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ORIGINAL ARTICLE

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Migration of transformed renal epithelial cells is regulated by K^+ channel modulation of actin cytoskeleton and cell volume

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Abstract Migration of transformed renal epithelial (MDCK-F) cells depends on the polarized activity of a Ca^{2+}-sensitive K^+ channel (IK channel; Pflügers Arch 432:R87–R93, 1996). This study was aimed at elucidating the functional link between the IK channel and the actin cytoskeleton which is required for cell locomotion. We monitored migration of MDCK-F cells with video microscopy, quantified filamentous actin with phalloidin binding, and measured the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) with the fluorescent dye fura-2/AM. We compared the effects of IK channel activation or inhibition with those of hypotonic swelling or hypertonic shrinkage. IK channel inhibition with charybdotoxin (CTX) or cell swelling (omission of up to 50 mmol/l NaCl) as well as IK channel activation with 1-ethyl-2-benzimidazolinone (1-EBIO) or cell shrinkage (addition of up to 100 mmol/l mannitol) reduce the rate of migration dose-dependently by up to 80%, i.e., to the same extent as cytochalasin D. Inhibition of migration is accompanied either by actin depolymerization (CTX and cell swelling) or by actin polymerization (1-EBIO and cell shrinkage). Changes of migration and phalloidin binding induced by CTX and cell swelling or by 1-EBIO and cell shrinkage, respectively, are linearly correlated with each other. CTX and cell swelling elicit a rise of $[Ca^{2+}]_i$ whereas 1-EBIO and cell shrinkage induce a slight decrease of $[Ca^{2+}]_i$ in most MDCK-F cells. Taken together IK-channel-dependent perturbations of cell volume and anisotonicity elicit virtually identical effects on migration, actin filaments and $[Ca^{2+}]_i$. We therefore suggest that cell volume – possibly via $[Ca^{2+}]_i$ – is the link between IK channel activity, actin filaments and migration. We propose a model for how temporal and local changes of cell volume can support the migration of MDCK-F cells.

Key words Ca^{2+}-sensitive K^+ channel · $[Ca^{2+}]_i$ · Charybdotoxin · 1-Ethyl-2-benzimidazolone (1-EBIO)

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Introduction

Migration of eukaryotic cells is involved in a wide variety of physiological and pathophysiological processes such as embryogenesis, wound healing, immune defense and formation of tumor metastases. As a cell migrates it is polarized along its longitudinal axis with the lamellipodium forming the front and the cell body the rear end. Amongst others, directed actin polymerization underlies the extension of the lamellipodium while gel-sol transitions and contraction of the cortical actomyosin are thought to play an important role in the retraction of the rear end of migrating cells (see [1, 18,20] for review). However, volume regulation also plays an important role in migration. In our previous studies we demonstrated that migration of transformed renal epithelial (MDCK-F) cells is impaired whenever ion channels and transporters involved in volume regulation are inhibited [28]. In particular, the polarized activity of a Ca^{2+}-sensitive K^+ channel is crucial for migration [2, 26, 28, 29]. This K^+ channel belongs to the class of recently cloned IK channels [12,14]. Similarly, migration of fibroblasts and human melanoma cells is also a K^+-channel-dependent process [30]. We concluded that efficient volume regulation, working in parallel with the cytoskeletal "migration machinery", is a prerequisite for migration. That cell volume may be linked to the cytoskeletal mechanisms of migration can also be deduced from experiments in which hypotonicity and hypertonicity were shown to be followed by depolymerization and polymerization of filamentous actin, respectively [11].

When the directed mechanical force for migration is provided by the cytoskeleton, inhibition of migration due to K^+ channel blockade implies that the organization and turnover of the actin cytoskeleton can be modulated by K^+ channel activity. Therefore, the present study was aimed at defining the relationship between IK channel activity, actin cytoskeleton and migration of MDCK-F cells. We wanted to elucidate whether inhibition of migration by K^+ channel blockade or activation also involves changes in the amount of F-actin in MDCK-F cells. To this end, we studied the effects of the specific IK channel blocker charybdotoxin (CTX; [12,14]) and the specific IK channel activator 1-ethyl-2-benzimidazolinone (1-EBIO; [3,14]) on migration and cellular F-actin content. If inhibition or activation of the IK channel affects the cellular content of F-actin by changing cell volume, these effects should be mimicked by anisosmotic manipulations of the volume of MDCK-F cells. Therefore, we compared the effects of CTX and 1-EBIO on MDCK-F cells with those of anisotonicity.

