

Expression of Potassium Channels in Epithelial Cells Depends on Calcium-activated Cell-Cell Contacts

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Abstract. Harvesting MDCK cells with trypsin-EDTA reduces potassium currents (I_K) to a mere 10%, presumably by hydrolysis of K^+ channels, but replating at confluence restores them in 12–18 hr, through a process that requires transcription, translation and exocytic fusion of intracellular membrane vesicles to the plasma membrane (Ponce & Cerejido, 1991; Ponce et al., 1991a). In the present work we find that this restoration of I_K also requires cell-cell contacts and the presence of 1.8 mM Ca^{2+} . The role of extracellular Ca^{2+} may be substituted by $2.0 \mu\text{M TRH}$, 10 nM PMA or $200 \mu\text{g/ml DiC8}$, drugs that stimulate the system of phospholipase C (PLC) and protein kinase C (PKC). Conversely, the recovery of I_K triggered by Ca-dependent contacts can be blocked by $110 \mu\text{M neomycin}$, $2.0 \mu\text{M H7}$, and $250 \text{ nM staurosporine}$, inhibitors of PLC and PKC. These results suggest that the expression of new K^+ channels depends on Ca^{2+} -activated contacts with neighboring cells and that the information is conveyed through PLC and PKC, a process in keeping with changes in its enzymatic activity and cellular distribution of PKC. Plasma membrane is also reduced and restored upon harvesting and replating, and depends on Ca^{2+} -activated contacts. However, the effects of the chemicals tested on I_K differ from the ones they elicit on the recovery of plasma membrane, suggesting that cells can independently regulate their population of K^+ channels and the surface of their membrane.

Key words: Epithelia — K^+ channels — Expression of ion channels — Ca^{2+} — Membrane area — Cell-cell contacts

Introduction

Cells of transporting epithelia establish tight junctions (TJs) and translocate substances vectorially from one compartment to another, thanks to a polarized distribution of transporting mechanisms in their apical and basolateral domains (Cerejido et al., 1980). The understanding of the mechanisms that form TJs and polarize the cells has been appreciably advanced by the use of model systems constituted by epithelial cell lines cultured as monolayers (Cerejido et al., 1978, 1980). In a previous work we found that MDCK cells (epithelioid of renal origin) express five types of K^+ channels and one of Cl^- , which are distributed in a strictly polarized manner: four types of K^+ channels are apical, a fifth is basolateral, and the only type of Cl^- channel found is exclusively located on the basolateral side (Bolívar & Cerejido, 1987; Ponce & Cerejido, 1991; Ponce et al., 1991a; Ponce, Contreras & Cerejido, 1991b).

Harvesting with trypsin-EDTA reduces the overall potassium current (I_K) to a mere 10% of pretrypsination levels, presumably by hydrolysis of the extracellular domains of the peptides constituting the channels. Restoration of I_K is achieved in 12–20 hr upon replating the cells at confluence, by a process that is blocked by inhibitors of transcription (actinomycin D), translation (cycloheximide), exocytic fusion (chloroquine), microfilaments (cytochalasin B) and microtubule assembly (colchicine), suggesting that it requires the synthesis of new K^+ channels (Ponce et al., 1991a). This indicates that the expression of new K^+ channels follows the same time course and depends basically on the same cellular mechanisms responsible for TJ formation (Contreras et al., 1992a). Junction formation also requires calcium-dependent contacts between neighboring cells (González-Mariscal, Chávez de Ramírez & Cerejido, 1985;

González-Mariscal et al., 1990). In the case of junction formation, Ca²⁺ is needed on an extracellular site (Contreras et al., 1992*a,b*) probably constituted by a uvomorulin molecule (Gumbiner & Simons, 1987), and the information that cell-cell contact has been established is transduced to the intracellular mechanisms that assemble and seal the TJ through a cascade of reactions involving phospholipase C (PLC) and protein kinase C (PKC) (AC) (Balda et al., 1991, 1993). Therefore, in the present work we investigate whether the expression of K⁺ channels depends likewise on cell-cell contacts and the presence of Ca²⁺, and involves PLC, and PKC.

Materials and Methods

CELL CULTURE

Starter MDCK cultures were obtained from the American Type Culture Collection (MDCK, CCL-34). In most experiments, cells were between 60–90th passage. They were grown at 36.5°C in disposable plastic bottles (Costar 3150, Cambridge, MA) with an air-5% CO₂ atmosphere (VIP CO₂ incubator 417, Lab. Line Instruments, New Brunswick, NY) and 20 ml of Dulbecco's modified Eagle's basal medium (DMEM, GIBCO, 430–1600, Grand Island, NY) with 100 U/ml of penicillin, 100 µg/ml of streptomycin (In Vitro, México, D.F.) and 10% fetal calf serum (Boehringer Mannheim, Germany).

