-rectifying K currents were the main currents in the supporting cells, and that DCs also have Ca-activated K currents, which were evoked at high depolarizing voltages.

Key words: supporting cells — voltage-dependent K current — Deiters' cells — Hensen's cells

It is well known that the cochlea hair cells of the mammalian inner ear; i. e. the outer hair cells (OHCs) and inner hair cells (IHCs), play an important role in the transduction process of mechanical sound stimuli into electrical nerve signals. These hair cells are located on the basilar membrane and held in place by a surrounding network of supporting cells, Deiters' cells (DCs) and Hensen's cells (HEs), in the organ of Corti. Although the physiological

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function of these hair cells has been extensively studied, and there have been many interesting reports regarding several of their electrical characteristics, little is known about the function of the supporting cells, which are believed to provide mechanical support to the sensory hair cells. The K channels in the OHCs of the guineapig are known to have outward currents which are activated by stimulation by depolarizing voltages, and inward currents which are activated by stimulation by hyperpolarizing voltages and Ca-activated K channels.[1-3] IHCs also have VDO-K channels.[4] The supporting cells are known to generate receptor potentials during sound stimulation, suggesting an electrical coupling with the hair cells.[5,6] Dulon[7] reported that DCs have an ATP receptor, characterized as the P_2-purinergic receptor, which has been suggested to exist in the sensory hair cells. Based on these findings, VD-K currents may reflect part of the communicative processes occurring between the sensory hair cells and the supporting cells in the cochlea. The aim of the present study was to determine whether VDO-K currents exist in the supporting cells. In this study, two types of supporting cells, DCs and HEs, were freshly isolated from the guinea pig's organ of Corti, and the physiological roles of their electrical responses and how the coupling between hair cells and supporting cells takes place were investigated.

MATERIAL AND METHODS

Cell preparation

Young adult pigmented albino guinea pigs (weight 250-400g) were killed by rapid cervical dislocation and then one of the bullae was transferred to a Petri-dish filled with cooled tissue culture medium and opened for easy access to the cochlea. It was stabilized in a miniature vice and rinsed in 10 ml of cooled tissue culture medium. After the isolated cochlea was microdissected in this medium, the stria vascularis of turns 2 to 4 of the cochlea was exposed in a normal extracellular solution (see solution in MATERIALS AND METHODS), removed together with Reissner's membrane. After removal of the tectorial membrane, the exposed part of turns 2 to 4 was loosened from the spinal lamina. The isolated coil was transferred into a small glass tube containing 2 ml of extracellular solution, and was triturated using a fire-polished Pasteur pipette in each solution at room temperature. The isolated cells were allowed to settle on the glass surface of the bottom of a patch chamber at room temperature for 20 minutes. Many of the isolated cells were DCs. To isolate HEs, the isolated coil was transferred to a small glass tube containing 2 ml of an extracellular solution with a 20 μl droplet of trypsine medium (type [IV] from Sigma; 1mg/ml). This was triturated using a vibrator at room temperature. After the HEs were allowed to settle on the bottom of the patch chamber, the trypsine medium was washed out with a normal extracellular solution.

Solution

The whole-cell normal extracellular solution (mM) consisted of 150 NaCl, 5 KCl, 2.2 $CaCl_2$, 1.0 $MgCl_2$, 10 HEPES and 10 Dextrose. The Ca-free solution (mM) consisted of 150 NaCl, 5 KCl, 5.0 EGTA (ethylene glycol-bis β-amino ethyl ether-N,N,N',N'-tetraacetic acid), 2.0 $MgCl_2$, 10 HEPES and 10

Dextrose. The extracellular 10mM TEA^+ (tetraethyl-ammonium) solution (mM) contained 140 NaCl, 5.0 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 10 TEA, 10 HEPES and 10 Dextrose. The pH of these solutions was adjusted to 7.4 with NaOH.

