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Chloride Influx Provokes Lamellipodium Formation in Microglial Cells

Susanna Zierler[1], Eva Frei[2], Stephan Grissmer[2] and Hubert H. Kerschbaum[1]

[1]Department of Cell Biology, Paris-Lodron- University of Salzburg, [2]Institute of Applied Physiology, University of Ulm

Key Words
Lamellipodium • Migration • Cell Volume • Microglia

Abstract
Lamellipodium extension and retraction is the driving force for cell migration. Although several studies document that activation of chloride channels are essential in cell migration, little is known about their contribution in lamellipodium formation. To address this question, we characterized chloride channels and transporters by whole cell recording and RT-PCR, respectively, as well as quantified lamellipodium formation in murine primary microglial cells as well as the microglial cell-line, BV-2, using time-lapse microscopy. The repertoire of chloride conducting pathways in BV-2 cells included, swelling-activated chloride channels as well as the KCl cotransporters, KCC1, KCC2, KCC3, and KCC4. Swelling-activated chloride channels were either activated by a hypoosmotic solution or by a high KCl saline, which promotes K^+ and Cl^- influx instead of efflux by KCCs. Conductance through swelling-activated chloride channels was completely blocked by flufenamic acid (200μM), SITS (1mM) and DIOA (10μM). By exposing primary microglial cells or BV-2 cells to a high KCl saline, we observed a local swelling, which developed into a prominent lamellipodium. Blockade of chloride influx by flufenamic acid (200μM) or DIOA (10μM) as well as incubation of cells in a chloride-free high K^+ saline suppressed formation of a lamellipodium. We assume that local swellings, established by an increase in chloride influx, are a general principle in formation of lamellipodia in eukaryotic cells.

Introduction

Lamellipodium formation is a fundamental property in most eukaryotic cells and propels migration in cells as diverse as amoebocytes in *Dictyostelium* or neurons in the developing brain of vertebrates [1, 2]. Advances in the study of cell migration over the last few years have revealed an asymmetric distribution of signaling molecules essential in regulation of the actin cytoskeleton, like Cdc 42 or Rac [3]. Despite the significance of polymerization and depolymerization of actin [3, 4], modulation of ion channel and transporter activity is equally important as cytoskeleton rearrangement to cell migration [5]. As

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Hubert H. Kerschbaum
Division of Animal Physiology, Department of Cell Biology
University of Salzburg, Hellbrunnerstr. 34, 5020 Salzburg (Austria)
Fax +43-662-8044-180, E-Mail hubert.kerschbaum@sbg.ac.at

migrating cells show substantial increase in their cell volume [6], and swelling-activated Cl channels are essential in regulatory volume processes, chloride conductance may control cell migration. Accordingly, chloride conductance has been identified as a driving force in migration in glioma cells [7, 8] and microglial cells [9]. Pharmacological blockade of Cl channels as well as substitution of Cl^- by impermeant anions, like glutamate, considerable reduced transwell migration in these cells [7-9]. In the present study, we asked whether modulation of chloride conductance is involved in an early phase of migration, namely formation of lamellipodia.

Microglial cells have a highly variable phenotype ranging from amoeboid to ramified [10-12]. Despite this overall plasticity, each of these phenotypes has been shown to extend lamellipodia. Upon local damage of capillaries, ramified microglial cells form small lamellipodia at the tips of their processes to seal damaged blood vessels [13, 14]. Amoeboid phenotypes form broad lamellipodia required for cell migration and phagocytosis of cell debris.

We monitored a swelling-activated Cl^- current using whole cell recording, identified KCCs via RT-PCR, and observed formation of lamellipdia by time lapse video microscopy. We document that (1) BV-2 cells express a swelling-activated Cl channel as well as KCC1 to KCC4 and (2) inhibition of chloride influx or exposure to chloride-free saline prevents formation of lamellipodia.

Materials and Methods

Cell culture

Experiments were done on BV-2 cells, a murine microglial cell line [15]. BV-2 cells were maintained in 25 cm^2 culture flasks in DMEM supplemented with 2.2 glucose/L and 10% FCS under standard culture conditions (37 ° C, 5% CO_2, 95% rel. humidity). 6 x 10^3 cells were seeded into each Petri dish on poly-D-lysine coated glass slides and after an initial 14 hours of culture in DMEM to promote adhesion, cells were incubated for 30 min in an extracellular solution without serum (in mM: 130 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 8 D-glucose; pH 7.4) and clamped into a special superfusion chamber for subsequent time-lapse microscopy. For RT-PCR cells were cultured in 25 cm^2 culture flasks in DMEM (10% FCS) until confluence.

