Heart Vessels (2011) 26:91–100
DOI 10.1007/s00380-010-0025-0

ORIGINAL ARTICLE

# Cell membrane stretch activates intermediate-conductance $Ca^{2+}$-activated $K^+$ channels in arterial smooth muscle cells

**Yasunobu Hayabuchi · Yutaka Nakaya · Kazuaki Mawatari · Miki Inoue · Miho Sakata · Shoji Kagami**

Received: 4 September 2009 / Accepted: 21 January 2010 / Published online: 10 November 2010


**Abstract** The aim of this study is to determine the signal transduction of membrane stretch on intermediate-conductance $Ca^{2+}$-activated $K^+$ (IKca) channels in rat aorta smooth muscle cells using the patch-clamp technique. To stretch the cell membrane, both suction to the rear end of patch pipette and hypotonic shock were used. In cell-attached and inside-out patch configurations, the open probability of IKca channels increased when 20- to 45-mmHg suction was applied. Hyposmotic swelling efficiently increased IKca channel current. When the $Ca^{2+}$-free solution was superfused, the activation of IKca current by the hyposmotic swelling was reduced. Furthermore, gadolinium ($Gd^{3+}$) attenuated the activation of IKca channels induced by hyposmotic swelling, whereas nicardipine did not. In the experiments with $Ca^{2+}$-free bath solution, pretreatment with GF109203X, a protein kinase C (PKC) inhibitor, completely abolished the stretch-induced activation of IKca currents. The stretch-induced activation of IKca channels was strongly inhibited by cytochalasin D, indicating a role for the F-actin in modulation of IKca channels by changes in cell stretching. These data suggest that cell membrane stretch activates IKca channels. In addition, the activation is associated with extracellular $Ca^{2+}$ influx through stretch-activated nonselective cation channels, and is also modulated by the F-actin cytoskeleton and the activation of PKC.

**Keywords** Smooth muscle cell · Intermediate-conductance calcium-activated potassium channel · Nonselective cation channel · Protein kinase C · Actin cytoskeleton

Y. Hayabuchi (✉) · M. Inoue · M. Sakata · S. Kagami
Department of Pediatrics, School of Medicine,
University of Tokushima, 3-18-15 Kuramoto-cho,
Tokushima 770-8503, Japan
e-mail: hayabuchi@clin.med.tokushima-u.ac.jp

Y. Nakaya · K. Mawatari
Department of Nutrition and Metabolism, School of Medicine,
University of Tokushima, 3-18-15 Kuramoto-cho,
Tokushima 770-8503, Japan

## Introduction

Blood vessels in vivo are continuously exposed to hemodynamic forces. These include shear stresses on the luminal surface generated by blood flow, cyclic distension due to the vascular wave caused by the pulsatility of the blood flow, and endocrine and local factors including angiotensin II (Ang II) and endothelin-1 [1–3]. The luminal surface of blood vessels is lined with endothelial cells so that shear stress is sensed predominantly by endothelial cells [4, 5]. However, both endothelial and vascular smooth muscle cells are subjected to cyclic stretch.

Under normal conditions, vascular smooth muscle cells are quiescent and contractile [1–3, 6, 7]. In response to pathologic stress, however, vascular smooth muscle cells develop a proliferative, hypertrophic, and secretory phenotype [1, 6, 7]. These alterations result in vascular remodeling characterized by cellular hyperplasia, hypertrophy, apoptosis, enhanced protein synthesis, and extracellular matrix reorganization.

Mechanical stretch stimulates the migration and proliferation of vascular smooth muscle cells. Recent studies indicate that $Ca^{2+}$-activated $K^+$ (Kca) channels, specifically intermediate-conductance $Ca^{2+}$-activated $K^+$ (IKca) channels, have an important role in cell migration and

proliferation. It is not known, however, whether the cell membrane stretch is linked to IKca channel regulation.

IKca channels are regulated through the influx of calcium ions. They are more sensitive to $Ca^{2+}$ than other types of $Ca^{2+}$-activated $K^+$ channels, such as BKca channels [8, 9]. In addition, each type of Kca channel has a distinct pharmacology, and can hyperpolarize the membrane potential. In contrast to the vasodilatory function of BKca channel, the role of IKca channels in vascular smooth muscle cells is not completely understood. IKca channels, however, play a role in many physiologic functions such as proliferation, epithelial transport, and cell migration [10–12].