The whole-cell pipette solution (intracellular solution) (mM) contained 140 KCl, 2.0 $MgCl_2$, 1.0 EGTA and 5.0 HEPES. The intracellular solution containing 4-AP (mM) was composed of 130 KCl, 2.0 $MgCl_2$, 1.0 EGTA, 5.0 HEPES and 10 4-AP (aminopyridine). The pH was adjusted to 7.4 with KOH.

The intracellular CsCl solution (mM) consisted of 140 CsCl, 2.0 $MgCl_2$, 1.0 EGTA, 5.0 HEPES. The pH was adjusted to 7.4 with CsOH.

The osmolality of both the extracellular and intracellular solutions was adjusted to near 300 mosmol/Kg H_2O before each experiment.

Recording methods

Conventional whole-cell recording techniques were employed, using an Axopatch 1D patch-clamp amplifier (Axon Instruments). Patch pipettes, which had tip resistances of 3-5 MΩ, were made of borosilicate glass (Inter Medical Co. Ltd) using a vertical pipette puller (Narishige pp-83). Command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments). The present experiments were approved by the Animal Research Committee of Kawasaki Medical School and conducted according to the "Guide for the Care and Use of Laboratory Animals" of Kawasaki Medical School.

RESULTS

Voltage-dependent outward currents (VDOCs) in DCs and HEs

The properties of voltage-dependent K (VD-K) channels in DCs and HEs were studied using normal extracellular and intracellular solutions. The whole-cell currents of DCs and HEs were recorded in response to depolarizing voltage step pulses of 10 mV starting from a holding potential of -80 mV. Typical current records of DCs and HEs are shown in Fig 1A, 2A. Voltage-dependent currents (VDCs) in both DCs and HEs developed outward from approximately -20 mV. The onset became faster with further depolarization and showed no resolvable delay. The current-voltage curves in both DCs and HEs developed linearly to approximately 60 mV. However, these currents in DCs did not develop from approximately 60 mV and formed a plateau phase, as shown in Fig 1D. Those in HEs, on the other hand, were evoked linearly with depolarizing voltages and did not form a plateau phase, as shown in Fig 2D. It is known that VDOCs develop with at least two types of components, fast and slow.[4] In both DCs and HEs, as shown in Fig 1, 2, the fast component in outward currents became more prominent with large depolarizations and occurred with a time course of not more than a millisecond, whereas the slow component lasted for 5-30 ms.

One feature of outward currents in both types of cells was the implication of VD-K currents. Outward K currents are reduced by intracellular CsCl solution. When 140 mM KCl in the intracellular solution was exchanged for CsCl, whole-cell currents were evoked in both DCs and HEs with depolarizing voltages of above -20 mV, similar to that in the normal extracellurar solution. Outward currents, which were slightly evoked depending on depolarizing

voltages, were suppressed by above 90% as shown in Fig 1B, 1D, 2B, 2D. These results indicated that the VDOCs in DCs and HEs were mainly carried by K currents. The dependence of Ca^{2+} in outward K currents on the supporting cells (DCs and HEs) was examined using a Ca-free extracellular solution containing 5 mM EGTA. As shown in Fig 1C, 2C, whole-cell currents in both DCs and HEs were evoked with depolarizing voltages of above -20 mV, and the size of the evoked currents was essentially unchanged as compared with that in the normal extracellular solution. The fast components of rapidly activating outward K currents which occurred with a time course of less than 1 ms became dependent more prominently on voltage, as was the case in the normal extracellular solution. Slow components in both DCs and HEs were enhanced, depending on depolarizing voltages.

The steady state values of the membrane currents at different potentials for both types of cells in the Ca-free extracellular solution are illustrated in Fig 1, 2. The current-voltage curves in both DCs and HEs were linear, starting from approximately -20 mV, and no plateau phase formed from about 60 mV. However, currents evoked with depolarizing voltages in HEs in the Ca-free extracellular solution were the same as those in the normal extracellular solution.