Primary microglial cells were isolated from forebrains of one to three day old wild type C57 black 6J mice as described elsewhere [16] and co-cultivated with astrocytes in Poly-D-Lysin coated 75 cm^2 culture flasks for at least 14 days in DMEM supplemented with 10 % FCS and 1% Pen/Strep (GIBCO) before they were harvested via shaking from an astrocyte monolayer [17]. Prior to experiments purity of microglial cells was determined (~97%) using IB_4 (I21411, Molecular Probes) and confocal laser scanning microscopy (LSM510, Zeiss).

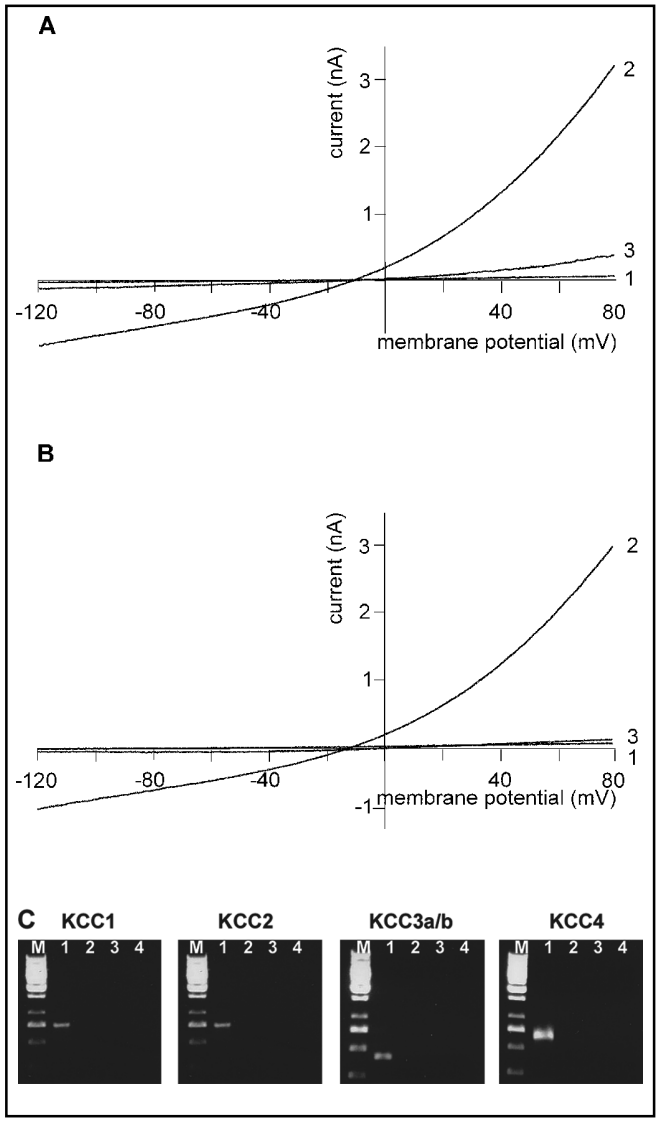
RT-PCR

Total RNA from BV-2 microglial cells was extracted using QIAshredder and RNeasy mini kit (Qiagen, Hilden, Germany) with RNase-free DNase treatment, according to manufacturer's instructions and reverse transcribed, using oligo(dT) primer (SuperScript first-strand synthesis system for RT-PCR; Invitrogen, Karlsruhe, Germany). cDNA was synthesized for 120 min at 37°C. cDNAs for each KCC isoform were amplified from the obtained ss-cDNA by PCR using *Taq* PCR DNA polymerase (Qiagen) with amplification protocols as follows: 35 cycles at 96 °C for 30 s, 64 °C for 30 s, and 72 °C for 1 min. RT-Mastermix (without RNA), BV-2 RNA only, and PCR-Mastermix (without cDNA) served as negative controls. In addition, amplification of a 687 bp fragment of β-Actin served as control; in case of genomic DNA contamination, a PCR fragment of 815 bp would have been generated. Following PCR primers were designed on the basis of the published murine cDNA sequences KCC2 (accession no. NM_020333.1), KCC3a (accession no. AF211854.1), KCC3b (accession no. AF211855.1), KCC4 (accession no. AF087436): Primer (5'-3'): KCC1 sense: *ctg cag gct ctc ctc att gtc c*, KCC1 antisense: *ggc cga ggg tgt ctg tgg ac*, (769 bp); KCC2 sense: *ctc acc tgc atg gcc acc gtt*, KCC2 antisense: *ccc acg cct ctc gac aat cac c*, (408 bp); KCC3ab sense: *gac ccg agt cag aac tcc atc ac*, KCC3ab antisense: *gac tcc ttg agt gca tcg tca ctc*, (62 bp) KCC4 sense: *cta cgc cgg gag atg gaa acc*, KCC4 antisense: *ggc cac aat gag gaa gga ctc cat*, (359 bp). To verify the sequences of the amplified PCR products, we gelpurified the via agarose gel electrophoresis separated PCR products, using the QIAquick gel extraction kit (Qiagen), cloned the pcDNA3.1/V5-HisTOPO TA vector, using the TA cloning kit (Invitrogen, Groningen, The Netherlands), and sequenced plasmid DNA from individual clones (MWG-Biotech).