We aimed to determine whether mechanical stretching of the cell membrane regulates the activity of IKca channels in cultured rat aorta smooth muscle cells using patch-clamp technique. Furthermore, we studied the signal transduction of membrane stretch on IKca channels. The present study demonstrates that cell-membrane stretch activates IKca currents, and the activation is associated with extracellular $Ca^{2+}$ influx through stretch-activated non-selective cation (SA) channels, and the modulation of F-actin cytoskeleton and the activation of protein kinase C (PKC).

## Methods

### Preparation of cultured smooth muscle cells

Embryonic rat thoracic aortic smooth muscle cells from normotensive Berlin-Druckrey IX (A10; ATCC CRL 1467) were obtained from the American Tissue Type Collection (Rockville, MD). A10 cells were cultured at 37°C in 95% air/5% $CO_2$ atmosphere in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY). Cells were passaged once every 4 days at a seeding density of $3 \times 10^5$/ml. In the patch-clamp experiments, the cells were cultured in DMEM with fetal bovine serum in 35-mm plates, as described above, until reaching approximately 80–90% confluency. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication NO. 85-23, revised 1996).

## Solution and chemicals

### Inside-out patch

The bath solution contained (in mM): 110 KCl, 30 KOH, 10 Hepes, 1 EGTA, 1 $MgCl_2$, and 0.54 $CaCl_2$, adjusted to pH 7.2. The free $[Ca^{2+}]$ calculated using the program Maxchelator (http://www.stanford.edu/~cpatton/webmaxc/webmaxcS.htm) was 215 nM. The patch pipette was filled with (in mM): 140 KCl, 1 $MgCl_2$, and 10 Hepes; adjusted pH to 7.4. Depending on the experiment, the free $Ca^{2+}$ was changed from 0 to 10 μM by changing the calcium concentration in the corresponding solution.

### Cell-attached patch

The 140 mM $K^+$ pipette solution for single channel recordings contained (in mM): 140 KCl, 1 $MgCl_2$, 10 Hepes, adjusted pH to 7.4. The bath solution was the physiological isotonic (310 mOsm/kg$H_2O$) solution consisted of (in mM): 134 NaCl, 6 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes, and 10 glucose, adjusted pH to 7.4, or the hypotonic (223 mOsm/kg$H_2O$) in which NaCl concentration was reduced (in mM): 90 NaCl, 6 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes, and 10 glucose, adjusted pH to 7.4. Depending on the experiment, $Ca^{2+}$-free bath solution was used.

### Chemicals

Charybdotoxin (ChTX), clotrimazole (CLT), TRAM-34, GF109203X, calcium ionophore A23187, 1,2-dioctanoyl-*sn*-glycerol (DOG), and cytochalasin D were purchased from Sigma. DOG was dissolved in dimethylsulphoxide. The final concentration of dimethylsulphoxide was less than 0.2%.

## Data recording and analysis

Standard patch-clamp recording techniques were used to measure single-channel currents in either the cell-attached or inside-out patch configuration [13].

Smooth muscle cells were placed in an experimental 1-ml chamber on the stage of an inverted microscope. All experiments were performed at 20–25°C. Soft glass pipettes were pulled (pp-83, Narishige, Tokyo, Japan), and the tips were coated with Silgard to reduce capacitance. The resistance of pipettes filled with solution and immersed in the bath solution ranged from 3 to 5 MΩ for whole cell recordings and from 7 to 9 MΩ for single-channel recordings. Channel currents were recorded with a List Electronics EPC-7 patch-clamp amplifier and stored on a personal computer disk with an analog-to-digital converter (DigiData 1200; Axon Instruments, Foster City, CA). High-frequency signal components (800 Hz) were eliminated using a four-pole Bessel filter and digitized at 2 kHz. The pClamp version 7 software (Axon Instruments) was used for data acquisition and analysis. The channel current amplitude was fitted using a Gaussian curve.

Average channel activity ($NP_o$) in patches was determined from current amplitude histograms and calculated as follows:

$$NP_o = \left(\sum_{j=1}^{N} t_j j\right)/T$$

where $P_o$ is the open probability, $T$ is the duration of the measurement, $tj$ is the time spent at the current level corresponding to $j = 1, 2 \ldots N$ channels in the open state, and $N$ is the maximum number of channels observed in the patch. $NP_o$ was determined over a 3- to 5-min period. The channel activity was expressed as $NP_o$.

## Statistics

Data were expressed as mean ± SEM. Statistical significance was determined by Student's $t$ test or analysis of variance followed by the Student–Newman–Keuls multiple range test as appropriate. Statistical analysis was performed using Prism version 5.0 (GraphPad Software, San Diego, CA). A $p$ value of less than 0.05 was considered statistically significant.