Our results show that inhibition and activation of the IK channel elicit virtually identical effects on migration, F-actin content and the $[Ca^{2+}]_i$ of MDCK-F cells as anisosmotic cell swelling or shrinkage. Since IK channel inhibition or activation elicits similar volume changes as anisotonicity [16, 24,33] we suggest that cell volume is one of the links between K^+ channel activity, the actin cytoskeleton and migration.

Materials and methods

Cell culture

Experiments were carried out on alkali-transformed Madin-Darby canine kidney (MDCK-F) cells [21]. Transformed MDCK-F cells were kept under standard cell culture conditions at 37°C in humidified air containing 5% CO_2. Cells were grown in bicarbonate-buffered Minimal Essential Medium (MEM) with Earle's salts (Biochrom, Berlin, Germany) supplemented with 10% fetal calf serum (Biochrom). For experiments cells were plated on poly-L-lysine-coated (0.1 g/l; Serva, Heidelberg, Germany) coverslips. Experiments were performed 1 or 2 days after seeding.

Migration experiments

Migration of individual MDCK-F cells was monitored in paired experiments with video microscopy as described previously [28]. Migration was quantitated from calibrated videoprints as the distance lamellipodia advanced with time. During the entire course of the experiments cells were superfused with prewarmed (37°C) Ringer solution which contained (in mmol/l): NaCl 122.5, KCl 5.4, $MgCl_2$ 0.8, $CaCl_2$ 1.2, NaH_2PO_4 1.0, D-glucose 5.5, HEPES 10.0, pH 7.4 titrated with 1 mol/l NaOH. The osmolarity of the superfusate was reduced by the omission of 12.5, 25 or 50 mmol/l NaCl. The effect of a reduced extracellular Na^+ concentration was tested by isosmotically substituting 50 mmol/l NaCl with mannitol. Hypertonic Ringer's solution was supplemented with 25, 50 or 100 mmol/l mannitol. The protocol of all migration experiments was such that a 15-min control period was followed by a 5-min experimental period. Cytochalasin D (Sigma) and 1-EBIO (Aldrich, Steinheim, Germany) were solved in dimethylsulfoxide (DMSO) while CTX (Alomone, Jerusalem, Israel) was dissolved in aqueous solution.

Quantification of phalloidin-labeled actin

We determined the amount of filamentous actin indirectly by quantifying binding of TRITC-coupled phalloidin (Sigma) in MDCK-F cells and referring it to cell protein. Cells seeded on coverslips were fixed for 10 min in 2% paraformaldehyde in PBS and permeabilized for 1 min with –20°C acetone. Cells were loaded for 30 min with TRITC-phalloidin (200 ng/ml in PBS). Bound TRITC-phalloidin was eluted by overnight incubation in methanol at –20°C and quantified fluorometrically (excitation at 542 nm, emission at 563 nm).

Cell protein was determined with naphthol blue black (Sigma) in those cells in which we had measured phalloidin binding [4]. Cells were stained for 10 min in 0.5% w/v naphthol blue black in 45% methanol, 45% water and 10% acetic acid. After removal of excess naphthol blue black (47.5% methanol, 47.5% water and 5% acetic acid) protein-bound dye was eluted by incubating cells for 30 min at 50°C in 80% formic acid, 10% acetic acid and 10% w/v trichlor-acetic acid. The supernatant was centrifuged for 10 min at 10,000 *g*, and the extinction of naphthol blue black was measured at 620 nm. Naphthol blue black extinction was calibrated against a protein standard (Sigma) which was treated in the same way as the cells seeded on coverslips. Determinations of phalloidin-binding and of cell protein were made in triplicate. Results obtained under experimental conditions were always referred to control experiments which were run in parallel.

Measurement of $[Ca^{2+}]_i$

The $[Ca^{2+}]_i$ of MDCK-F cells was measured as described previously [25] by using video imaging techniques and the fluorescent Ca^{2+} indicator fura-2/AM (Molecular Probes, Eugene, Ore., USA). For dye-loading MDCK-F cells were incubated in culture medium containing 4 µmol/l fura-2/AM for 30 min. Coverslips were placed on the stage of an inverted microscope (×40 oil-immersion objective; Axiovert TV 100, Zeiss, Oberkochen, Germany) and continuously superfused with prewarmed (37°C) Ringer solution. Excitation wavelength alternated between 334 and 380 nm, and emitted fluorescence was monitored at 500 nm with an ICCD camera (Atto Instruments, Rockville, Md., USA). Filter change and data acquisition were controlled by Attofluor software (Atto Instruments). Several areas were placed over the projected cell surface and average fluorescence intensities in these areas were measured in 5- to 10-s intervals.