Video microscopic documentation of lamellipodium formation

Cells were seeded on poly-D-lysine coated cover slides (Ø 32mm) in petridishes and cultured in DMEM supplemented with 10% FBS over night in a humidified incubator (37° C, 5% CO_2). 30 minutes before the high KCl solution experiments (in mM: 135 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 8 D-glucose; pH 7.4; 333 mosmol/l) and high Kglut saline (in mM: 135 KGlut, 10 HEPES, 8 D-glucose; pH 7.4; 331 mosmol/l) medium was substituted with extracellular solution (in mM: 130 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 8 D-glucose; pH 7.4; 340 mosmol/l). Osmolority was adjusted using a 2 osmol/l glucose solution. A special heat-able chamber for superfusion of cells seeded on glass cover slips was provided by Zeiss (Germany). Visual evaluation of BV-2 cells superfused with high potassium chloride saline, high potassium chloride saline supplemented with 10µM or 100µM DIOA, 200µM flufenamic acid and 1mM amiloride, respectively, hypoosmotic 60% sodium chloride saline, hypoosmotic 60% sodium chloride saline supplemented with 10µM DIOA and 200µM flufenamic acid, respectively, sodium chloride saline, sodium chloride saline supplemented with 10µM or 100µM DIOA, 200µM flufenamic acid and 1mM amiloride, respectively, high potassium glutamate saline and serum containing as well as serum free media, was performed

Fig. 1. BV-2 cells express swelling-activated chloride channels as well as KCC. A swelling-activated Cl^- current was either activated by a hypoosmotic solution (60% standard saline) (upper panel) or by a high KCl solution (lower panel). Pipette solution [mM]: 150 K aspartate, 1 $CaCl_2$, 2 $MgCl_2$ 10 HEPES, 10 EGTA, 3 MgATP, 0.5 GTP. The contaminating inwardly rectifying K^+ current in the high KCl external solution was eliminated by 3mM Ba^{2+} and the delayed outwardly rectifying current was inactivated by holding the membrane potential at 0 mV. Cl^- currents were monitored during 400ms voltage ramps from -120mV to +80mV elicited every 10 seconds. In the upper panel, trace 1 represents the current before exposure to hypoosmotic solution, trace 2 represents Cl^- current following exposure to 60% standard solution, and trace 3 visualizes the block of the Cl^- current by 10µM DIOA. In the lower panel, trace 1 is the current trace in the standard external solution, trace 2 represents Cl^- current following exposure to 135mM KCl solution, and trace 3 visualizes the block of the Cl^- current by 10µM DIOA, respectively. (B) Expression of KCl cotransporters (KCCs) in BV-2 cells. (A-D) Lane 1 shows RT-PCR reaction of individual mRNA samples. As a control for each, the RT-PCR reaction samples without template mRNA (lane 2), with RNase-free DNase (lane 3), and without cDNA (lane 4) were run in parallel. Lane M: 1kb DNA ladder. In order to exclude genomic DNA contamination a 551bp fragment of β-Actin served as control.



via time laps video microscopy using a confocal laser scanning microscope form Zeiss (LSM510Meta). To exclude mechanical stimulation superfusion of BV-2 cells with serum containing media served as control. A Helium Neon laser with λ = 543nm and a Plan-Apochromat 63x/1.4 oil DIC objective was used to visualize BV-2 cells. Every 10 seconds a differential interference contrast image was collected. The software being used for evaluation was provided by Zeiss especially for LSM 510 Meta.