MEMBRANE CURRENTS AND CAPACITANCE

Monolayers of cells cultured on glass coverslips were deposited on a flat chamber fixed to the stage of an inverted Diavert microscope (Leitz, Wetzlar) equipped with Hoffman optics. Membrane currents were studied in the whole-cell clamp configuration as previously described (Bolívar & Cerejido, 1987). Recordings were made through a Dagan 8900 amplifier (Minneapolis, MN) using micropipettes pulled from borosilicate glass (Corning, I.D. 1.1–1.2 mm) prepared in a Brown-Flaming type micropipette puller (Sutter Instruments, Novato, CA). Micropipettes were fire-polished and had a resistance of 2–3 MΩ. They were mounted in a pipette holder attached to a hydraulic micromanipulator (Narishige MF83, Tokyo, Japan). All recordings reported were obtained after seals of at least 5 GΩ. Sealing and recordings were monitored with a Tektronix 2212 oscilloscope (Beaverton, OR). Whole-cell currents were recorded through Ag-AgCl electrodes and filtered at 1 kHz. Signals were digitalized through an analogue/digital converter (Labmaster TM-125, DMA, Scientific Solutions) and analyzed with a 80486-33 MHz based PC type computer. Stimulation was made with the Clampex program and analysis was performed with the Clampan program (pCLAMP version 5.5, Axon Instruments, Foster City, CA). Capacitive currents used to estimate the area of plasma membrane were filtered at 10 kHz and recorded at 100 kHz.

PROTEIN KINASE C (PKC) ASSAY

PKC specific activity of cells extracts was determined with the Protein Kinase C Assay System (GIBCO BRL, 3161SA), which measures the transference of γ phosphate from [γ-³²P]ATP (neg-002A, DuPont) to a synthetic peptide containing the PKC substrate consensus sequence, as described by Yasuda et al. (1990).

FLUORESCENCE CYTOLOGICAL DETECTION OF PKC

We used a rhodamine-conjugated bisindolylmaleimide PKC inhibitor (rim-1) (Teflabs, Austin, TX) that targets the catalytic site of this enzyme. Glass coverslips containing MDCK monolayers, cultured under several of the conditions described below, were rinsed twice with PBS, fixed and permeabilized with –20°C methanol for 45 sec, washed with PBS, incubated with 3% fetal bovine serum in PBS for 30 min, and treated with rim-1 for 1.0 hr. They were then washed twice with PBS and mounted in *p*-phenyldiamine-glycerol (1:9) and examined with a confocal microscope (MRC-600, Bio-Rad).

SOLUTIONS, ABBREVIATIONS AND SOURCE OF CHEMICALS

Intracellular solutions contain (mM): K⁺ 141, Na⁺ 5.0, Ca²⁺ 1.0, EGTA 2.3 (Free Ca²⁺ 3 × 10⁻⁷), Mg²⁺ 1.0, glucose 14, HEPES 10, methanesulfonic acid 153, pH 7.4. The bathing medium contains (mM): K⁺ 5.0, Na⁺ 141, Ca²⁺ 5.0, Mg²⁺ 1.0, glucose 16, HEPES 10, methanesulfonic acid 158, pH 7.4.

DiC8 (1,2-dioctanoylglycerol) and EGD (ethylene glycol dioctanoate) were from Molecular Probes (Eugene, OR). dB-cAMP (N⁶,-O²-dibutyryl adenosine 3'-5'-cyclic monophosphate) and staurosporine were from Sigma Chemical (St. Louis, MO). NC and LC refer to media with low (1–5 µM) and normal Ca²⁺ (1.8 mM); respectively.

Results are expressed as mean ± SE (number of observations).

Results

As mentioned above, MDCK cells harvested from confluent monolayers with trypsin-EDTA lose half of their surface membrane and 90% of their *I*_K, but recover them in 12–20 hr after replating at confluence. Accordingly, to investigate the effect of cell-cell contacts and Ca²⁺ on this recovery, we delivered the protocol of pulses shown in Fig. 1A to cells that had been just plated, and to cells that had been allowed to recover for a given length of time. Protocols consisted of two parts. In the first one, the holding potential was suddenly switched from –80 to –75 mV to elicit a transient of capacitive current, and sampling was made at 100 khz (Fig. 1B, left). This maneuver was repeated 10 times and averaged. By dividing the value of the area under the transient by the voltage step, we calculated membrane capacitance. Using the equivalence of 1 µF/cm² of membrane we then transformed capacitance into membrane area. The second protocol consisted of a series of voltage steps, each lasting 500 msec, delivered to record the current response. A P/4 procedure was used to eliminate linear components (Fig. 1A, right). Figure 1 shows capacitive transients (left) and ion currents recorded from a cell in a mature monolayer (B), a cell recently plated (C), and a cell plated and allowed to recover for 18 hr (D). Considering that (i) records exhibited a series of outward currents; (ii) experiments were performed in the absence of permeable anions; (iii) the concentrations of K⁺ in the cell and the bathing solution were 141 and 5 mM, respectively, and those of Na⁺ were 5 and 141 mM, currents