$[Ca^{2+}]_i$ was calculated according to the equation $[Ca^{2+}]_i = K_d \times \beta \times (R - R_{min})/(R_{max} - R)$ [9]. K_d is the dissociation constant of 225 nmol/l, R_{min} and R_{max} are fluorescence ratios at zero and saturating Ca^{2+} concentrations after excitation with 334 and 380 nm, respectively. β is a factor obtained by dividing fluorescence intensity after excitation with 380 nm obtained at zero Ca^{2+} by the 380 nm value measured at saturating Ca^{2+}. R_{max}, R_{min} and β were determined by application of ionomycin-containing (2 µmol/l) Ringer solutions with either 5 mmol/l Ca^{2+} or with 5 mmol/l EGTA. $[Ca^{2+}]_i$ is given as the averaged value for the entire cell.

Statistics

All data are presented as mean ± SEM. Paired or unpaired Student's *t*-test were performed where applicable. Significance was assumed when $P<0.05$.

Results

Migration experiments

Figure 1 depicts a single migrating MDCK-F cell which is polarized in the plane of movement along its longitudinal axis with the lamellipodium and cell body representing

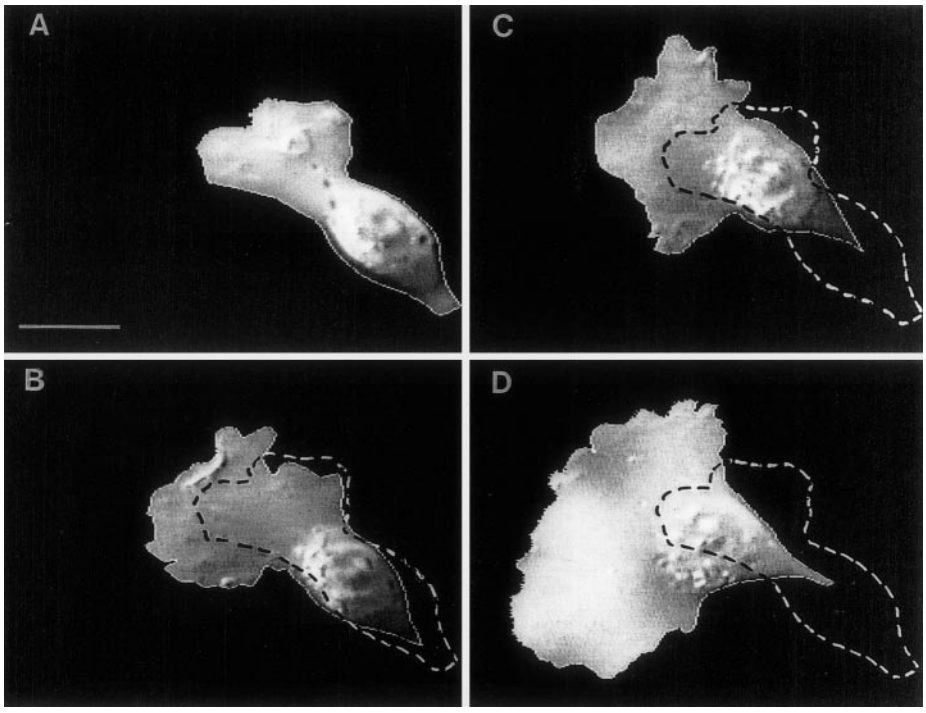

Fig. 1A–D Migrating MDCK-F cell. Videomicrographs **A–D** of this migrating MDCK-F cell were taken at 5-min intervals. The *dotted line* indicates the outlines of the cell at the beginning of the experiment. *Bar*: 20 µm

front and rear end, respectively. Under control conditions MDCK-F cells migrate at a rate of 1.0±0.03 µm/min (n=225).

Figure 2A shows that both inhibition of the IK channel with CTX, and its activation with 1-EBIO impair migration in a dose-dependent manner. Whereas 5 nmol/l CTX slows down MDCK-F cells to 19.7±6.6% of the control migration rate, 0.5 nmol/l CTX elicits no statistically significant effect on migration (88.6±9.5% of control). At concentrations of 50 µmol/l and 500 µmol/l, 1-EBIO reduces the rate of migration to 76.8±10.6% and 57.2±11.4%, respectively.

If IK channel activity modulates the migration of MDCK-F cells by controlling cell volume [2,26], "direct" swelling or shrinking of MDCK-F cells with anisosmotic Ringer's solution will also reduce the rate of migration. As shown in Fig. 2B this is the case. Small changes of the osmolarity (–12.5 mmol/l NaCl or + 25 mmol/l mannitol) do not affect migration (105.6± 14.9% and 97.0±9.7% of control). However, omitting 25 mmol/l and 50 mmol/l NaCl slows down MDCK-F cells to 70.7±12.9% and 27.5±5.3% of control. Isosmotically reducing the NaCl concentration (replacement by 100 mmol/l mannitol) has no effect on migration (101.2±10% of control; n=17; data not shown). Increasing the osmolarity by adding 50 mmol/l or 100 mmol/l mannitol to the superfusate elicits the same inhibition of migration as hypotonicity. MDCK-F cells are slowed down to 61.2±7.3% and 38.9±8.7%, respectively. Taken together these experiments show that there is a narrow optimal range of the extracellular osmolarity for migration and that anisotonicity impairs locomotion of MDCK-F cells in a similar way as modulating the IK channel.