Electrophysiology

BV-2 cells were plated on poly-L-lysine (1mg/ml) coated glass coverslips. Ion currents were monitored in a standard whole-cell recording mode [18, 19] with an EPC-9 patch clamp amplifier (HEKA Elektrotechnik, Lambrecht, Germany). The cells were superfused with standard external solution containing (in mM): 164.5 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 HEPES; titrated to pH 7.4 with NaOH. A swelling-activated chloride current was elicited either by a hypoosmotic solution (60% extracellular saline) or by an iso-osmotic KCl solution [20, 21]. Dilution of the external standard solution with distilled water was used to prepare a hypoosmotic external solution (60% external saline). The iso-osmotic KCl solution contained (in mM): 164.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 HEPES; titrated to pH 7.4 with KOH. The recoding pipette was filled with a solution containing (in mM): 150 K-aspartate, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 10 EGTA, 3 Mg_2ATP, 0.5 GTP; titrated to pH 7.2 with KOH. Resistance of electrodes filled with internal solution ranged between 2 and 4 MΩ. Electrodes were pulled from soft glass capillaries and fire polished. Membrane and pipette capacitance were compensated using an electronic feedback via the patch clamp amplifier immediately after break in and formation of stable whole-cell recording mode. Liquid junction potential between pipette and bath solution (< 5mV) was not corrected for. In all experiments, the holding potential was kept at 0mV. Chloride current was monitored during a 400ms voltage ramp from -120mV to +80mV and the voltage ramp was elicited every 10 second. All experiments were done at room temperature. Ion channel and transporter blockers were dissolved in EtOH and diluted in external solutions.

Statistical analysis

All data were presented as mean ± SD. Statistical analysis occurred via Excel and GraphPad. Each experiment was conducted at least for 5 times. Student's double sided t-test for independent samples was applied to calculate the levels of significance, $P < 0.5$ and $P < 0.05$.

Results

Hypoosmotic extracellular solution as well as high extracellular K^+ activate a swelling activated chloride channel

In the absence of osmotically challenging solutions, Cl^- currents were not detectable. Exchange of the high KCl saline either by a hypoosmotic saline (60%) or by iso-osmotic KCl solution provoked the development of Cl^- currents. Regardless of the osmotic challenge, Cl^- currents were completely blocked by SITS (1mM) and flufenamic acid (200μM). Superfusion of BV-2 cells with high KCl solution containing DIOA (10μM) prevented development of Cl^- currents. This observation is in line with the documented inhibitory effect of DIOA on KCC [21]. However, DIOA suppressed high K^+ - as well as hypoosmotic-induced Cl^- current (Fig. 1). Therefore, DIOA not only inhibits KCC activity, but is also a potent blocker of swelling-activated Cl channels.

Among the four identified mammalian KCC, KCC1 and KCC4 are osmolarity-sensitive and involved in volume regulation (reviewed in [22]). To characterize the repertoire of KCC in BV-2 cells, we used RT-PCR. As visualized in Fig. 1, BV-2 cells express each of the four KCC.

High extracellular K^+ and hypoosmotic saline induces lamellipodium formation

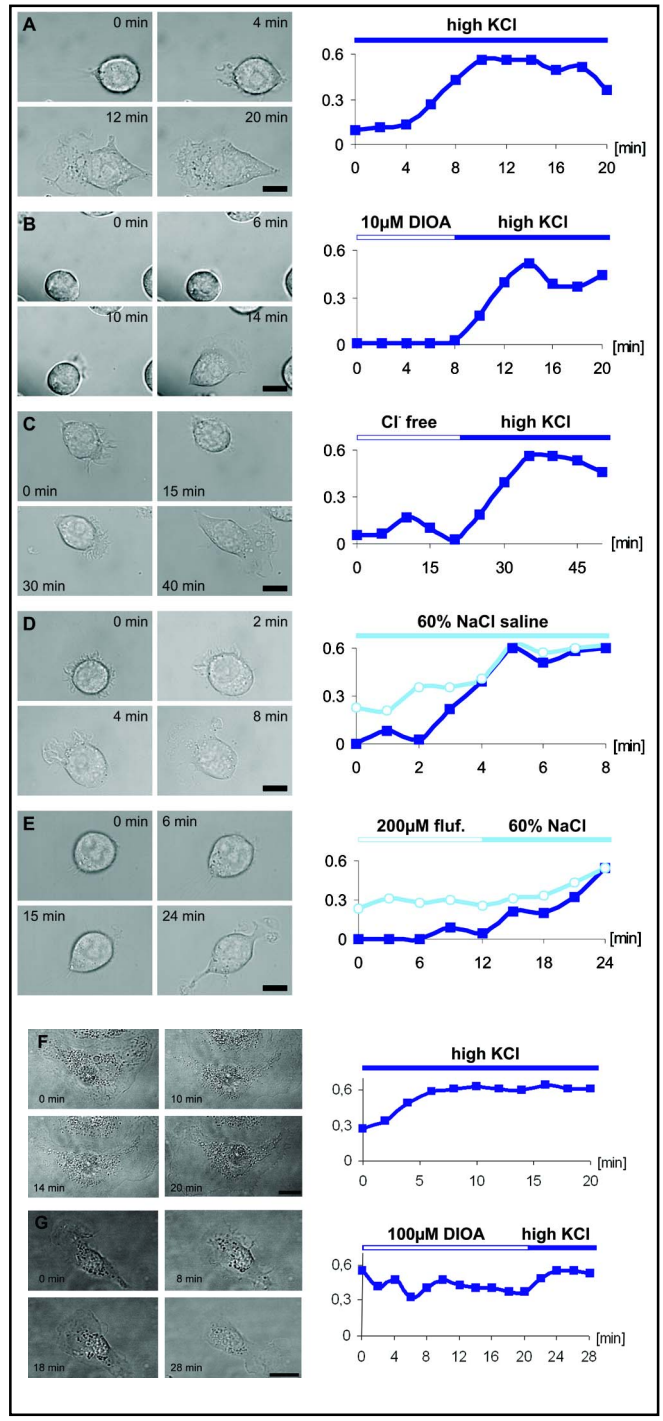
Because chloride conductance is associated with migration [23] as well as volume regulation [5], we speculated that formation of lamellipodia is nothing but a localized swelling that depends on chloride conductance and influx of water. Response of a cell to a hypoosmotic challenge may be subdivided in an early phase, where the cells swell via influx of osmolytes and water, and in a late phase, where the original volume is reestablished, via an efflux of osmolytes, like chloride and organic ions, followed by an efflux of water. In the present study, we evoked cell swelling by superfusion (1) of BV-2 cells and primary microglial cells, respectively, with high KCl solution and (2) of BV-2 cells with 60% saline. In the experiments described below, cells were cultured in serum-containing medium for 12 hours. Before exposure of cells to osmotically challenging solutions (60% saline or high KCl solution) cells were cultured in saline for 30 minutes. Cell swelling as well as migration was documented by video-microscopy using a video acquisition rate of six frames every minute.