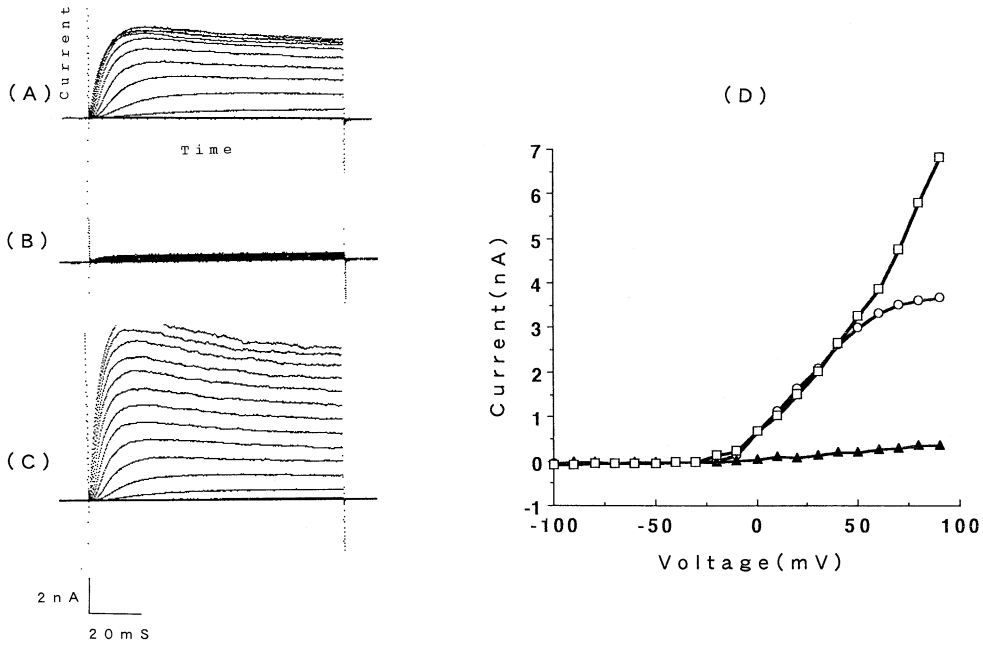

Fig 1. Voltage-dependent outward currents in DCs
Stimulation methods, as described in "Recording methods", are shown.
Sample records of voltage-dependent outward currents obtained with normal intracellular and extracellular solutions (A), with the intracellular solution containing 140mM CsCl instead of 140mM KCl (B), and with a Ca-free solution in place of the normal extracellular solution (C).
Current-voltage relation curves (D) obtained with the normal intracellular and extracellular solutions (○), with 140mM CsCl in the intracellular solution (▲), and with the Ca-free extracellular solution (□).

TEA-resistant component of the outward currents in DCs and HEs

Whole cell currents of the supporting cells in the extracellular solution containing 10 mM TEA appeared slightly with depolarizing voltages of above -20 mV, similar to the results obtained in the normal extracellular solution, but these currents rose more frequently than the control ones. Currents with large depolarizations were more strongly suppressed than those with small depolarizations, as can be seen in Fig 3B, 4B. Although outward currents in the normal extracellular solution activated fast and slow components, in the presence of 10 mM TEA most of the fast components of outward currents disappeared and activation of the currents was also delayed. These TEA-resistant currents had almost linear current-voltage curves, as can be seen in Fig 3D, 4D. However, these resistant currents in DCs did not develop from approximately 60mV and the current-voltage curves formed a plateau phase. Those in HEs, on the other hand, were evoked linearly with depolarizing voltages and did not form a plateau phase. The principal effect of TEA was to remove the fast component of outward K currents.