For comparison we tested the effect of cytochalasin D: 100 nmol/l and 1 µmol/l reduce migration dose de-

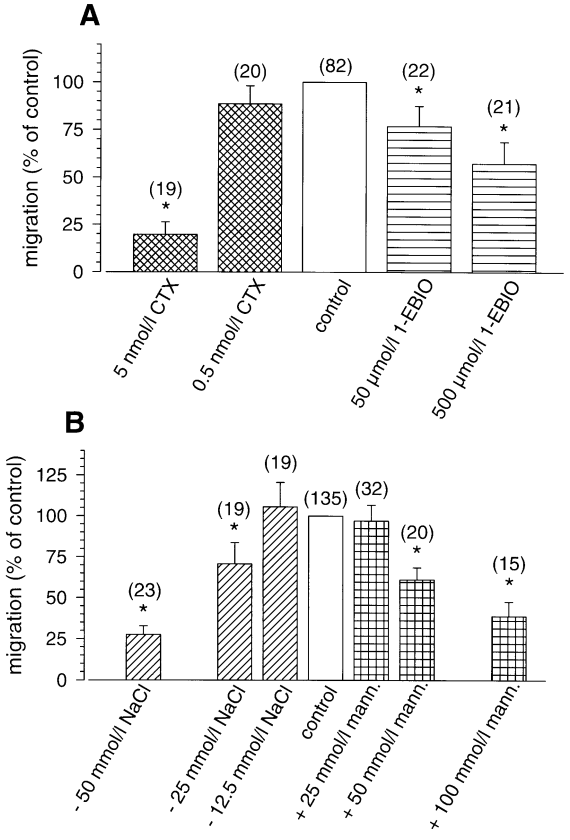

Fig. 2A,B Summary of migration experiments. Migration is normalized with respect to the control rate of migration which is taken as 100%. *Statistically significant difference from control (P<0.05). **A** Effect of K^+ channel inhibition with charybdotoxin (*CTX*) and K^+ channel activation with 1-ethyl-2-benzimidazolone (*1-EBIO*) on migration of MDCK-F cells. **B** Effect of anisotonicity on migration of MDCK-F cells

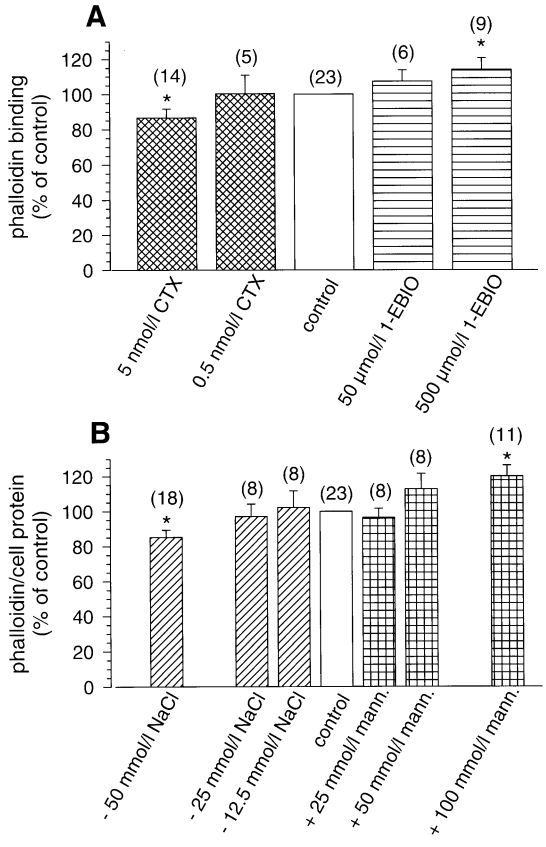

Fig. 3A,B Summary of phalloidin binding experiments. Phalloidin binding is normalized to the respective control experiments which were always run in parallel. Statistically significant changes of phalloidin labeling are indicated by *asterisks* ($P<0.05$). **A** Phalloidin binding after inhibition or activation of K^+ channel activity with CTX or 1-EBIO. **B** Phalloidin binding after hypotonic swelling or hypertonic shrinkage

pendently to a similar extent as modulating K^+ channel activity or the extracellular osmolarity. MDCK-F cells are slowed down to 39.3±11.9% (100 nmol/l; n=19) and 11.5±6.2% of control (1 µmol/l; n=9) (data not shown).