High KCl mediates cell swelling by decreasing the K^+ gradient across the plasma membrane [21]. Under physiological conditions, KCl cotransporter export K^+ and Cl^- from the cytoplasm into the environment. When the extracellular K^+ concentration is increased, KCl cotransporter mediates an influx of K^+ and Cl^-. Cytoplasmic accumulation of these ions increases intracellular osmolarity and, consequently, an influx of water and cell swelling. BV-2 cells as well as primary microglial cells exposed to a high KCl solution did not show an equal swelling in each direction, but, surprisingly, developed a delicate extension at one pole of the cell, which proceeded rapidly into a vigorously moving lamellipodium within seconds. To quantify the extension of the lamellipodium, we encircled the light microscopically organelle-free area using LSM 5 Image Browser (Zeiss) (Fig. 2). Following formation of a lamellipodium, the cell started to migrate. We observed lamellipodium formation, when using 135mM as well as 70mM KCl solution (Fig 3). In contrast to most BV-2 cells, primary microglial cells had at least a small lamellipodium at the beginning of the experiment. Nonetheless, exposure of primary microglial cells to 135mM KCl enhanced their lamellipodium impressively. Whereas BV-2 cells used their newly formed lamellipodium to migrate, primary microglial cells subsequently formed several lamellipodia. Consequently, they migrated only a small distance, before changing their direction.

To investigate a second cell-swelling paradigm, we exposed BV-2 cells to hypoosmotic 60% saline. When the high KCl saline was replaced by 60% saline, the cell displayed three distinct responses. Initially, the cell started to swell equally to a maximum of 172% of its initial cell volume within two minutes. Subsequently, a lamellipodium emerged at one pole of the cell, and the cell started to migrate.

In a series of experiments, we investigated the impact of (1) the chloride conductance blockers, DIOA and flufenamic acid and (2) chloride-free high K^+ solution, where Cl^- has been substituted by glutamate, on formation of lamellipodia. DIOA (10μM) consistently suppressed formation of lamellipodium in BV-2 cells exposed to high K^+ (n = 12) or to 60% saline (n = 14). Flufenamic acid (200μM) had a similar suppressing effect (KCl: n = 21, 60% saline: n = 16). 100μM flufenamic acid as well as 1μM amiloride did not prevent lamellipodium formation (Fig 3). Also superfusion with chloride-free high K^+ solution did not provoke lamellipodium formation (n = 30). Superfusion of cells with KCl, following exposure to DIOA, flufenamic acid, or chloride-free solution resulted in the formation of a lamellipodium (Fig. 2). Moreover lamellipodia formation and rapid undirected cell migration