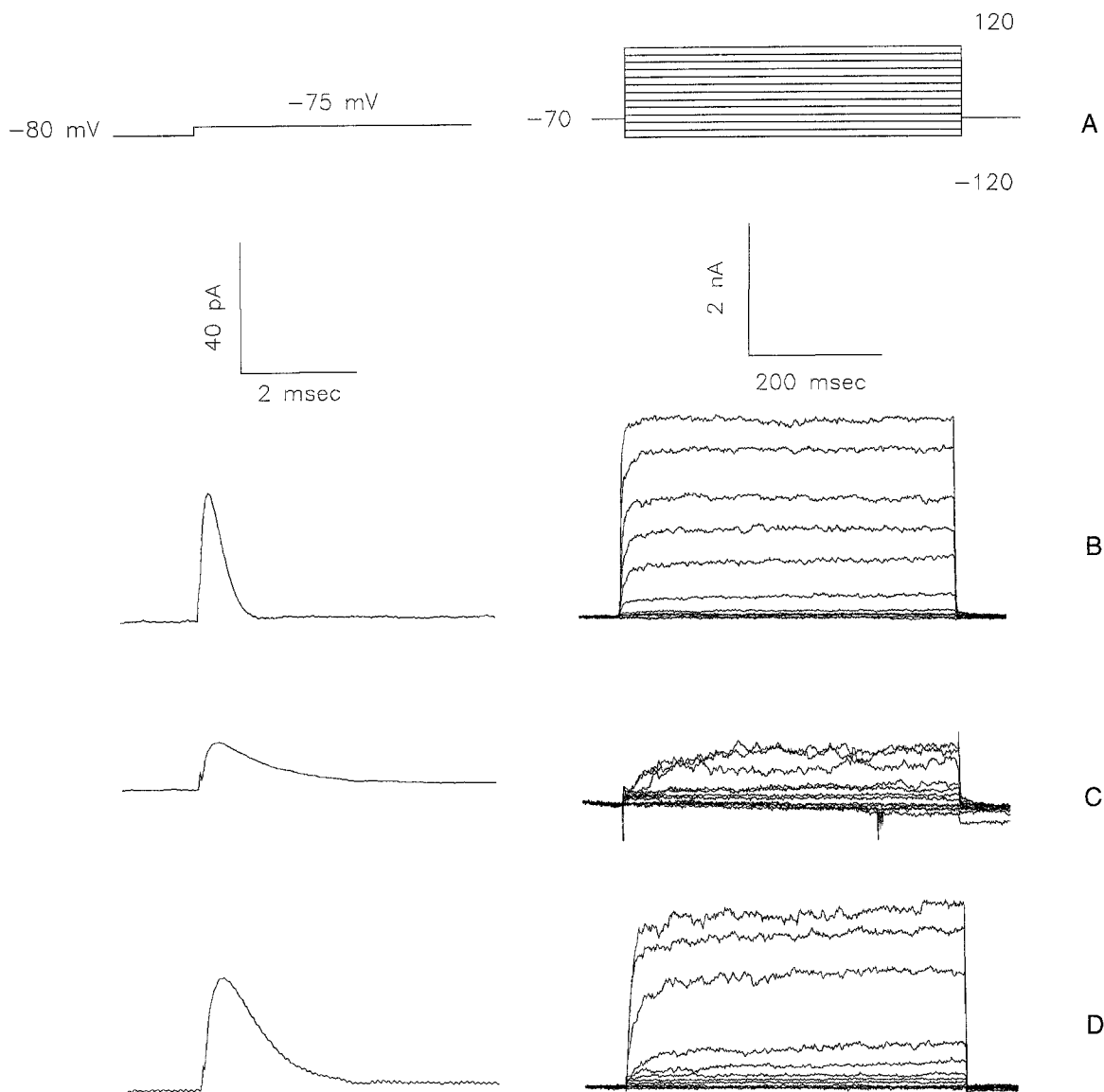


Fig. 1. Measurement of surface membrane and potassium currents in MDCK cells. Cells harvested with trypsin-EDTA are seeded on a glass coverslip, which is then placed in a Lucite chamber on the stage of an inverted microscope. Once the whole-cell configuration is achieved, the membrane potential is held at -80 mV (A). A step change to -75 mV elicits a capacitive current that is used to evaluate the area of the plasma membrane. The speed is then slowed (right) and a protocol of 0.5 sec voltages pulses is delivered. (B) Recordings of a mature cell in a confluent monolayer before trypsination, and a cell (C) that has been harvested and plated for 30 min. Notice the reduction of the amount of plasma membrane (a function of the area under the capacitive transient), and the sharp decrease in the levels reached by I_K . (D) A cell that has recovered its surface and I_K after being plated at confluence for 18 hr.

shown in this and following figures may be attributed to potassium outflux; (iv) in previous studies we observed that these currents were reduced by known inhibitors of K⁺ channels (Bolivar & Cerejido, 1987).