Quantification of phalloidin binding

In order to quantitatively correlate the changes of the actin filament network with the inhibition of migration we determined the amount of phalloidin binding in MDCK-F cells. Phalloidin binding was quantitated after a 5-min treatment with the same experimental solutions used in migration experiments. Under control conditions phalloidin binding amounts to 0.22±0.01 ng/µg cell protein. Figure 3A summarizes the effect of modulating IK channel activity on phalloidin binding. When migration is inhibited by 5 nmol/l CTX phalloidin binding is reduced to 86.6±5.1% of control. At 0.5 nmol/l CTX does not inhibit migration, and neither does it affect phalloidin binding (100.3±10.6%). Activating the IK channel with 1-EBIO elicits the opposite effect on phalloidin binding: 50 µmol/l and 500 µmol/l 1-EBIO increase phalloidin binding to 107.2±6.5 and 113.9±6.6%, respectively.

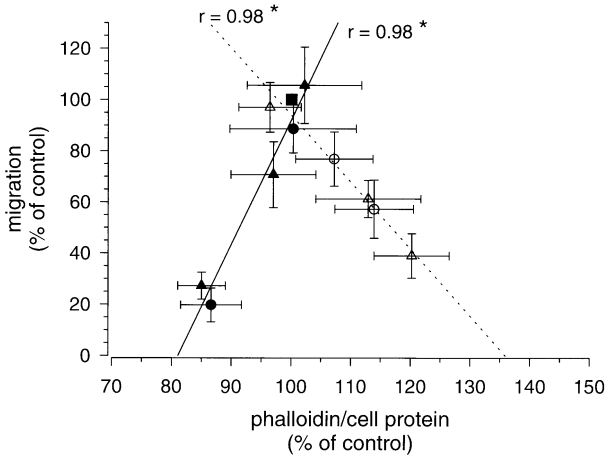

Fig. 4 Migration of MDCK-F cells is plotted as a function of phalloidin binding. Both parameters are normalized to the respective control experiments (■). Data are pooled into two groups: CTX (•) and hypotonic swelling (▲), and 1-EBIO (○) and hypertonic shrinkage (Δ). Within both groups data are linearly correlated pointing to a common mechanism of action of anisotonicity and K^+ channel modulation

If CTX- or 1-EBIO-induced changes of phalloidin binding are due to cell swelling or cell shrinkage, respectively, these effects should be reproduced by the corresponding changes of extracellular osmolarity. The results of these experiments are summarized in Fig. 3B. Omission of 50 mmol/l NaCl is followed by the same reduction of phalloidin binding as treatment with 5 nmol/l CTX (85.2±4.0% of control). In contrast, addition of 100 mmol/l mannitol elevates phalloidin binding to 120.2±6.3%. Omission of 25 mmol/l NaCl or addition of 50 mmol/l mannitol also elicits a decrease or an increase of phalloidin binding (97.0±7.1% and 112.9±8.8%, respectively), but these effects do not reach statistical significance. As expected from migration experiments small changes of the extracellular osmolarity (–12.5 mmol/l NaCl or + 25 mmol/l mannitol) induce no statistically significant change of phalloidin binding (102.2±9.6% and 96.4±5.2% of control).

Figure 4 combines the results from Figs. 2, 3. Migration is plotted as a function of phalloidin binding, i.e., as a function of the F-actin content. We pooled experiments involving cell swelling (hypoosmolarity and CTX) or cell shrinkage (hyperosmolarity and 1-EBIO). It is apparent that migration depends biphasically on the amount of F-actin since both a decrease and an increase of phalloidin binding are associated with impaired migration. In addition, hypoosmolarity- and CTX-induced changes of migration and phalloidin binding are linearly correlated. The same holds true for hyperosmolarity and 1-EBIO. This correlation is consistent with cell volume being a mediator between IK channel activity and migration.