Effect of TEA in a Ca^{2+}-free solution

The Ca^{2+} dependence of TEA-resistant outward K currents in supporting cells (DCs and HEs) was examined using a Ca-free extracellular solution

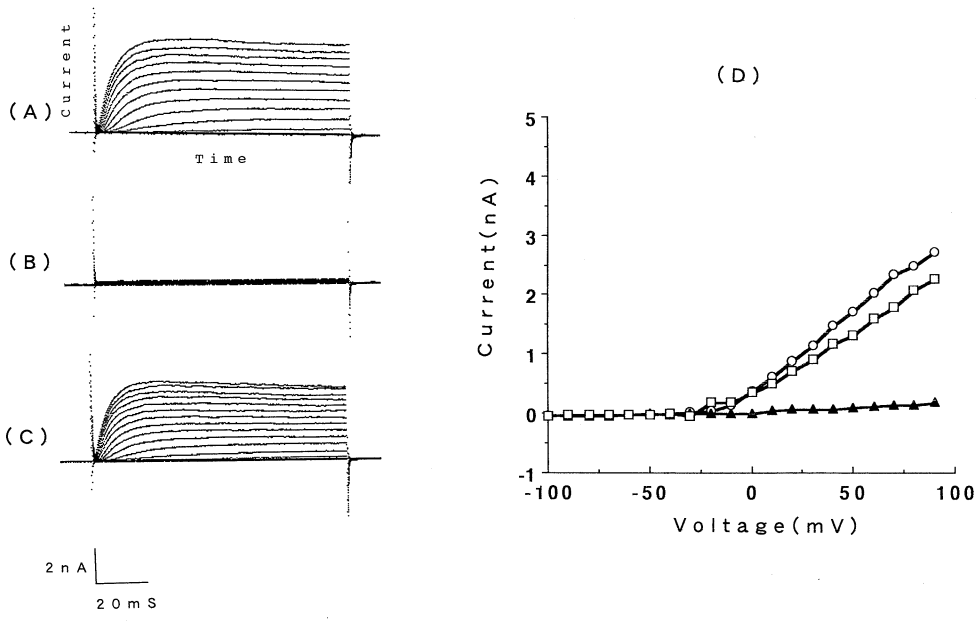

Fig 2. Voltage-dependent outward currents in HEs

Sample records of voltage-dependent outward currents obtained with the normal intracellular and extracellular solutions (A), with the intracellular solution containing 140mM CsCl instead of 140mM KCl (B), and with a Ca-free solution in place of the normal extracellular solutions (C).

The current-voltage relation curves (D) obtained with the normal intracellular and extracellular solutions (○), with 140mM CsCl in the intracellular solution (▲), and with a Ca free extracellular solution (□).

containing 5 mM EGTA and 10 mM TEA. The results for both DCs and HEs are shown in Fig 3C, 4C. The currents began to activate at about -20 mV, similar to those in the normal extracellular solution. Membrane currents were essentially unchanged as compared with those in the normal extracellular TEA solution, and the fast component of rapidly activated outward K currents disappeared in similar manner to those in the normal extracellular TEA solution. The TEA-resistant slow currents in both DCs and HEs had almost linear instantaneous current-voltage curves. These results in DCs suggested that the plateau phase of outward K currents, as shown in Fig 1, depended on the slowly activated Ca^{2+}-sensitive K currents with large depolarization. However, 10 mM TEA blocked from 10 to 30% of these slowly activated Ca^{2+}-sensitive K currents. On the other hand, the currents evoked with depolarizing voltages in HEs in the Ca-free extracellular solution were the same as those in the normal extracellular solution. Therefore, the HEs could also maintain VDO-K currents, but did not have voltage-gated Ca channels, like the DCs.