Fig. 2. Lamellipodium formation of BV-2 and primary microglial cells depends on extracellular Cl^- and chloride conductance. Imaging data were collected every 10 seconds using video microscopy (LSM510Meta, Zeiss, Germany). The relationship between the degree of lamellipodium formation and external conditions was assessed by correlating the change in the extension of the lamellipodium with either (A) high external KCl, (B) high external KCl in the presence of 10µM DIOA, (C) Cl^- - free external solution (Cl^- was substituted by glutamate), (D) 60% standard external solution (hypoosmotic solution), and (E) and with hypoosmotic NaCl saline supplemented with 200µM flufanamic acid. The y-axis shows the ratio of the area of the lamellipodium (LA) compared to the cell area (CA) (LA/CA). Application of 10µM DIOA (B), as well as a chloride free solution (C), reversibly prevented the isoKCl-induced lamellipodium formation. Hypoosmotic solution triggers rapid cell swelling, followed by the formation of a lamellipodium (D). The formation of a lamellipodium, but not cell swelling, is blocked by 200µM flufenamic acid (E) as well as 10µM DIOA (data not shown). The blue graphs (squares) again show the ratio of the area of the lamellipodium (LA) compared to the cell area (CA) (LA/CA). The light blue graph (circles) directly shows the changes in cell area (x 10 µm). Data are representative for 12 to 30 cells for each experimental condition. Scale bar, 10µm. Additionally, we investigated the impact of high external KCl on lamellipodia formation in primary microglial cells (F, G). Similar to BV-2 cells, primary microglia respond to high external KCl with rapid lamellipodia formation and undirected cell migration (F), which is reversibly inhibited by 100µM DIOA (G). The blue graphs (squares) again show the ratio of the area of the lamellipodium (LA) compared to the cell area (CA) (LA/CA). Data are representative for 5 cells for each experimental condition. Scale bar, 10µm



of primary microglial cells treated with high K^+ is inhibited by the addition of 100µM DIOA (Fig 2F,G).

An amiloride-sensitive conductance is involved in RVD [24] as well as extension of lamellipodia [25]. Amiloride blocks the epithelial Na channel, ENaC, as well as the Na^+/H^+ exchanger, NHE1, [23, 25, 27]. In BV-2 cells, amiloride (1mM) significantly suppressed NaCl saline - induced lamellipodium formation (n = 11), whereas lower concentrations of amiloride (1µM, n = 6 and 100µM, n = 8) did not significantly inhibit lamellipodium formation (Fig. 3B). Because amilorde blocks ENaC with a K_d of about 3 nM [28], but we found a significant effect only at 1mM, it is unlikely that ENaC plays a significant role in lamellipodium formation in BV-2 cells. Thus, formation of lamellipodia may be based on redundant systems in different cell types; one depends on chloride conductance and a second on an amiloride sensitive conductance, like epithelial Na channels.