Measurement of $[Ca^{2+}]_i$

$[Ca^{2+}]_i$ is a critical parameter in regulating actin filament turnover [13], and a rise of $[Ca^{2+}]_i$ is part of the regulatory

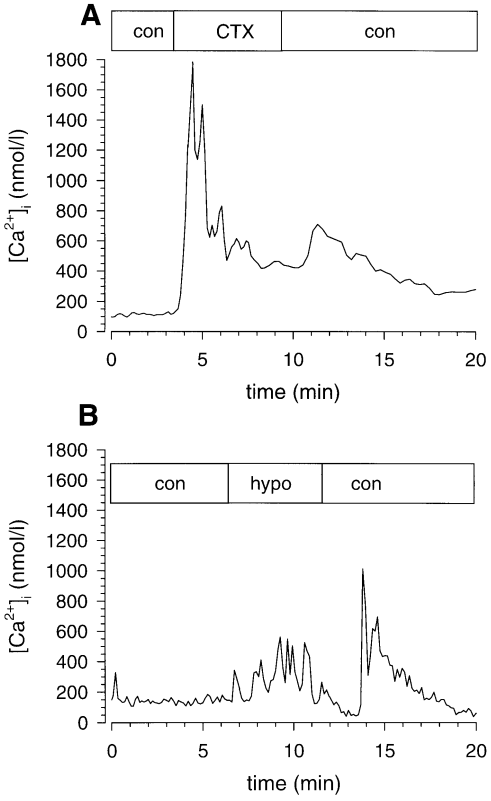

Fig. 5A,B Measurements of $[Ca^{2+}]_i$ in MDCK-F cells. **A** At 5 nmol/l, CTX elicits a sharp rise of $[Ca^{2+}]_i$ in MDCK-F cells. Upon washout of CTX $[Ca^{2+}]_i$ only gradually returns towards baseline levels. **B** Under control conditions there are two spontaneous peaks of $[Ca^{2+}]_i$. Reducing the extracellular osmolarity (omission of 50 mmol/l NaCl) increases their frequency and amplitude. The rise of $[Ca^{2+}]_i$ during secondary regulatory volume decrease is representative for approximately 50% of the cells

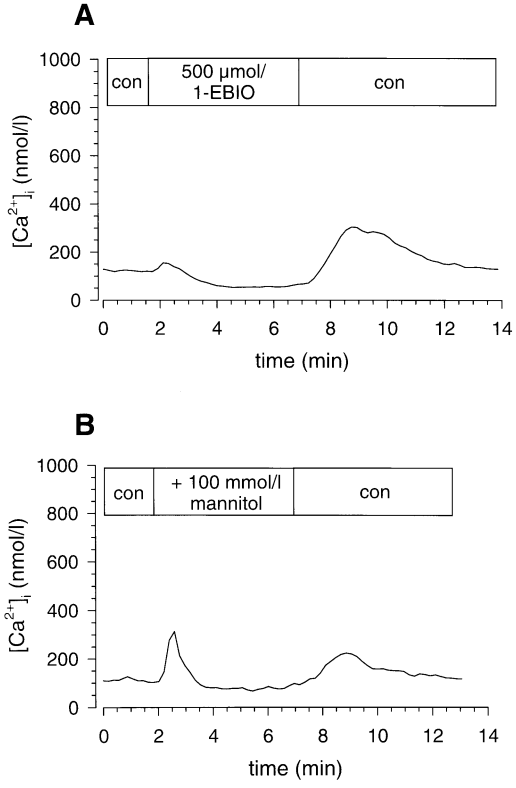

Fig. 6A,B Measurements of $[Ca^{2+}]_i$ in MDCK-F cells. **A** 1-EBIO induces a decrease of $[Ca^{2+}]_i$ in MDCK-F cells. Upon washout $[Ca^{2+}]_i$ rises transiently. **B** Hypertonic shock induces a biphasic response of $[Ca^{2+}]_i$. After an initial transient elevation there is a slight decrease of $[Ca^{2+}]_i$. Upon returning to normotonicity $[Ca^{2+}]_i$ rises transiently

volume decrease in MDCK cells [35]. Therefore, we examined the effect of CTX on $[Ca^{2+}]_i$ in MDCK-F cells. The average Ca^{2+} concentration in untreated MDCK-F cells is 125±26 nmol/l (*n*=45). Application of CTX (5 nmol/l) to MDCK-F cells elicits a sharp rise of $[Ca^{2+}]_i$ to a peak concentration of 1140±150 nmol/l (*n*=12). Figure 5A depicts a typical experiment. $[Ca^{2+}]_i$ only gradually (over a time course of several minutes) returns towards baseline levels upon washout of CTX. $[Ca^{2+}]_i$ rises only to 264±48 nmol/l when extracellular Ca^{2+} is removed (*n*=5) and pretreatment with Ca^{2+}-free solution (nominally Ca^{2+} free plus 2 mmol/l EGTA) precludes the elevation of $[Ca^{2+}]_i$ upon application of CTX (*n*=15; data not shown).