4-AP-resistant component of the outward currents in DCs and HEs

Since the report of Kenyon and Gibbons,[8] 4-AP has been used widely as a selective inhibitor of transient outward K currents, and it is known that 4-AP suppresses the slow component of outward K currents. In this experiment, we investigated whether the VDO-K currents in both DCs and HEs were sensitive or insensitive to 4-AP. 4-AP is a membrane permeant and acts on the intra-

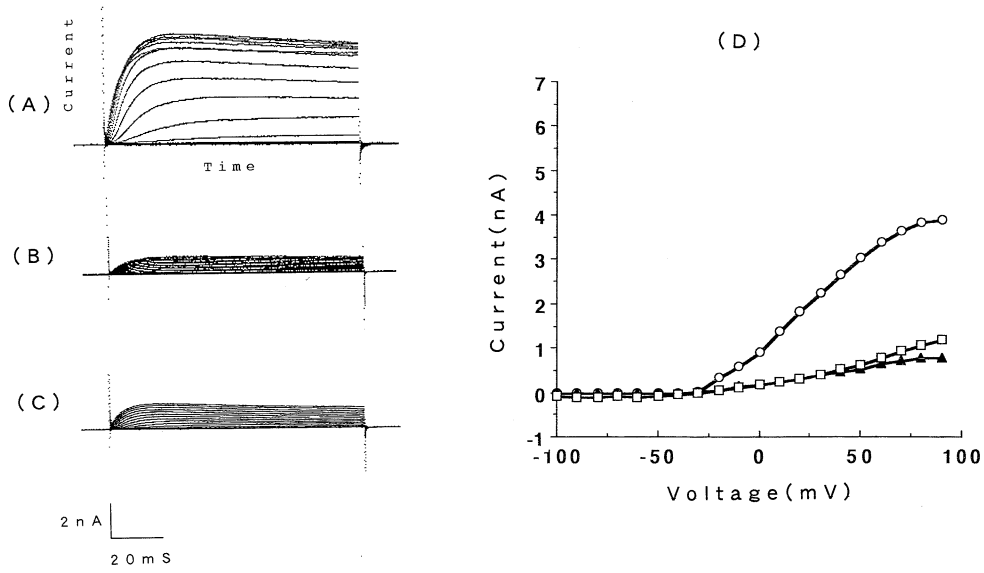

Fig 3. Effect of TEA on voltage-dependent outward currents in DCs

Sample records of voltage-dependent outward currents obtained with the normal intracellular and extracellular solutions (A), with the normal extracellular solution containing 10mM TEA (B), and with a Ca-free extracellular solution containing 10mM TEA (C).

Current-voltage relation curves (D) obtained with the normal intracellular and extracellular solutions (○), and with the normal (▲) and Ca-free extracellular solutions (□) containing 10mM TEA.

cellular side of the membrane. When 10 mM 4-AP was applied in the normal pipette solution, the whole-cell currents in DCs were slightly evoked with depolarizing voltages of above -20 mV and were activated with a delay, as shown in Fig 5.

As shown in Fig 5B, the slow component of the outward K currents in DCs was suppressed with depolarizing voltages by 4-AP, but 4-AP did not affect the fast components. These currents evoked with depolarizing voltages had almost linear instantaneous current-voltage curves. However, these curves were suppressed more than those in the normal intracellular solution, and no plateau phase occurred from approximately 60 mV, as shown in Fig 5B, 5D. In DCs, this 4-AP-resistant component increased with depolarizing voltages, and was partially blocked by an external 10 mM TEA solution, as shown in Fig 5C, 5D.

On the other hand, the slow components of the VDO-K currents in HEs were rarely evoked by 4-AP, as shown in Fig 6B, 6C. The slow components of the K currents in HEs were inhibited by 90% of those in Fig 6A. The TEA-resistant VDO-K currents were completely blocked by 10 mM 4-AP in the normal pipette solution. Therefore, it seems that nearly all of the K channels in HEs can be blocked by a combination of TEA and 4-AP at these concentrations.