The earliest time after plating we can record is at 20 – 40 min, i.e., the time taken by cells to deposit and attach to the support with sufficient strength to withstand the threefold gentle rinsing with filtered bathing medium, which is in turn an absolute requirement to obtain seals of more than 5 G Ω . Under this condition, the membrane surface was 614 ± 232 (22) μm^2 , and I_K at $+80$ mV and

at 250 msec from the onset of the voltage pulse was 99 ± 25 (25) pA. If on the contrary, cells are plated at confluence and incubated for 12 – 18 hr these parameters increase to $1,190 \pm 70$ (67) μm^2 and 519 ± 44 (33) pA, respectively.

Figure 2 illustrates current curves obtained under control conditions (Top), in a sparse culture, i.e., having no cell-cell contacts but in the presence of 1.8 mM Ca²⁺ (Middle), and a cell completely surrounded by neighboring ones, but incubated with “calcium free” medium (actually 1 – 5 μM Ca²⁺) (Bottom). Figure 3 depicts the

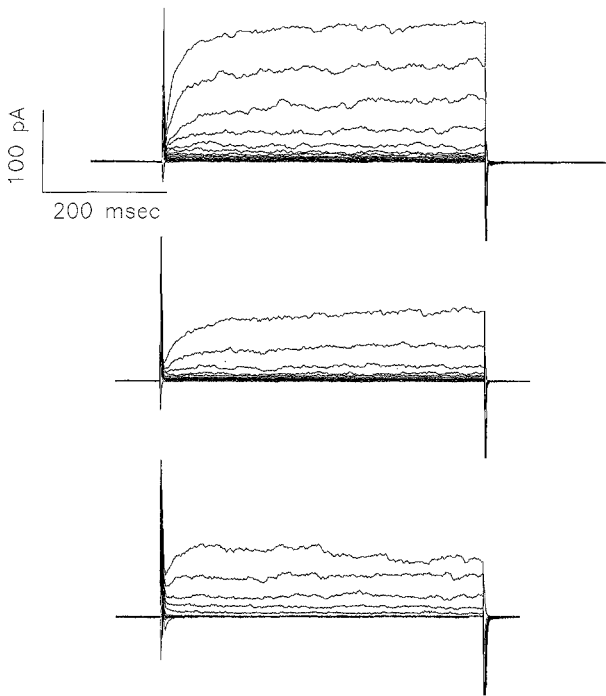


Fig. 2. (Top) Potassium currents recorded in cells that have been plated for 18–20 hr as confluent monolayers, and incubated in a medium with 1.8 mM Ca²⁺ (NC). (Middle) Potassium currents recorded in a cell in a sparse culture, i.e., without neighbors, but in NC. (Bottom) I_K recorded in a cell plated at confluence, but incubated thereafter in the absence of Ca²⁺ (actually 1–5 μ M).

current/voltage relationship under these three experimental conditions. It may be noticed that their left branch is essentially identical, indicating that harvesting does not increase the leakage, and differences are almost exclusively observed in the voltage-stimulated part. The size of the currents in the last two experimental conditions is much smaller. Even the shape of the current curves varies (Fig. 2), as in the condition of confluency without Ca²⁺ (Fig. 2, Bottom) the kinetic of activation appears faster and I_K shows some tendency to inactivate.

Figure 4 summarizes the measurements of membrane surface (Top) and potassium currents (Bottom). It is clear that their expression depends on the simultaneous presence of cell-cell contacts and Ca²⁺. The requirement of Ca-dependent cell-cell contacts was also observed in the *de novo* assembly and sealing of TJs (González-Mariscal et al., 1990; Contreras et al., 1992b). The effect of these contacts on the expression of TJs is mediated by PLC, PKC via G proteins (Balda et al., 1991). To explore whether PLC and PKC are also involved in the expression of K⁺ channels, we used inhibitors and stimulators of these enzymes. To facilitate comparisons, we express the values obtained as percentage of control, a procedure that requires two specifications: (1) Control values are different for inhibitors than for stimulators, because inhibitors were tested in conflu-

