Two Types of K\(^+\) Currents Modulated by Arachidonic Acid in Bovine Corneal Epithelial Cells

Masayuki Takahira, Norimasa Sakurada, Yasunori Segawa, and Yutaka Shirao

**PURPOSE.** Fenamate sensitivity of the large-conductance K\(^+\) current in the corneal epithelium suggests that K\(^+\) transport could be modulated by arachidonic acid (AA) and/or its metabolites, which also regulate corneal epithelial migration. The main purpose of this study was to investigate AA-induced modulation of K\(^+\) currents expressed in the bovine corneal epithelium.

**METHODS.** Freshly isolated bovine corneal epithelial cells were perfused with Ringer solution. Whole-cell currents were recorded by using either the conventional whole-cell–patch or the perforated-patch configuration.

**RESULTS.** Two distinct types of K\(^+\) currents dominated the whole-cell current. The first was a voltage-gated K\(^+\) current that was inactivated completely by membrane depolarization. The inactivating voltage-gated K\(^+\) current was largest in presumptive basal cells. The second was a noisy, sustained K\(^+\) current that was never inactivated and seemed to be a counterpart of the large-conductance K\(^+\) current reported in the rabbit corneal epithelium. External application of AA (5–20 \(\mu\)M) inhibited the inactivating voltage-gated K\(^+\) current and augmented the noisy, sustained K\(^+\) current. Identical dual modulation was induced by other fatty acids (e.g., palmitoleic acid) that are not substrates for enzymes in the AA cascade.

**CONCLUSIONS.** An inactivating voltage-gated K\(^+\) channel was identified for the first time in the corneal epithelium. AA and some fatty acids may directly activate the large-conductance K\(^+\) channel to augment its housekeeping functions in corneal epithelial cells. (Invest Ophthalmol Vis Sci. 2001;42:1847–1854)

The corneal epithelium plays crucial roles in corneal function, providing a refractive surface and serving as a barrier. Epithelial migration and differentiation are important processes, not only for epithelial turnover but also for corneal wound healing. Recently, Watksy\(^1\) reported that a K\(^+\) current activated by fenamates is absent during corneal wound healing in rabbits. This nonactivating K\(^+\) current derives from a large-conductance K\(^+\) channel identified in freshly dissociated rabbit corneal epithelium, which constitutes the major K\(^+\) conductance in the basal membrane.\(^2\)\(^–\)\(^7\) A recent study of cultured human corneal epithelium demonstrated that fenamates activate two distinct types of K\(^+\) current.\(^8\) The sensitivity of these K\(^+\) channels to fenamates suggests that arachidonic acid (AA) and/or its metabolites, which have been reported to regulate corneal epithelial migration,\(^9\)\(^–\)\(^10\) could modulate corneal K\(^+\) channel activities, but details of this effect are unknown.

From studies of other tissues, it is thought that voltage-gated K\(^+\) channels, which play a role in generating the action potentials in excitable cells,\(^1\) may also participate in developmental regulation,\(^1\) including cell differentiation,\(^1\) proliferation,\(^1\)–\(^1\) apoptosis.\(^1\) However, to our knowledge no voltage-gated K\(^+\) current has been identified in the corneal epithelium.

In the present study, we discovered that in addition to a counterpart of the large-conductance K\(^+\) current, an inactivating voltage-gated K\(^+\) current dominated the whole-cell current in freshly isolated bovine corneal epithelial cells. Modulation of these K\(^+\) currents by AA and by some fatty acids was also investigated.