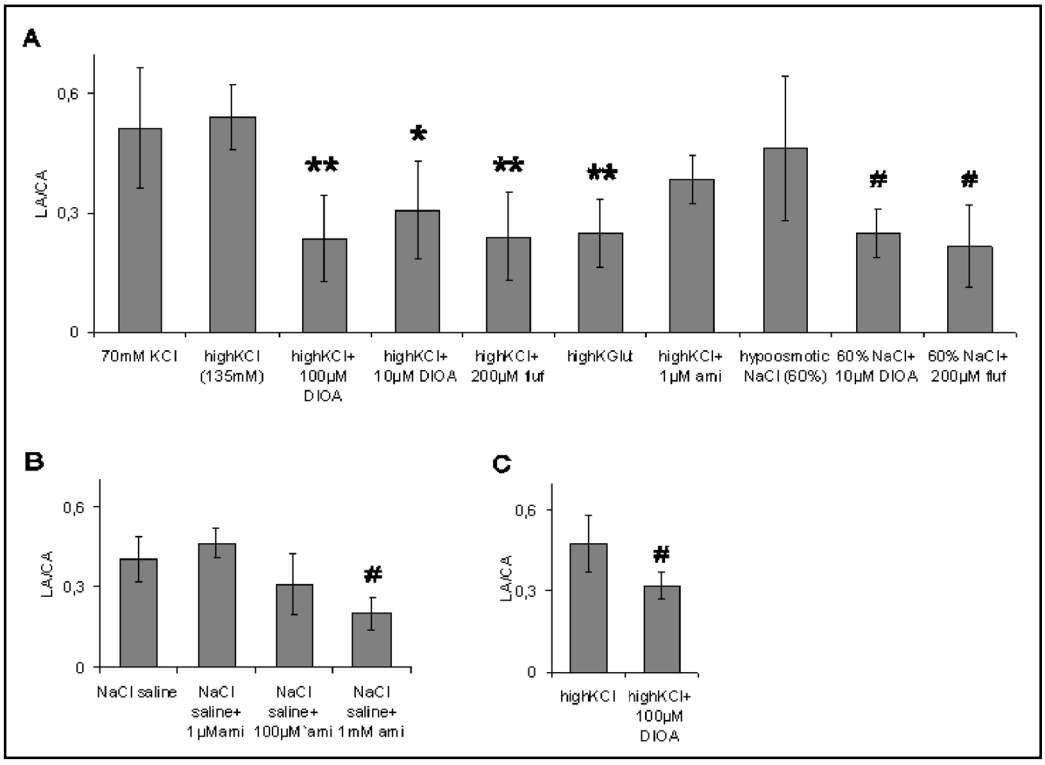


Fig. 3. Quantitative Analysis of different ion channel blockers on lamellipodia formation. (A) Summary of inhibitors of chloride conductance and chloride-free saline on lamellipodia formation of BV-2 cells. (B) Summary of different concentrations of the Na/H exchange and NHE1-3 blocker amiloride on NaCl saline - induced formation of lamellipodia in BV-2 microglial cells. (C) Effect of 135mM KCl solution and blockade of chloride conductance on lamellipodia formation in primary microglial cells. Maximal lamellipodia area was assessed visually according to the criteria mentioned in the text and quantified by calculating the ratio of the lamellipodium area (LA) to the entire cell area (CA) LA/CA. Error bars represent SD, n between 5 and 30 cells, P = 0.01 was considered as highly significant (**) and P = 0.025 as significant (*) in (A), and P = 0.1 as significant in (B) and (C), respectively (#).

Adaptation of BV-2 cells to saline before the experiment was required because immediate change of serum-containing media with isotonic saline provoked a transient formation of lamellipodia. Although the lamellipodium formation is sensitive to DIOA (10µM), flufenamic acid (200µM), and amiloride (1mM), the mechanism of this effect had not been pursuit further in the present study.

Discussion

In migration assays, chloride conductance is essential for migration in glioma cells, human monocytes, transformed renal epithelial cells, carcinoma cells (CNE-2Z), and microglial cells [7, 8, 29-31]. Despite these convincing reports on the correlation between chloride conductance and migration, little is known on the impact of chloride conductance on lamellipodium formation.

Detailed cellular maps on the asymmetric distribution and activation of cytosolic signaling proteins, like Cdc 42, PI3Ks, PTEN, or Rac, as well as integrins, integral membrane proteins, are available (for review see [3]). In addition, several studies reveal that different transporters and ion channels participate in the molecular regulation of cell migration. According to one model, a migrating cell shows a regulatory volume decrease at its rear end and a regulatory volume increase at its front end [5]. This model has been substantiated by identification of the asymmetric distribution of transporters and ion channels in the polarized migrating cell. At the rear end, intracellular Ca^{2+} oscillations evoke K^+-efflux by concomitant activation of Ca^{2+}-activated K channels, whereas in the lamellipodium Na/H exchanger and Na-K-2Cl cotransporters have been identified [5]. Stimulation of Na/H exchanger leads to local swelling, which is required for migration [5]. Interestingly, Schwab and coworkers postulate that different ion channels such as K channels and cotransporters, like the $NaHCO_3$ cotransporter NBC1, are distributed in a polarized way [25]. Our findings extend this model by showing that chloride influx promotes lamellipodium formation. Furthermore, our study suggests a polar distribution of KCC transporters before the cell is morphologically polarized (Fig. 4).