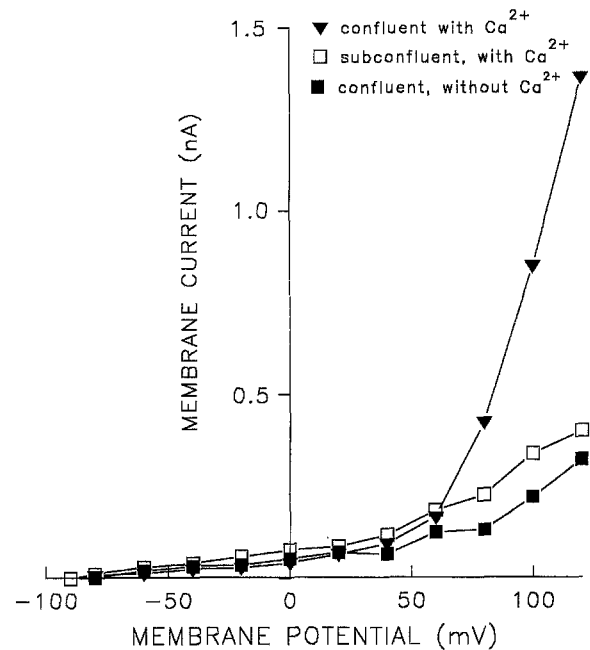


Fig. 3. Current/voltage relationship in cells treated as in Fig. 2.

ent monolayers incubated with 1.8 mM Ca²⁺, but stimulators were added instead to isolated cells with 1.8 mM Ca²⁺ or to confluent cells in the absence of this ion, to bypass the step in which Ca-activated cell-cell contacts should have acted. Therefore, 100% in the first condition corresponds to a higher value. Controls are shown in Fig. 4. (2) As stated in the Introduction, full recovery of K⁺ channels takes 12–18 hr; therefore, the longer the interval between recordings in recently plated cells and those in cells allowed to recover, the more significant the difference between values obtained. However, preliminary experiments indicated that cells could not withstand exposure to some of the chemicals used (e.g., staurosporine) for more than some 6 hr (*results not shown*). Accordingly, the length of time allowed for recovery was decided as a compromise between the “longest period of recovery” and the “shortest exposure to a toxic.” Since every experiment included control recordings (i.e., without inhibitors or stimulators), data were normalized as follows:

$$v = v_m(C_{all}/c)$$

where v is the normalized value, v_m is the value actually measured, c is the average of control experiments run in parallel, and C_{all} is the mean value of all controls of the same conditions (i.e., one of the three shown in Fig. 4).

PLC transforms phosphatidyl 4,5-diphosphoinositol into inositol 1,4,5-triphosphate (IP₃) and diacylglycerol (DAG), and this substance stimulates PKC. To explore whether the effect of cell-cell contacts and Ca²⁺ on the recovery of surface membrane and I_K is mediated by

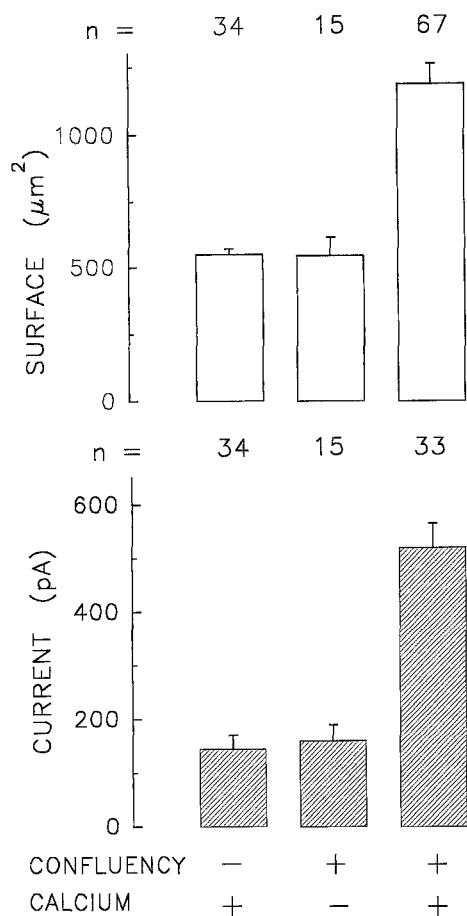


Fig. 4. Membrane surface (Top) and potassium currents (Bottom) of cells that have been plated at subconfluence in 1.8 mM Ca²⁺ (left), at confluence without Ca²⁺ (center), and cells at confluence with NC (right). The surface of the plasma membrane was obtained by dividing the area under the capacitive curve by the size of the voltage step (5 mV), and using the equivalence 1 μF = 1 cm². I_K is the value taken at +80 mV and at 250 msec of starting the voltage pulse.

PLC, we assayed the effect of 2.0 μM TRH, a stimulator of this enzyme (Martin et al., 1986), and observed a significant increase in the expression of K channels (Fig. 5, hatched bar) without affecting membrane area (open bar). Correspondingly, 110 μM neomycin, a substance that binds to PIP₂ and prevents its conversion to IP₃ plus DAG by PLC, produces a drastic inhibition of channel expression. However, this drug does block restoration of membrane area. This lack of effect of the stimulator may not be attributed to insufficient concentration, as the use of 4.0 μM TRH, i.e., doubling the dose, fails to increase the amount of plasma membrane (control: 648 ± 83 [10] vs. TRH: 456 ± 48 [8] μm²).