We compared the effect of CTX on $[Ca^{2+}]_i$ with that of lowering the extracellular osmolarity (–50 mmol/l NaCl). Upon hypotonic swelling $[Ca^{2+}]_i$ remains unchanged in 6 out of 19 MDCK-F cells. In the remaining 13 MDCK-F cells hypotonic swelling elicits an increase of $[Ca^{2+}]_i$ to an average concentration of 220±50 nmol/l (peak concentration 660±195 nmol/l). A representative example of these experiments is illustrated in Fig. 5B. Isosmotic reduction of the extracellular Na^+ concentration elicits no increase of $[Ca^{2+}]_i$ (data not shown).

In contrast, activation of IK channels with 500 µmol/l 1-EBIO is followed by a decrease of $[Ca^{2+}]_i$ in most cells (15 out of 21) to 62±9 nmol/l (see Fig. 6A). In the remaining cells there is either a transient doubling of $[Ca^{2+}]_i$ or there is no change. However, upon washout of 1-EBIO all cells respond with a transient increase of $[Ca^{2+}]_i$ to 381±73 nmol/l. Treating MDCK-F cells with hypertonic solution (+100 mmol/l mannitol) is ensued in most cells (six out of nine) by a decrease of $[Ca^{2+}]_i$ to 58±7 nmol/l which may be preceded by a transient elevation of $[Ca^{2+}]_i$ (Fig. 6B). As is the case for 1-EBIO, $[Ca^{2+}]_i$ rises (to 214±14 nmol/l) upon returning to the control solution, i.e., during a secondary volume regulatory increase. Taken together, these experiments show that both hypotonic swelling and IK channel inhibition as well as hypertonic shrinkage and IK channel activation elicit qualitatively similar changes of $[Ca^{2+}]_i$ in MDCK-F cells.

Discussion

We combined migration experiments with the indirect determination of F-actin and measurements of $[Ca^{2+}]_i$ in order to disclose the relationship between the activity of a Ca^{2+}-sensitive K^+ channel belonging to the class of IK

channels [12,14] and locomotion of MDCK-F cells. At first sight the results appear contradictory since both IK channel blockade with CTX and its stimulation with 1-EBIO are ensued by inhibition of migration. Moreover, inhibition of migration by IK channel blockade or IK channel stimulation is associated with opposite effects on actin filament turnover: disassembly of F-actin in the presence of CTX and an increase of F-actin in the presence of 1-EBIO. Similar behavior is seen when MDCK-F cells are treated with anisosmotic solutions. Both hypotonicity and hypertonicity inhibit the migration of MDCK-F cells while phalloidin binding is either decreased or increased, i.e., migration depends biphasically on IK channel activity, the extracellular osmolarity and on the cellular F-actin content.

We interpret the biphasic dependence of migration on F-actin as a common biological behavior: whenever a given parameter (here the F-actin content) is shifted from its optimum cell function (here migration of MDCK-F cells) is impaired. Such a general interpretation would be in line with observations of CHO cells [23]. Migration of these cells depends biphasically on the cell substratum level and on cellular integrin expression levels. Similarly, a related process, the outgrowth of neurites, responds biphasically to changes of $[Ca^{2+}]_i$ [15]. Thus, a simple explanation of our phalloidin binding experiments is that the level of F-actin in MDCK-F cells is at its optimum under control conditions. Any perturbation due to either IK channel modulation or anisosmotic solutions or treatment with cytochalasin D therefore reduces the speed of migration.

It is known that cell volume is inversely related to F-actin. F-actin was shown to decrease in HL60 cells upon hypotonic shock and to increase upon hypertonic shock [11]. These changes of F-actin were seen amongst others in the context of volume-dependent changes in the overall affinity of actin-binding proteins in the cell. One of the regulators of actin assembly is $[Ca^{2+}]_i$. A rise and a decrease of $[Ca^{2+}]_i$ have been related to actin depolymerization and polymerization, respectively [5,13]. Therefore, it is feasible that the CTX- and hypotonicity-induced increase of $[Ca^{2+}]_i$ is one of the steps leading to actin depolymerization. An elevation of $[Ca^{2+}]_i$ could have activated Ca^{2+}-sensitive actin-severing proteins such as gelsolin, thereby promoting actin disassembly [5,13]. Along these lines, the decrease of $[Ca^{2+}]_i$ in the presence of 1-EBIO could account for actin polymerization [5]. Presently we cannot rule out that in particular during anisotonicity other intracellular signaling molecules such as polyinositol phosphates are involved, too. Polyinositol phosphates are important regulators for actin assembly [13] and they are also linked to volume regulatory mechanisms (see [17] for review).