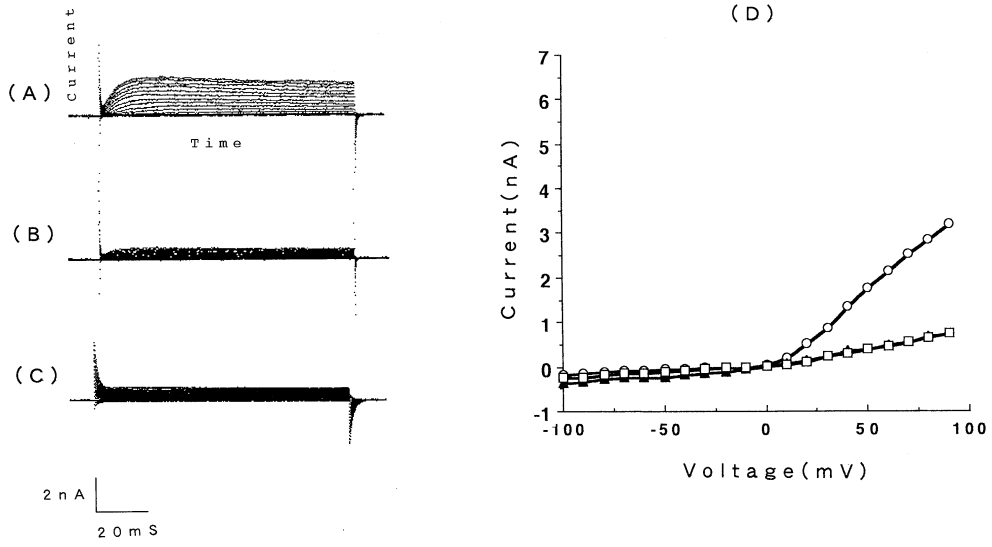

Fig 4. Effect of TEA on voltage-dependent outward currents in HEs

Sample records of voltage-dependent outward currents obtained with the normal intracellular and extracellular solutions (A), with the normal extracellular solution containing 10mM TEA (B), and with a Ca-free extracellular solution containing 10mM TEA (C).

Current-voltage relation curves (D) obtained with normal intracellular and extracellular solutions (○), and with the normal (▲) and Ca-free extracellular solutions (□) containing 10mM TEA.

DISCUSSION

The aim of this study was to determine whether VD-K currents exist in the supporting cells, which are related mechanically to the hair cells. These currents have previously been detected in hair cells, and they are inhibited by CsCl, TEA and 4-AP, which is a specific K channel blocker. Using conventional whole-cell recording techniques, we also investigated whether the VDO-K currents in the supporting cells of two types of cells (DCs and HEs) were the same as the VD-K currents in the hair cells.

As shown in Fig 1, 2, both types of cells had VDOCs which were activated by depolarizing voltages from approximately -20 mV. These outward currents were considered to be K currents, because they were almost completely suppressed by intracellular Cs ion. In previous DCs experiments, as shown in Fig 1, the formation of a plateau phase with depolarizing voltages from approximately 60 mV in current-voltage curves implicated VD-inward Ca currents or Ca-activated K currents, because a plateau phase of outward K currents with large depolarization disappeared in Ca-free extracellular solutions, as shown in Fig 1C, 1D. Therefore, depolarization and hence external Ca^{2+} may control K currents.

Dulon[9] showed that the application of isotonic high K solution (20-150 mM) to isolated DCs produced a rapid and transient increase in $[Ca^{2+}]_i$. The