**METHODS**

**Solutions**

External and internal solutions were buffered with HEPES, as in previous patch–clamp studies of corneal epithelium\(^8\)\(^–\)\(^8\) and essentially the same as the solutions used in a patch–clamp study on the bovine retinal pigment epithelium (RPE).\(^1\) Briefly, the standard Ringer solution consisted of (in mM) 135 NaCl, 5.0 KCl, 10 HEPES, 10 glucose, 1.8 CaCl\(_2\), and 1.0 MgCl\(_2\) and was titrated to pH 7.4 with NaOH. The standard pipette solution consisted of (in mM) 30 KCl, 83 potassium gluconate, 5.0 HEPES, 5.5 EGTA-KOH, 0.5 CaCl\(_2\) (\(-10^{-8}\) M free Ca\(^{2+}\)), calculated using Calcium, a computer program in Basic\(^1\), and 2.0 MgCl\(_2\) and was titrated to pH 7.2 with KOH. In the whole-cell–patch configuration, 2 mM adenosine triphosphate (ATP; Mg\(^{2+}\) salt) was added to the pipette solution. In the perforated-patch configuration, 20 \(\mu\)M amphotericin B stock solution (1.2 mg amphotericin B/50 \(\mu\)l dimethyl sulfoxide (DMSO)) was added to 2.0 ml of the pipette solution to give a final concentration of 240 \(\mu\)g/ml. The cell isolation medium was similar to that used in a previous study of RPE\(^1\) and contained (in mM) 135 N-methyl-D-glutamine (NMDG)-Cl, 5.0 KCl, 10 HEPES, 3.0 EDTA-KOH, 10 glucose, 3.0 cysteine, 1.0 glutathione, 1.0 \(\alpha\)-ascorbic acid, 1.0 taurine, and 0.2 mg/ml papain (type III) and was titrated to pH 7.4 with NaOH.

In experiments in which the concentrations of K\(^+\), Cs\(^+\), or tetraethylammonium\(^+\) (TEA\(^+\)) were varied, NaCl was replaced by an equimolar amount of the appropriate Cl\(^-\) salt. Diltiazem was dissolved in DMSO and diluted in Ringer solution (DMSO <0.1%). Perfusates containing AA and other fatty acids were made just before the experiments by dilution of the stock solutions, in either methanol or DMSO. Throughout the experiments, final concentrations of methanol and DMSO in perfusates were no more than 0.1% and 0.2%, respectively, which by themselves did not affect the whole-cell currents in this study.

NaCl, KCl, CaCl\(_2\), and \(\alpha\)-ascorbic acid were obtained from Wako Chemical Co. (Osaka, Japan). The remaining reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

**Cell Isolation**

All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Bovine eyes were enucleated at a local abattoir just after death and

---

From the Department of Ophthalmology, Kanazawa University School of Medicine, Japan.

Supported by Grant-in-Aid 09771414 and 11771043 from the Ministry of Education, Japan (MT).

Submitted for publication October 6, 2000; revised February 27, 2001; accepted March 9, 2001.

Commercial relationships policy: N.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Masayuki Takahira, Takara-Machi 13-1, Department of Ophthalmology, Kanazawa University School of Medicine, Kanazawa 920-8640, Japan. takahira@kenroku.kanazawa-u.ac.jp
transferred to the laboratory within 30 minutes. A 5 × 5 to 8 × 8 mm square piece of the epithelium–stroma was cut from the central cornea and was incubated in the cell isolation medium for 10 minutes. The tissue was transferred to standard Ringer solution containing 0.1% bovine serum albumin for 3 minutes and incubated in the standard Ringer solution for 10 minutes followed by gentle vortexing. This series of incubations was repeated two or three times before isolated cells were seen in a sample of the suspension under microscopy. The cell suspension was stored for up to 24 hours at 4°C before use.

**Cell Perfusion**

The isolated corneal epithelial cells were transferred to a lucite perfusion chamber (RC-5/25; Warner Instruments, Hamden, CT) and settled for 10 to 20 minutes before perfusion. The perfusate flowed into the chamber under gravity at a flow rate of 0.7 ml/min by a perfusion system (BPS-4; ALA Scientific Instruments, Westbury, NY) and was continuously removed by suction. Fluid height was adjusted to give a chamber volume of approximately 0.5 ml and a complete solution exchange within 2 minutes. All experiments were conducted at room temperature (20–25°C).