Next, we explored the participation of PKC by assaying the effect of 200 μg/ml DiC8 (a permeable analogue of DAG) in cells in confluent monolayers cultured without Ca²⁺. Since this substance is rapidly metabolized to phosphatidic acid (May, Lapetina & Cuatrecasas,

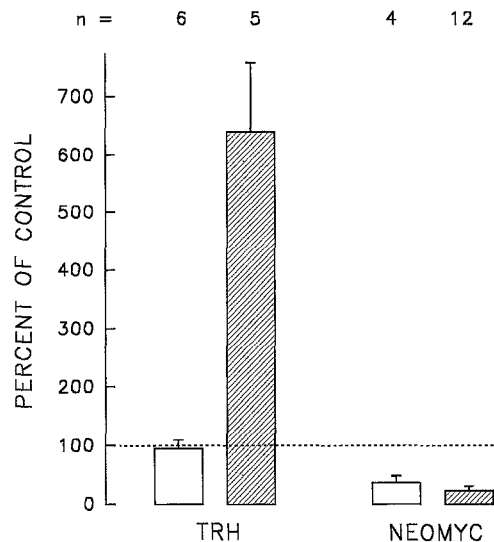


Fig. 5. Effect of stimulation and inhibition of PLC in the recovery of plasma membrane (open bars) and I_K (shaded bars). In this and following figures the horizontal dashed line represents the control value obtained for each condition (Fig. 4). The first two bars correspond to cells incubated at confluence without Ca²⁺, treated with 2.0 μM TRH. The last two bars correspond to cells incubated at confluence with Ca²⁺.

1986) we protected its decay with 200 μM EGD (Balda et al., 1991). Figure 6 shows that 200 μg/ml of DiC8 permits a recovery of the amount of plasma membrane, and stimulates the restitution of ion channels. PMA (10 nM) another inhibitor of PKC, produces essentially the same effect, albeit at a lower degree. In another series of studies (Fig. 6) we tested the effect of 250 nM staurosporine and 2.0 μM H7, inhibitors of PKC (Tamaoki et al., 1986; Knight, Sugden & Baker, 1988). In this case cells were plated at confluence and in the presence of 1.8 mM Ca²⁺, i.e., a condition in which cells recover their plasma membrane and I_K. It may be noticed that the blockade of PKC suppresses the expression of both parameters.

The Table shows the content of PKC under the three experimental conditions. The absence of Ca²⁺ increases the content of PKC as if the cell were posed to phosphorylate a crucial molecular component upon arrival of this ion, so that in confluent cells with Ca²⁺, having performed its task, the amount of PKC was reduced. Furthermore, Ca²⁺ and cell-cell contacts modify the distribution of this enzyme (Fig. 7). Thus, in subconfluent cells incubated in the presence of Ca²⁺ or at confluence but in the absence of this ion, the enzyme remains in the perinuclear area. On the contrary, in confluent cells with Ca²⁺, PKC seems to be contained in vesicles spread over the cytoplasm and presents a distinct accumulation in the membrane region, a position that in some cells was shown to be required for the participation of this enzyme in membrane events.

The amount of plasma membrane, measured in the

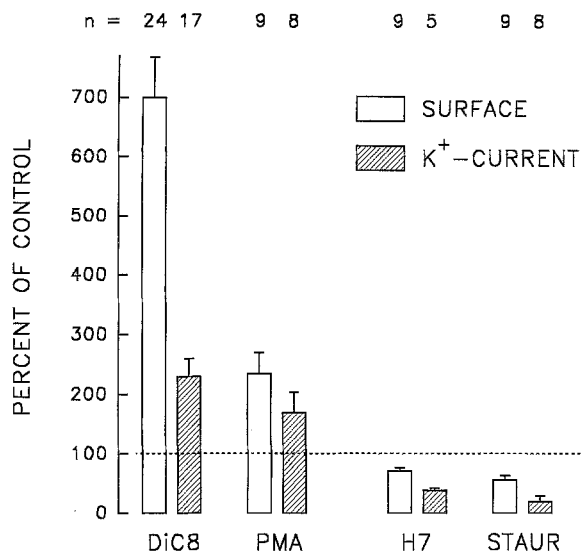


Fig. 6. Membrane surface and potassium currents of confluent cells incubated without Ca²⁺, in the presence of PKC stimulators (200 µg/ml DiC8 plus 200 µM EGD, and 100 nM PMA) and with Ca²⁺ in the presence of PKC inhibitors (2.0 µM H7 and 250 nM staurosporine).