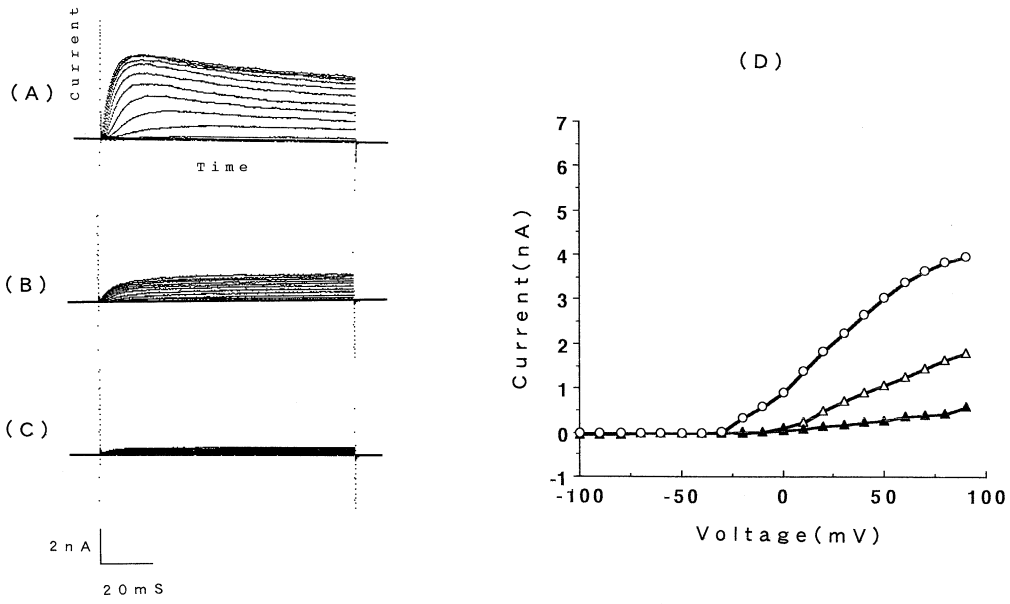

Fig 5. Effect of 10mM 4-AP and 10mM TEA on voltage-dependent outward currents in DCs

Sample records of voltage-dependent outward currents obtained with the normal intracellular and extracellular solutions (A), with the intracellular solution containing 10mM 4-AP (B), and with the intracellular solution containing 10mM 4-AP and the extracellular solution containing 10mM TEA (C).

Current-voltage relation curves (D) obtained with the normal intracellular and extracellular solutions (○), with the normal extracellular solution (△) and the extracellular solution containing 10mM TEA (▲) and with the intracellular solution containing 10mM 4-AP.

increment in $[Ca^{2+}]_i$ during K stimulation most likely came from a Ca^{2+} influx, because removal of extracellular free Ca^{2+} entirely suppressed the response. These findings suggest the presence of voltage-geted Ca channels in DCs. Therefore, it is suggested that VDO-K currents from approximately 60 mV in normal extracellurar solution are strongly dependent on Ca^{2+} entry, and that they disappear with Ca^{2+} influx. These results in DCs suggested the presence of VDO-K currents and voltage-gated Ca channels. On the other hand, the main currents in HEs were the VDO-K currents, but there were no voltage-gated Ca channels as with DCs. Thus, the main feature of membrane currents in the supporting cells seems to be VDO-K currents, and only in DCs were VD-Ca currents or Ca-activated K currents implicated with large depolarization. Therefore, the present results indicate a differential K sensitivity in terms of the extracellular Ca^{2+} response between the two types of supporting cells. It seems likely that the membrane currents in supporting cells are carried mainly by the K ion.

In general, TEA and 4-AP are used as blockers of VD-K channels and act by different mechanisms and possibly at different sites in the channels. TEA acts like an open channel blocker that can be displaced by permeant ions and by the closing of activation gates.[10] Fast and slow outward K currents can also be distinguished by their differential sensitivity to the K channel blocker TEA. When DCs were exposed to 10 mM TEA, the residual VDOCs were activated slightly at above -20 mV (Fig 3D), and there was an almost linear instaneous current-voltage curve. While these TEA-resistant outward currents showed approximately 20% of the control outward K current (Fig 3B), the current-voltage curve formed a plateau phase with large depolarizing voltages.