**Electrophysiological Methods**

Patch pipettes were pulled from borosilicate glass tubing (BF150–110-3; Sutter Instruments, San Rafael, CA) with a multistage programmable puller (P-97; Sutter Instruments). The pipette input resistance was between 1 and 3 MΩ. Under phase-contrast microscopy (Eclipse TE300; Nikon, Tokyo, Japan), a target cell was selected (see the Results section), and the pipette tip was pressed onto the cell membrane by using a micromanipulator (MP-285; Sutter Instruments) to establish a gigaohm seal. Currents under voltage clamp were recorded by an amplifier system (EPC-8; Heka, Lambrecht, Germany). The built-in low-pass filter was set to 3 kHz, unless noted otherwise. Recordings were referenced to an Ag-AgCl electrode (EP-2; WPI, Sarasota, FL). The membrane capacitance was compensated by built-in circuits. The apparent membrane potential was corrected by the pipette tip potential (10 mV). Statistical data are presented as mean ± SD. Data were fitted by a nonlinear least-squares fitting on computer (IGOR Pro software; Wavemetrics, Lake Oswego, OR).

**RESULTS**

**Isolated Corneal Epithelial Cells**

Cells with thick membrane and dense intracellular material under phase-contrast microscopy (magnification, ×400) were selected for recording. Relatively large (>25 μm in diameter) and flat cells, indicating superficial cells, were excluded. Cells most commonly observed were relatively small (15–20 μm in diameter) and round, showing poor polarity (Fig. 1). Some cells, however, appeared columnar with distinct polarity (Fig. 1). The latter were morphologically similar to the basal cells in a vertical slice of the intact bovine cornea (not shown). However, we could not determine precisely which layer of the corneal epithelium (superficial, midepithelial, or basal) the round cells came from. Therefore, data from cells with and without polarity were pooled in this study.

**Electrophysiological Parameters**

In the perforated-patch configuration, the series resistance ($R_s$) and the membrane capacitance ($C_m$) were calculated from uncompensated capacitative transients. $\frac{R_s}{C_m}$ averaged respectively 32 ± 13 MΩ and 14 ± 4 pF for the perforated-patch recordings ($n = 154$). $R_s$ and $C_m$ could not be precisely determined, because capacitative transients were too fast ($\tau < 0.1$ msec), although $R_s$ was usually less than 10 MΩ. The zero-current potential ($V_0$) measured after establishment of the

![Figure 1](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933589/)  
Photomicrograph of isolated bovine corneal epithelial cells. A columnar cell was clamped by a patch pipette. Another round cell is also seen. Bar, 30 μm.

![Figure 2](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933589/)  
Representative whole-cell currents expressed in the bovine corneal epithelial cells. In each cell (A, B, and C), whole-cell currents were elicited by 200-msec voltage steps ranging from +50 to −50 mV in −20-mV increments from a holding potential (HP) of −70 or −10 mV. The different scales for the current amplitudes are shown. Current-voltage (I-V) relationships are depicted for each cell (bottom panels) with HP of −70 (●) and −10 mV (○). Current amplitudes were averaged between 100 and 200 msec for the I-V plots in (A) and (C). For the I-V plots in (B), the peak amplitudes were measured when the inactivating voltage-gated K⁺ current activated. Otherwise, steady state current amplitudes (180–200 msec) were used.
whole cell mode was $-24.2 \pm 9.9$ mV in 188 cells bathed with the standard Ringer solution. These values were less negative than those of the rabbit corneal epithelium reported in several studies.$^{3-7}$ In another study in rabbit, Watanabe et al.$^{20}$ reported that dissociated corneal epithelial cells had considerably less negative potentials ($>-10$ mV), which might be caused by cell dissociation or storage.

**FIGURE 3.** Activation and inactivation of the voltage-gated $K^+$ current. (A) Activation kinetics. Membrane depolarization in response to the voltages indicated activated currents with a sigmoid time course. Currents were recorded in the conventional whole-cell configuration with a low-pass filter of 5 kHz. The smooth curve is the fit of the data to the Hodgkin-Huxley $n^2$ model as follows:

$$I = I_{\text{max}}(1 - e^{-t/\tau_{\text{act}}})^2,$$

with $\tau_{\text{act}}$ of 4.60 (+20 mV), 6.49 (+10 mV), and 9.85 msec (0 mV). (B) Inactivation kinetics. The smooth curves are exponential fits of the data at voltages ranging from +40 to −10 mV in −10-mV increments with $\tau_{\text{inact}}$ of 498, 505, 692, 775, 1137, and 1708 msec, respectively. (C) Steady state inactivation. Peak currents elicited by voltage pulses to −40 mV after 30-second prepulses to various test potentials were measured (inset) and the normalized mean values were plotted (n = 3). The smooth curve is the least-square fit of the data to a Boltzmann function as follows:

$$I/I_{\text{max}} = 1 / [1 + \exp((-V - V_{1/2})/k_n)],$$

with $V_{1/2}$ (the prepulse voltage at which the current is half-maximum) of −30.0 mV and $k_n$ (the steepness of the slope) of +6.3 mV.