## Results

### Characteristics of IKca channels in cultured smooth muscle cells

The electrophysiologic properties of the IKca channels expressed on cultured smooth muscle cells by whole-cell and single-channel recordings were determined previously in our laboratory [14]. We performed whole-cell patch-clamp experiments on cultured A10 cells to measure functional IKca channel expression. Whole-cell recording was performed with a holding potential of −60 mV. The intracellular solution contained 140 mM $K^+$ and 100 nM free $Ca^{2+}$, and the extracellular $K^+$ concentration was 140 mM. The average cell capacitance was 12.4 ± 0.3 pF ($n = 6$). We examined the effect of CLT, a selective blocker of IKca channels. CLT (1.0 μM) inhibited the current by 71 ± 5% ($n = 6$). The subsequent addition of ChTX, the blocker of both BKca and IKca channels, caused a decline in the remaining current (Fig. 1a). Next, we examined the effect of TRAM-34, a selective inhibitor of IKca channels (Fig. 1b). TRAM-34 (1.0 μM) inhibited the current by 81 ± 6% ($n = 6$). CLT-sensitive and TRAM-34-sensitive current was predominantly expressed in cultured A10 cells.

The single channel current–voltage relationship in a series of inside-out patch clamp experiments using symmetrical 140 mM $K^+$ solution is shown in Fig. 1c. The channel showed inwardly rectifying behavior. The mean values of six independent experiments were 3.0 ± 0.1 and 2.1 ± 0.1 pA at −80 and +80 mV, respectively, corresponding to chord conductances of 38 ± 1 and 26 ± 1 pS, respectively.

Inside-out single channel recordings measured in symmetrical 140 mM $K^+$ conditions for internal $Ca^{2+}$ concentrations ranging from 0 to 10 μM are shown in Fig. 1d. In these experiments, the membrane voltage was maintained at −80 mV. Raising the internal $Ca^{2+}$ concentration from 0 to 10 μM significantly increased the single channel activity. The sigmoidal curve was computed using the Hill equation with an ED50 of 2.41 ± 0.11 μM and a Hill coefficient of 2.38 ± 0.07 ($n = 6$). The channel showed no obvious voltage dependence because $NP_o$ remained unchanged when the holding potential was varied between −80 mV ($NP_o = 0.170 \pm 0.035$; $n = 6$) and +80 mV ($NP_o = 0.190 \pm 0.055$; $n = 6$). These properties are consistent with IKca channels expressed in immature and de-differentiated smooth muscle cells [15].

### Effect of membrane stretching on IKca channels

Stretch-induced activation of IKca channels was observed in smooth muscle cells. The effect of suction application of 25 and 45 mmHg was tested at +40 mV in cell-attached mode. Figure 2a shows a sample recording from one of the patches containing one channel that are activated during the application of negative pressure and subsequently inactivated after removal of the pressure. The application of 25 mmHg increased $NP_o$ from 0.009 ± 0.003 to 0.090 ± 0.020 and the application of 45 mmHg increased $NP_o$ from 0.009 ± 0.003 to 0.203 ± 0.081 ($n = 7$; $p < 0.01$).

To determine if IKca channel activity is enhanced by the release of $Ca^{2+}$ from intracellular stores or entrance of $Ca^{2+}$ through the plasma membrane by stretch stimulus application, we performed experiments in the inside-out mode. At constant $Ca^{2+}$ concentration (215 nM), application of pressure increased the member of channel openings at 25 and 45 mmHg (Fig. 2b). Application of negative pressure to the pipette caused activation of channels that produced outward unitary currents. The $NP_o$ without the negative pressure was 0.135 ± 0.027. The $NP_o$ after application of 25 and 45 mmHg were 0.281 ± 0.041 and 0.440 ± 0.101, respectively, which were significantly higher than the control condition ($n = 6$; $p < 0.01$). After restoring atmospheric pressure to the pipette, open probability returned to the control level. Figure 2c, d display the amplitude histograms showing the effect of the negative pressure on IKca channel activity in the cell-attached and in the inside-out configuration, respectively.