Table. Effect of Ca²⁺ and cell-cell contacts on PKC activity

Ca ²⁺	Confluency	n	Activity
+	+	12	1
-	+	10	1.99 ± 0.28
+	-	4	0.77 ± 0.21

MDCK cells were harvested, plated in medium with 1.8 mM Ca²⁺ for 1.0 hr and subsequently transferred to the same Ca-containing medium or to a "Ca-free" one (actually 1–5 µM). After 18 hr, monolayers were scraped and PKC activity was measured with the GIBCO BRL kit as described in Materials and Methods.

same cells, is also dependent on Ca-activated cell-cell contacts, and is sensitive to the chemicals used to modify the effect of PLC and PKC (open bars in Figs. 4–6), a phenomenon expected from the fact that, as demonstrated in previous work (Ponce et al., 1991a), channels are inserted in the plasma membrane through a process of exocytic fusion. However, exocytic fusion is a common mechanism for the insertion of all other transporting mechanisms, and is not expected to vary to the same extent as I_K . Furthermore, the amount of plasma membrane in a given moment is the balance of exocytic fusion and retrieval, which are processes that may have different sensitivity to the several experimental conditions tested in the present work. This is reflected in Fig. 8, where I_K and membrane area measured in all series of experiments reported, show little correlation.

Discussion

Cation currents in the strain of MDCK cells that we use are almost exclusively accounted for by K⁺ channels, as

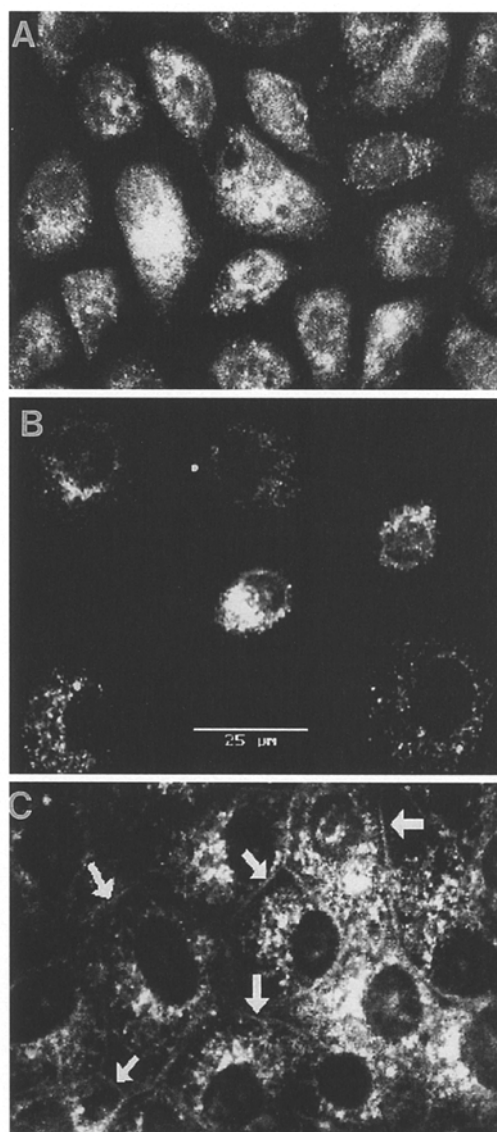


Fig. 7. Fluorescence cytological detection of PKC. Cells stained with rhodamine conjugated rim-1 were observed with a confocal fluorescence microscope. (A) In confluent cells incubated without Ca²⁺, PKC remains accumulated in the perinuclear area. (B) In sparse cells incubated in a Ca-containing medium PKC appears accumulated around the perinuclear area. (C) In cells incubated at confluency in the presence of Ca²⁺ PKC appears to be contained in vesicles spread over the cytoplasm, and along the cell borders (arrows). Intensity was adjusted to show details and is not proportional to the amount of enzyme the cells showed. B is a composite of different fields, grouped to show six sparse cells at the same magnification as in the other two pictures.

Na⁺ and Ca²⁺ currents and the corresponding channels for these ions were never detected (Stefani & Cerejido, 1983; Ponce & Cerejido, 1991). Likewise, studies from other laboratories have exclusively found K⁺ currents and K⁺ channels (Kolb, Paulmichl & Lang, 1987; Friedrich et al., 1988). MDCK cells also have Cl⁻ currents (Lang, Defregger & Paulmichl, 1986; Ponce et al.,

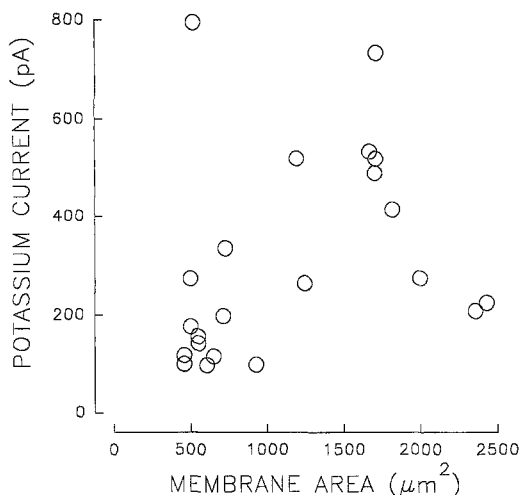


Fig. 8. Relationship between I_K and the amount of plasma membrane. Each circle represents the value of both parameters measured in the same group of cells under the same experimental conditions described in the present work. Accordingly, the graph includes cells with high and low Ca^{2+} , confluent and sparse, and with and without drugs, to show that the different experimental situations may affect I_K and surface independently.