**FIGURE 4.** Voltage dependence of the inactivating voltage-gated $K^+$ current. (A) Tail-current analysis to determine the reversal potential. Tail currents due to channel deactivation were produced by membrane repolarization (range: −75 to −55 mV) after a brief depolarization that fully activated the channel. The filter was set to 5 kHz. The reversal potential was determined from plots of tail-current magnitudes calculated by exponential fit of the data, as representatively shown (inset). In this cell, the reversal potential was $-68$ mV. (B) Conductance-voltage relationship. Conductance ($G$) of the inactivating voltage-gated $K^+$ channel in each cell was calculated using the equation: $G = I_p / (V - V_r)$, where $I_p$ is the peak current and $V_r$ is the reversal potential determined by tail-current analysis as in (A). Conductance-voltage plots were well fitted by a Boltzmann function:

$$G = G_{\text{max}} / [1 + \exp((-V - V_{1/2})/k_p)],$$

with $V_{1/2}$ of −5.5 mV and $k_p$ of −7.6 mV.
though the relationship between $K_1$ large in amplitude when we selected the columnar cells, al-
at $K_1$ age-gated $K_1$ the inactivating current seemed to derive from a type of volt-
was negligible (Fig. 2B). In voltage dependence and kinetics, the inactivating current was dominant, whereas the noisy current
than 500 pA (no leak subtraction, mean $944 \pm 6$ elicited by a voltage step to
analyzed systematically.

cell types (superficial, midepithelial, or basal) could not be
Currents generated by membrane depolarizations to $+30$ mV from a
potential of $-70$ mV in absence and presence of 0.5 mM TEA$^+$.
$K_1$ial function (Fig. 3B). The time constant of inactivation ($\tau_{\text{inact}}$) of 375 (control) and 395 msec (TEA$^+$).

Whole-Cell Current

Figure 2A shows an example of whole-cell currents recorded from a bovine epithelial cell using the standard Ringer and pipette solutions. When the membrane was held at $-70$ mV, voltage steps to test potentials greater than 0 mV (upper panel) generated noisy currents that rose to a peak and decreased slowly, indicating channel inactivation. In contrast, when the holding potential was set at $-10$ mV (lower panel), the inactivating component was not elicited by voltage steps to the same potentials (>0 mV), but noisy outward currents remained. Therefore, the bovine corneal epithelium apparently exhibited two types of outwardly rectifying currents: The first was an inactivating, voltage-dependent current, and the other was a noisy current that was independent of the holding potential. The latter seems to be a counterpart of the large-conductance $K^+$ current in rabbit corneal epithelial cells$^2$-$^7$ (described later).

Magnitudes of these two types of $K^+$ currents varied considerably between cells. In 40 cells where the peak current elicited by a voltage step to $+50$ mV from $-70$ mV was larger than 500 pA (no leak subtraction, mean $944 \pm 333$ pA), the inactivating current was dominant, whereas the noisy current was negligible (Fig. 2B). In voltage dependence and kinetics, the inactivating current seemed to derive from a type of voltage-gated $K^+$ channel that has been reported in other unexcitable cells.$^1$ The inactivating voltage-gated $K^+$ current was expressed in 150 of 188 total cells. In the remaining 38 cells, only the noisy current was expressed (Fig. 2C). Currents elicited by membrane depolarization from a holding potential of $-70$ mV showed no decay, indicating no inactivation. Of interest, the inactivating voltage-gated $K^+$ current was often large in amplitude when we selected the columnar cells, although the relationship between $K^+$ current expression and cell types (superficial, midepithelial, or basal) could not be analyzed systematically.

General properties of the two $K^+$ currents are shown separately in the following sections.