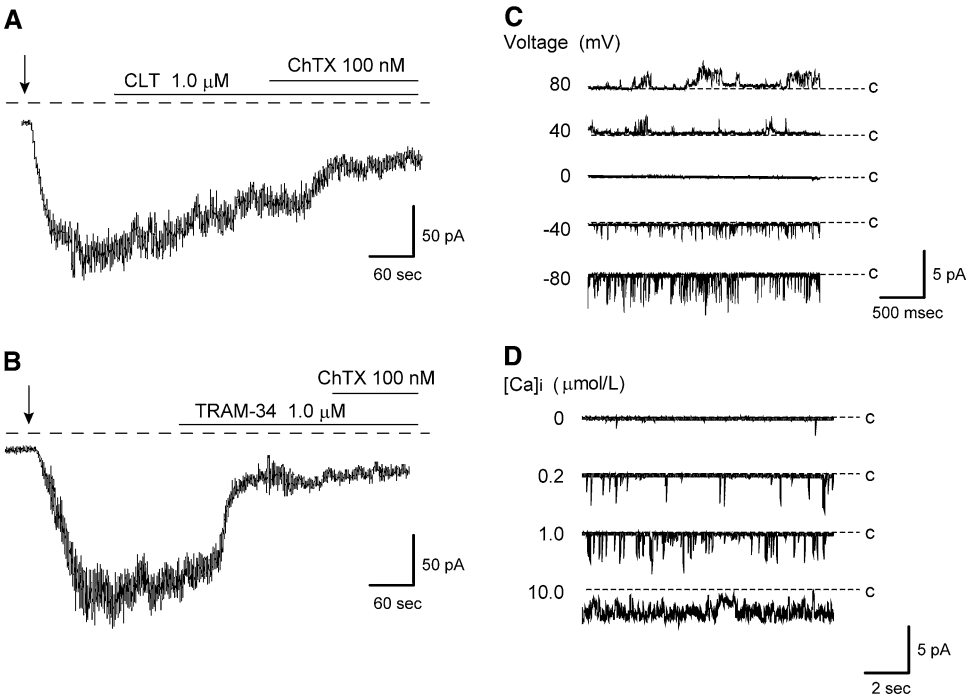


**Fig. 1** Channel properties of IKca channels expressed in A10 cells. **a** Representative recording of whole-cell current from an A10 cell held at −60 mV. The current shows clotrimazole (CLT)-sensitive $K^+$ currents. CLT inhibited the current by 65% in this cell, and charybdotoxin (ChTX) inhibited the remaining current. The *vertical arrow* indicates when the whole-cell configuration was established, and the *dashed line* shows the zero current level. The cell was dialyzed with a solution containing 140 mM $K^+$, and the extracellular $K^+$ concentration was 140 mM. CLT and ChTX were added as indicated. **b** Representative recording of whole-cell current from an A10 cell held at −60 mV. The current shows TRAM-34-sensitive $K^+$ currents. TRAM-34 inhibited the current by 92% in this cell, and charybdotoxin (ChTX) inhibited the remaining current. TRAM-34 and ChTX were added as indicated. **c** IKca single-channel currents from an inside-out patch exposed to symmetrical (140 mM) $K^+$ solutions. Intracellular free $Ca^{2+}$ concentration = 610 nM. Membrane potential was stepped from −80 to +80 mV in 10-mV increments. Closed state is represented by *c*. **d** Intracellular $Ca^{2+}$ activation of IKca channels. Representative traces show single-channel activity of inside-out patches exposed to 0, 0.2, 1.0, and 10.0 μmol/l free $Ca^{2+}$. Channel openings are shown as downward deflections. Voltage was −80 mV, and $K^+$ concentration was symmetrical at 140 mM. Closed state is represented by *c*