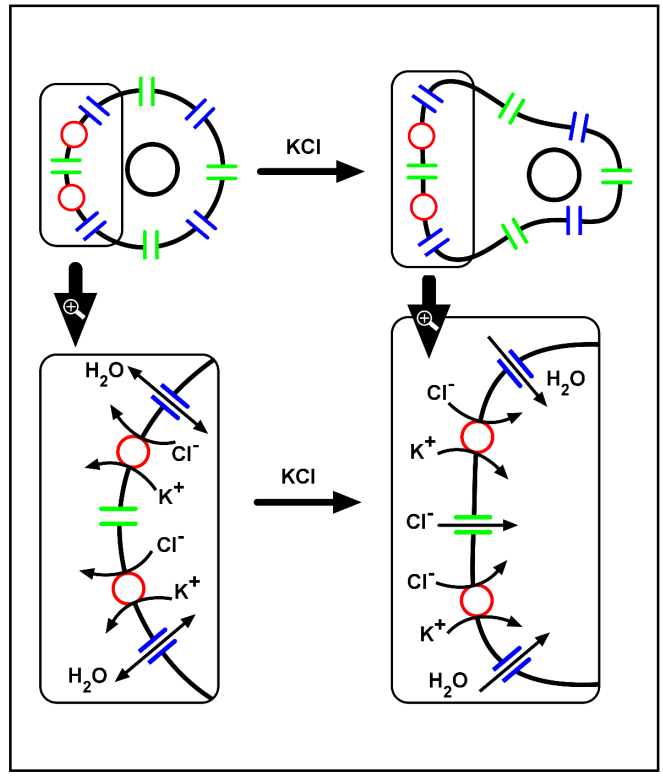


Fig. 4. Hypothetical mechanism on high K^+ - induced lamillipodium formation. Our model depicts (1; upper panel) the distribution of swelling – activated Cl-channels, aquaporins, and KCC, and (2; lower panel) the mechanism of local swelling. Upper panel: In morphologically non-polarized cells, swelling - activated Cl-channels and aquaporins are evenly distributed, whereas KCC are accumulated on one pole. This assumption is based on our experimental data that (1) hypoosmotic saline initially triggers an equal swelling of the cell, indicating the swelling – activated Cl-channels and aquaporins are equally distributed and (2) that high K^+ triggers lamellipodium formation right away. Lower panel: Superfusion of the cell with high K^+ reverses the flux direction of K^+ and Cl- from outward to inward. Influx of K^+ and Cl- increases locally the osmolarity and evokes an influx of water via aquaporins.

At least three factors may activate chloride influx: (1) an increase in extracellular K^+ concentration, (2) hypoosmotic conditions, or (3) induction of chloride conductance by endogenous factors. Extracellular K^+ increases transiently during neuronal activity, but is prolonged elevated during epileptic seizures [32]. Furthermore, cell death by injuries or neurodegenerative diseases liberates intracellular K^+. For instance, following ischemia the extracellular K^+ concentration increases from the range of 2.5 – 3.5mM to 50 – 80mM [33]. According to our study, extracellular K^+ increase reverses flux direction of KCC and evokes cell swelling. Whereas transient increases in osmolarity are common in the brain, for example during synaptic transmission, persistent hypoosmotic conditions have not been documented. However, hypoosmotic swelling increases migration in neutrophils [6]. Interestingly, endogenous factors are known which induce migration in microglial cells via activation of Cl channels [9]. Moreover the activation of purinergic receptors (P2Y) and canabinoid receptors (CB2) triggers lamellipodium formation [34-36]. This list suggest that increase in extracellular K^+ concentration as well as endogenous factors are relevant stimuli in cell migration and, presumably, in lamellipodium formation.

In microglial cells activation of chloride influx and concomitant local volume changes may explain why these cells don't swell homogenously in response to osmotic changes in their microenvironment. Because we could not pharmacologically distinguish between Cl channels and KCC transporters, we cannot estimate the contribution of these membrane proteins to lamellipodium formations. The extracellular osmolarity changes when plasma of injured capillaries leaks into the CNS tissue [13, 14] or when the high intracellular K^+ and ATP concentrations are discharged [34, 35]. The remarkable feature of microglial cells to respond to distinct changes in their microenvironment with formation of vibrant lamellipodia, either at the tip of their processes to seal injured capillaries or with a prominent lamellipodium in the case of amoeboid phenotypes to migrate to the site of injury, marks these cells as significant and flexible players in neuroinflammatory diseases.

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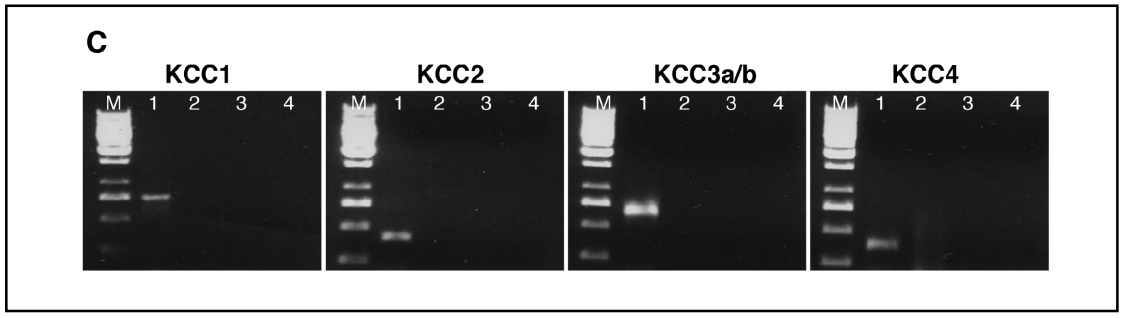
Erratum

Cell Physiol Biochem 2009;24:635

In the article by Zierler et al. entitled “Chloride influx provokes lamellipodium formation in microglial cells” published in Cell Physiol Biochem 2008;21(1-3):55-62 the Fig. 1C is incorrect. KCC1 inadvertently was shown twice instead of KCC1 and KCC2. The corrected figure below now visualizes KCC1 and KCC2. The change of the figure does not affect the original figure legend.



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