Inactivating Voltage-Gated $K^+$ Current

The activation threshold of the inactivating voltage-gated $K^+$ current in the bovine corneal epithelium was usually approximately $-30$ mV (Fig. 2B), which is similar to that of native delayed rectifier-type $K^+$ currents in other cells.$^1$ The activation kinetics became faster as the membrane potential was made more positive (Fig. 2B). Although current activation was too fast to model kinetics precisely, the activation time course seemed to be well fitted by the Hodgkin-Huxley $n^2$ model$^4$ (Fig. 3A).

The inactivation time course was well fitted by an exponential function (Fig. 3B). The time constant of inactivation ($\tau_{\text{inact}}$) at $+40$ mV for 2 seconds was $615 \pm 135$ msec in seven cells recorded with the conventional whole-cell configuration. Considering this value of $\tau_{\text{inact}}$, we would call this delayed rectifier, rather than A-type $K^+$ current, because of its slow rate of inactivation ($\tau_{\text{inact}} > 100$ msec).

The voltage dependence of steady state inactivation (Fig. 3C) indicates that maximal conductance of the inactivating voltage-gated $K^+$ current could be obtained when the membrane was depolarized from a holding potential of $-70$ mV and that the inactivation was almost complete when the holding potential was $-10$ mV. Because the complete recovery from inactivation after full activation took approximately 10 seconds at $-70$ mV (data not shown), the interval between voltage steps was set at 15 seconds in all trials.

Membrane repolarization around the peak of the inactivating voltage-gated $K^+$ current produced a tail current, due to channel deactivation (Fig. 4A). In seven cells in which the tail current was analyzed (Fig. 4A, inset) the reversal potential was $-71.9 \pm 4.2$ mV, indicating high selectivity to $K^+$ ($E_K = -80$ mV). The conductance–voltage relationship of the inactivating voltage-gated $K^+$ current (Fig. 4B) shows that its activation threshold was positive to $-30$ mV and that the voltage at which conductance was half maximum ($V_{1/2}$) was $-7.6$ mV. Conductance declined at a membrane potential more positive than $+30$ mV.

FIGURE 5. TEA$^+$-induced inhibition of the voltage-gated $K^+$ current. Currents generated by membrane depolarizations to $+30$ mV from a holding potential of $-70$ mV in absence and presence of 0.5 mM TEA$^+$. Smooth traces are exponential fits of inactivating current decays with $\tau_{\text{inact}}$ of 375 (control) and 395 msec (TEA$^+$).

FIGURE 6. Effects of niflumic acid (NA) on $K^+$ currents. Families of whole-cell currents elicited by voltage steps (range: $+50$ to $-50$ mV) from a holding potential of $-10$ mV in absence and presence of 0.5 mM NA. Currents in the current–voltage graph represent averaged values between 100 and 200 msec at each voltage. (●) Control; (○) NA. Continuous curves are data generated by a voltage ramp protocol. Note crossover of curves near $E_K$.
External application of TEA\(^+\), one of the classic K\(^+\) channel blockers, inhibited the inactivating voltage-gated K\(^+\) current in a dose-dependent manner. The inhibition was almost complete at a concentration of 5 mM (seven of seven cells, data not shown). At a lower concentration (0.5 mM), TEA\(^+\) inhibited the peak amplitude partially without changing the inactivation kinetics (Fig. 5, similar results in the other four cells). Another classic K\(^+\) channel blocker, 4-aminopyridine (4-AP), inhibited the inactivating voltage-gated K\(^+\) current completely at a concentration of 2 mM (\(n = 4\)). The inactivating voltage-gated K\(^+\) current was relatively insensitive to external 20 \(\mu\)M ampin (6.7% \pm 6.5% inhibition in three cells), a known blocker of some A-type K\(^+\) currents.\(^{11}\) External 20 \(\mu\)M diltiazem, which completely blocks the large-conductance K\(^+\) current in the rabbit corneal epithelium, partially inhibited the inactivating voltage-gated K\(^+\) current (29% \pm 18% inhibition of peak amplitudes in four cells). The inactivating voltage-gated K\(^+\) current was sensitive to 2 mM Ba\(^{2+}\) (100% inhibition in two cells) but not to 2 mM Cs\(^+\) (1% \pm 2% inhibition in three cells).