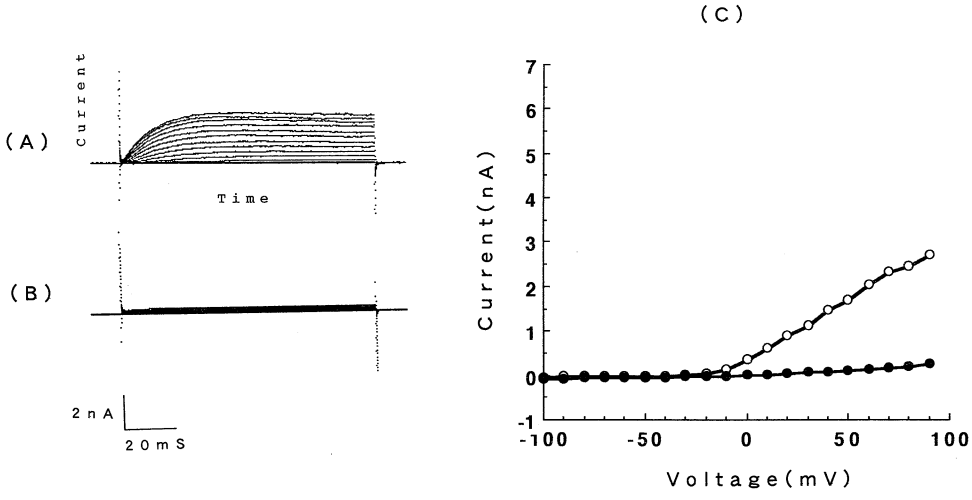

Fig 6. Effect of 10mM 4-AP on voltage-dependent outward currents in HEs
Sample records of voltage-dependent outward currents obtained with the normal intracellular and extracellular solutions (A), and with the intracellular solution containing 10mM 4-AP (B).
Current-voltage relation curves (C) obtained with the normal intracellular and extracellular solutions (○), and with the intracellular solution containing 10mM 4-AP (●).

These results indicate that the TEA-resistant outward currents, with the exception of slow components of outward K currents, developed with other currents. However, the VDO-K currents of DCs in the Ca-free extracellular solution had an almost linear instantaneous current-voltage curve (Fig 3C).

The size of these currents was same as that of the TEA-resistant currents. This result in DCs suggests that the plateau phase of outward K currents, as shown in Fig 1, depended on the slow activating Ca-sensitive K currents with large depolarizations. This may implicate VD-Ca currents or Ca-activated K currents. Thus, depolarization and hence external Ca^{2+} may control TEA resistant K currents.

Therefore, the rapidly activating outward K currents of guineapig DCs were Ca^{2+} dependent and relatively sensitive to TEA. There were similar in this respect to Ca-activated K currents observed in the OHC of lower vertebrates[11,12] and will be referred to as IK (Ca). The slow outward currents did not require Ca^{2+} influx for activation, and they were relatively insensitive to TEA. The TEA-resistant currents in HEs as shown in Fig 4, on the other hand, had an almost linear instantaneous current-voltage curve regardless of Ca ion. These results indicate that the outward K currents in DCs have components differ from those of HEs. Dulon[9] reported that a large portion of the outward K currents in DCs originates from Ca-activated K currents (KCa), and that these currents are similar to those described in OHC. However, in this study, the voltage-dependent outward K current was not suppressed by a Ca-free solution containing 5 mM EGTA, as shown in Fig 3. These results suggest that large portions (above 80%) of the outward K currents in DCs are not dependent on the Ca ion.

It is known that 4-AP is complicated by variable interaction with closed, open and inactivated channel states. As result, 4-AP is a slow open channel blocker. In this experiment, suppression of the slow components of the VDO-K currents in DCs increased with depolarizing voltages. Therefore, it is suggested that a portion of the slow components in the currents activated with depolarizing voltages in DCs was suppressed by 4-AP, and that TEA completely suppressed these components. It is proposed that inhibition of the Ca-activated K currents in hair cells by 4-AP[13] depressed outward K currents to a plateau phase in DCs with large depolarizing voltages.

From these results, it was concluded that the main currents in the supporting cells were voltage-dependent outward K currents. DCs have Ca-activated K currents evoked with high depolarizing voltage.

ACKNOWLEDGMENTS

Sincere thanks are due to Dr. H. Nishijima for helpful suggestions and discussions.

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