1991b) but these are absent in the experimental conditions used in the present work, because Cl^- has been replaced by nonpermeable methanesulfonic acid.

Harvesting with trypsin-EDTA abolishes most of the I_K . It is not clear whether the small amount of I_K measured as soon as one can start making recordings (20–40 min) is due to old channels that have escaped clipping by the enzyme, or whether it is due to channels reinstalled during the 20–40 min period in which, for technical reasons (waiting for cell attachment to the substrate, gentle washing to permit gigasealing), we cannot make recordings. Anyhow, the extent of recovery during the 6–18 hr period chosen for the present studies is sufficiently large to study the factors involved.

Judging on the basis of conductance, voltage dependence and other kinetic parameters, we have previously found five different types of K⁺ channels in the mature stage (1–3 days after plating at confluence) in the clone of MDCK cells used in the present work (Ponce & Cerejido, 1991). Since the physiological role of each type of channel in these cells is still unknown, it is not certain that each type would be needed in the same proportion along the whole-cell cycle. While the fact that trypsinization reduces I_K by 90% suggests that all of them are almost totally eliminated by harvesting, the kinetics of re-expression of the different types might not be necessarily the same for each channel type.

In previous work we found that restoration of K⁺ channels requires synthesis of mRNA and proteins, the participation of microtubules and microfilaments, and depends also on exocytic fusion of membrane vesicles to the plasma membrane, presumably to incorporate

newly synthesized channels (Ponce et al., 1991a). In the present work we observe that restoration of K⁺ channels requires also Ca²⁺-dependent cell-cell contact and a chain of reactions in which PLC and PKC are involved.

Therefore, the mechanism for the expression of K⁺ channels seems to be part of a general mechanism that results in the making of a cell from transporting epithelia. Thus, Vega-Salas, Salas and Rodríguez-Boulan (1987) have shown that MDCK cells plated as monolayers, but incubated without Ca²⁺, accumulate apical markers in a cytoplasmic compartment (VAC = vesicular apical compartment). Upon transferring these cells to Ca²⁺-containing media, the VAC is fused to the plasma membrane and becomes their apical domain. Likewise, in a previous work, we have demonstrated that MDCK cells incubated without Ca²⁺ have their Na-K-ATPases distributed at random in the plasma membrane, and a large fraction of this enzyme is in a cytoplasmic compartment; yet when 1.8 mM Ca²⁺ is added, Na-K-ATPase molecules are directed to and fused with the plasma membrane (Contreras et al., 1989). Finally, monolayers kept at confluence in the absence of Ca²⁺ do not assemble and seal TJs, but these start to form as soon as the monolayers are transferred to media with 1.8 mM Ca²⁺ (González-Mariscal et al., 1985).

In the case of the expression of TJs, the requirement of Ca²⁺ is very specific, as it cannot be replaced by any of the other multivalent cations tested: Mn²⁺, Mg²⁺, Sr²⁺, Cd²⁺ and La³⁺ (Contreras et al., 1992b). Ca²⁺ elicits this effect by acting on the outside of the cell membrane (González-Mariscal et al., 1990; Contreras et al., 1992a), probably on an ovomorulin molecule (Gumbiner & Simons, 1987), and the information that Ca²⁺ has activated this cell-attaching-molecule (CAM) is conveyed to the cytoplasmic side through a cascade involving at least two G proteins (one excitatory and one inhibitory) acting on the PLC-PKC system (Balda et al., 1991). In the present work we observe that recovery of membrane area and ion channels depends also on cell-cell contacts and Ca²⁺, and involves PLC and PKC, as the chemicals affecting these molecules have a clear effect on the expression of I_K .

Figure 7 and the Table show that the distribution and activity of PKC require the presence of cell-cell contacts and Ca²⁺. Interestingly, Kraft and Anderson (1983) and Myers, Lazo and Pitt (1989) have shown that in order to phosphorylate membrane components in EL4 mouse thymoma cells and cells from the pulmonary artery of calves, part of the PKC must be transferred from the cytoplasm to the plasma membrane.

Taken together, the present results plus those we have obtained in earlier studies (Ponce & Cerejido, 1991; Ponce et al., 1991a,b), suggest that to express most of its K⁺ channels, a newly plated cell requires transcription, translation, exocytic and a fusion of membrane vesicles guided by the cytoskeleton, and that PLC, PKC and AC participate in these processes.

Our results also show that cells are able to control independently the amount of surface membrane and their population of channels. This is not surprising because epithelial cells and renal cells, in particular, have to adapt the expression of translocating mechanisms to acidification, dehydration, K overload, and other physiological requirements that pose entirely different problems and require different cellular responses.

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