**Sustained K\(^+\) Current**

In some cells the only major current was a noisy, outwardly rectifying current that was never inactivated (Fig. 2C). Previous studies of rabbit corneal epithelium have shown that external fenamates enhance a noisy K\(^+\) current known as the large-conductance K\(^+\) current.\(^4\) In bovine corneal epithelial cells, external application of the fenamate, nilfumic acid (500 \(\mu\)M), markedly augmented a noisy current that reversed near \(E_K\), identifying it as a K\(^+\)-selective current (Fig. 6, similar results in the other three cells). Another fenamate, flufenamic acid (100 \(\mu\)M), also stimulated the noisy, sustained K\(^+\) current (\(n = 3\), data not shown). These results indicate that bovine corneal epithelial cells express a counterpart of the large-conductance K\(^+\) current.

**Modulation by AA**

Fenamates are well-known inhibitors of the cyclooxygenase pathway in the AA cascade, which suggests that the fenamate-induced activation could be caused by the buildup of AA and/or changes in the levels of certain AA metabolites of the cytochrome P-450 oxygenase and lipoxygenase pathways. External application of AA (10 \(\mu\)M) mimicked the effect of fenamates (Fig. 7A). Again, the reversal potential was near \(E_K\). Similar results were obtained in all 23 cells perfused with AA (5 or 10 \(\mu\)M). The current at +50 mV increased from 99 \pm 79 pA in the standard Ringer solution to 491 \pm 424 pA after exposure to AA (5 or 10 \(\mu\)M in 23 cells, Fig. 7B) for 10.0 \pm 2.5 minutes, which is significant by a paired \(t\)-test (\(P = 0.000046\)).

![Figure 7](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933589/)

**Figure 7.** Effects of AA on K\(^+\) currents. (A) Families of whole-cell currents elicited by voltage steps (range: +50 to –50 mV) from a holding potential of –10 mV in absence and presence of 10 \(\mu\)M AA. The average currents between 100 and 200 msec are depicted in the current-voltage graph. (B) Control; (C) AA. (A, bottom) Time course of the current augmentation (at +40 mV) in the same experiment. (B) AA-induced current augmentation in all the trials (\(n = 23\)). Paired data of the current at +50 mV in absence and presence of AA in each cell are connected by the line. Cells were exposed to AA for 10.0 \pm 2.5 minutes (range: 6–18 minutes, \(n = 23\)). Note the logarithmic current scale. (C) Inhibition by diltiazem. Current-voltage relationships were obtained in 80 mM [K\(^+\)] Ringer solution (●), in additional 10 \(\mu\)M AA (●), and in AA with 20 \(\mu\)M diltiazem (○). The theoretical \(E_K\) was approximately –18 mV. (D) Currents produced by a voltage step from –70 to +60 mV in the control Ringer solution and in the presence of 2 and 10 \(\mu\)M AA. The inactivating voltage-gated K\(^+\) current was inhibited partially in 2 \(\mu\)M AA and almost completely in 10 \(\mu\)M AA. The increase in current caused by increasing AA from 2 to 10 \(\mu\)M was due to the augmentation of the noisy, sustained K\(^+\) current.
potent inhibitor of the inactivating voltage-gated K+ current, did not affect the noisy K+ current (n = 4). These results regarding the voltage-dependence, kinetics, and pharmacologic properties of the noisy, sustained K+ current enhanced by AA further support the idea that it is a counterpart of the large-conductance K+ current described in the rabbit corneal epithelium.2–7

AA-induced activation of the sustained K+ current was unaffected by preincubation of cells with either lipoxygenase inhibitors (10 μM 5,8,11-eicosatrienoic acid [ETI], n = 2 and 10 μM nordihydroguaiaretic acid [NDGA], n = 3) or a cytochrome P-450 oxygenase inhibitor (10 μM clotrimazole, n = 3; data not shown), indicating that metabolites of these pathways did not mediate the response.