We suggest for the following reasons that the rise of $[Ca^{2+}]_i$ in the presence of CTX is not due to changes of the cell membrane potential. Oscillating IK channel activity is paralleled by intermittent hyperpolarizations of the cell membrane potential. In the presence of IK channel blockers the cell membrane potential remains depolarized [21,28]. Since there is no voltage-dependent Ca^{2+} conductance [34] Ca^{2+} influx into MDCK-F cells should be attenuated in the presence of CTX and $[Ca^{2+}]_i$ should be lowered. However, the opposite is the case, $[Ca^{2+}]_i$ rises. Since we showed that MDCK-F cells swell by up to 20% in the presence of CTX [24] we propose that cell swelling triggers Ca^{2+} influx through mechano-sensitive channels. In previous experiments we showed that mechanical stimulation of MDCK-F cells elicits a CTX-inhibitable K^+ efflux through IK channels [2].

The question remains as to whether the anisosmotic treatment is of any physiological relevance. The volume of MDCK cells is increased by 25% after a hypotonic shock (omission of 50 mmol/l NaCl; [16]) and it is decreased by 16% after a hypertonic shock (addition of 150 mmol/l mannitol; [33]). Since MDCK-F cells and MDCK cells are likely to express the identical set of ion channels and transporters [27] it is reasonable to assume that the anisosmotic treatment changed the volume of MDCK-F cells to a similar degree. By measuring the cell volume of MDCK-F cells with the atomic force microscope we could show that inhibition or maximal activation of the IK channel is followed by cell swelling (+20%) or cell shrinkage (–19%), respectively [24]. Hence, when IK channel activity is fluctuating under physiological conditions MDCK-F cells experience changes of their volume which are of similar magnitude as those induced by anisotonicity.

Finally, we would like to present a model for how K^+ channel activity affects cell migration. We suggest the following cycle of events. Starting after a burst of IK channel activity the volume and $[Ca^{2+}]_i$ of MDCK-F cells have reached a transient minimum favoring actin filament polymerization and outgrowth of the lamellipodium. At the same time the cell volume of MDCK-F cells is "replenished" by Na^+/H^+ and Cl^-/HCO_3^- exchangers as well as by the $Na^+/2Cl^-/K^+$ cotransporter [28]. Interestingly, the Na^+/H^+ exchanger is localized at the leading edge of fibroblasts [8], i.e., salt and water uptake at the front of a migrating cell act in concert with the gelosmotic swelling at the leading edge of the lamellipodium [22]. Consequently, inhibitors of Na^+/H^+ and Cl^-/HCO_3^- exchangers impair migration only when they are applied to the lamellipodium of MDCK-F cells [31]. The extending lamellipodium and the gradual cell swelling stretch the cell membrane, activate Ca^{2+}-permeable mechano-sensitive channels which are found in other motile cells too [32], thereby triggering the rise of $[Ca^{2+}]_i$. Accordingly, mechanical stimulation of MDCK-F cells induces a CTX-inhibitable K^+ efflux through IK channels [2]. (The IK channel itself is not mechano-sensitive.) The rise of $[Ca^{2+}]_i$ which is more pronounced in the cell body than in the lamellipodium [25] can have several effects: it loosens cell-matrix connections at the rear end [19], it isolates the cortical actomyosin gel allowing a contraction at the rear end [18] and it activates the IK channel at the rear end of MDCK-F cells [29]. Local cell shrinkage of the cell body due to IK channel activation [24] is accompanied by a large K^+ efflux [2]

and supports the cytoskeletal mechanisms that retract the rear end of MDCK-F cells. After K^+ and volume loss mechano-sensitive Ca^{2+} entry stops, $[Ca^{2+}]_i$ returns to basal levels and the cycle starts over again. IK channel inhibition or permanent IK channel activation thus impair migration by interrupting this cycle and preventing the alternating reorganization of the actin filament network.

According to our model periodic bursts of IK channel activity lead to cyclic actin polymerization and depolymerization. In neutrophils such oscillations of F-actin occur in phase with shape oscillations which are closely associated with neutrophil locomotion [36] and which can be partially blocked by impairing volume regulatory mechanisms [6]. Since we determined phalloidin binding in a population of cells which are not synchronized with respect to their K^+ channel activity, we are presently unable to detect the inferred spontaneous changes of F-actin content. However, the effects of CTX and 1-EBIO provide evidence for the assumption of K^+ channel modulation of the actin cytoskeleton. Interestingly, the mechanical force required for locomotion also oscillates in single macrophages at a frequency similar to that of bursts of K^+ channel activity in macrophages and in MDCK-F cells [2, 7, 10, 27]. Force generation is abolished by treating macrophages with cytochalasin which points to the central role of actin filaments for this process. Further experiments are required in order to find out whether there is a direct correlation between oscillating K^+ channel activity, cyclic actin assembly and oscillations of the mechanical force.

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