In cells expressing the inactivating voltage-gated K+ current, external application of 10 μM AA blocked it completely before the augmentation of the noisy, sustained K+ current developed (in seven of seven cells, Fig. 7D). A lower concentration of 2 μM AA inhibited the peak current partially and accelerated the inactivation kinetics markedly (n = 4, Fig. 7D). This inhibition was qualitatively different from that of TEA+, which did not affect inactivation kinetics (Fig. 5). The AA-induced activation of the noisy, sustained K+ current is also shown in Figure 7D.

Modulation by Fatty Acids

To date, many studies have reported that AA modulates K+ channels.22 In smooth muscle, several fatty acids that are not substrates for enzymes in the AA cascade mimic the AA-induced K+ channel activation, suggesting that AA activates the K+ channel as a direct mediator.23 We also investigated whether fatty acids affect the two types of K+ channels in the bovine corneal epithelium. Figure 8A shows a representative example in which palmitoleic acid (20 μM) mimicked the effect of AA on the inactivating voltage-gated K+ current—that is, the peak current decreased and the inactivation kinetics accelerated. In 10 cells, 20 μM palmitoleic acid inhibited the peak current at +50 mV by 51% ± 22% and decreased the inactivation time constant (τinact) to less than 50 msec. The effects of AA and other fatty acids on the inactivating voltage-gated K+ current are summarized in Table 1. Palmitoleic acid and linolelaidic acid, as well as AA, inhibited the peak current but accelerated the inactivation. Myristic acid and oleic acid inhibited the peak current partially without changing the kinetics (data not shown).

In addition to the inhibitory modulation of the inactivating voltage-gated K+ current, these fatty acids had a stimulatory effect on the noisy, sustained K+ current that mimicked the AA-induced activation. Figure 8B shows an example in which palmitoleic acid (50 μM) augmented the sustained K+ current markedly. In 12 of 17 cells, the sustained K+ current increased in the presence of 20 or 50 μM palmitoleic acid. Effects of other fatty acids on the sustained K+ current are summarized in Table 1. Fatty acids that accelerated inactivation of the voltage-gated K+ current were potent stimulators of the sustained K+ current as well.

DISCUSSION

We identified two types of dominant macroscopic K+ currents in the bovine corneal epithelial cells. The first is an inactivating K+ current with characteristics identifying it as a member of the voltage-gated K+ channel family. So far, this is the first description of an inactivating voltage-gated K+ current in the corneal epithelium. Another is a noisy, sustained K+ current that resembles the large-conductance K+ current in the rabbit. Previous studies have shown that the rabbit large-conductance K+ current was un abbreviated by pre-incubation of cells with either lipoxygenase inhibitors (10 μM 5,8,11-eicosatrienoic acid [ETI], n = 2 and 10 μM nordihydroguaiaretic acid [NDGA], n = 3) or a cytochrome P-450 oxygenase inhibitor (10 μM clotrimazole, n = 3; data not shown), indicating that metabolites of these pathways did not mediate the response.

In cells expressing the inactivating voltage-gated K+ current, external application of 10 μM AA blocked it completely before the augmentation of the noisy, sustained K+ current developed (in seven of seven cells, Fig. 7D). A lower concentration of 2 μM AA inhibited the peak current partially and accelerated the inactivation kinetics markedly (n = 4, Fig. 7D). This inhibition was qualitatively different from that of TEA+, which did not affect inactivation kinetics (Fig. 5). The AA-induced activation of the noisy, sustained K+ current is also shown in Figure 7D.

**FIGURE 8.** Effects of palmitoleic acid (PA) on K+ currents. (A) Effects of PA on the inactivating voltage-gated K+ current. The voltage-gated K+ current was elicited by a voltage step to +50 mV from −70 mV in absence and presence of 20 μM PA. Subsequent addition of 2 mM 4-AP blocked the inactivating voltage-gated K+ current completely. (B) PA-induced augmentation of the noisy, sustained K+ current. Families of whole-cell currents were generated by voltage steps (range: +40 to −60 mV) from a holding potential of −10 mV in absence and presence of 50 μM PA. The average currents between 100 and 200 msec are depicted in the current-voltage graph. (●) Control; (○) PA.
The large-conductance K\(^+\) channel participates in cell migration, proliferation, and differentiation, not only in excitable cells 