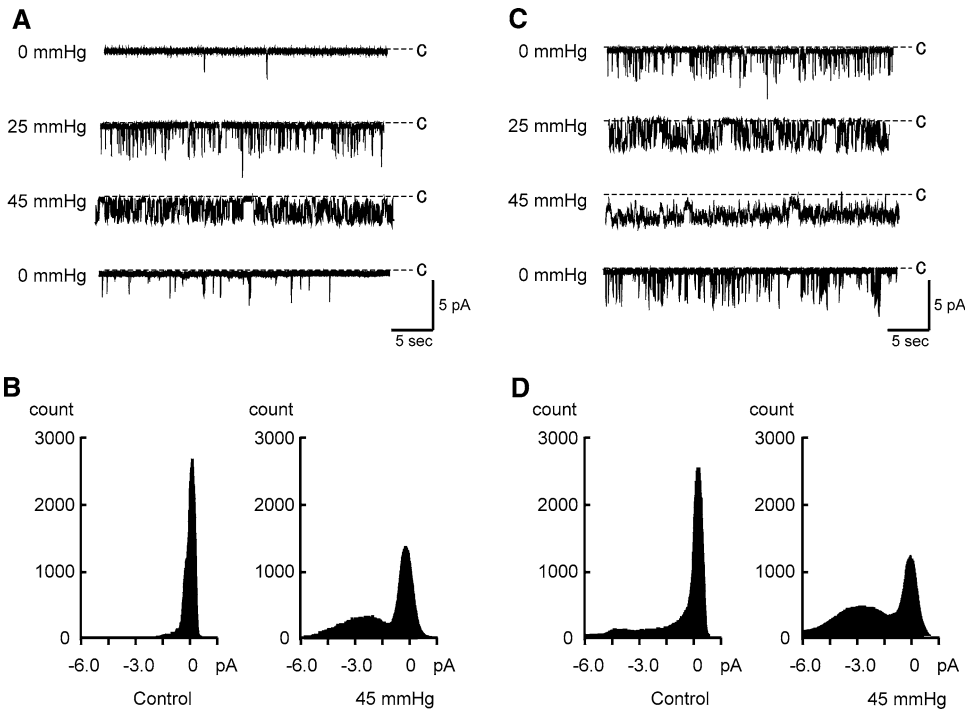


**Fig. 2** Effect of cell membrane stretching on IKca channels. **a** Representative recordings showing the effect of membrane stretching on IKca channels in A10 cells. IKca channel currents are recorded from cell-attached patches at a pipette voltage of +40 mV. **b** Amplitude histograms showing the activation of IKca channels in the same experiments as those of **a**. **c** Representative recordings showing the effect of membrane stretching on IKca channels in inside-out patch configuration. IKca channel currents are recorded at an intracellular $Ca^{2+}$ concentration of 215 nM, and at a pipette voltage of −80 mV. **d** Amplitude histograms showing the activation of IKca channels in the same experiments as those of *c*

### Effect of hyposmotic stretching on IKca channels

Hypotonic shock has been used in other many cell types to stretch the cell membrane. This procedure increases cell membrane tension due to cell swelling. We evaluated the effect of a hypotonic stimulus on the cell-attached mode. IKca currents were first recorded in isotonic medium (control; 310 mOsm/kg$H_2O$) and afterward, the bath solution was replaced by hypotonic medium (223 mOsm/kg$H_2O$).

Figure 3a is a typical recording from ten experiments showing the effects of hyposmotic stress on the IKca channel in cultured smooth muscle cells. IKca single-channel currents were recorded from cell-attached patches at a pipette voltage of +40 mV with a constant perfusion of the experimental chamber. The pipette solution contained 140 mM $K^+$, and the extracellular $K^+$ concentration was 6 mM. The superfusion with hyposmotic solution significantly stimulated IKca channel activity.

The mean open probability increased from 0.009 ± 0.006 in control solution to 0.480 ± 0.012 at 5 min of hypotonic stress. Subsequent application of 1.0 μM TRAM-34 induced a significant inhibition in IKca channel current. In other recordings, channel activity usually decreased when the hypotonic solution was removed and the cells were again bathed in control solution.

### Effect of extracellular $Ca^{2+}$ on the activation of IKca current induced by hyposmotic swelling

It is well known that the IKca channel is activated by intracellular free $Ca^{2+}$ and that extracellular $Ca^{2+}$ is necessary for efficient control of $Ca^{2+}$ homeostasis. To determine if $Ca^{2+}$ influx is involved in the hyposmotic stretch-induced activation of the current, we removed extracellular $Ca^{2+}$ and observed the effect of hyposmotic swelling on IKca channels. When $Ca^{2+}$-free isosmotic solution was replaced with $Ca^{2+}$-free hyposmotic solution, the activation of IKca was attenuated significantly compared with the recordings using physiological $Ca^{2+}$ solution (Fig. 3b). Although the mean open probability increased from 0.009 ± 0.005 in control solution to 0.120 ± 0.006 at 5 min of hypotonic stress ($n = 6$; $p < 0.01$), it was significantly lower than the $NP_o$ with the physiological $Ca^{2+}$ hyposmotic solution ($p < 0.01$).

### L-type $Ca^{2+}$ channel is not involved in the pathway

First, to assess the contribution of L-type $Ca^{2+}$ channels to the hyposmotic stretch-induced activation of IKca channels, we tested the effect of hyposmotic solution on the IKca current in the presence of nicardipine, an L-type $Ca^{2+}$ channel blocker (Fig. 3c).

Nicardipine was added to the extracellular solution for 10 min before the application of hyposmotic solution. The $NP_o$ after pretreatment with nicardipine was 0.007 ± 0.005, which was not significantly different from the $NP_o$ without the pretreatment. The $NP_o$ of IKca channels was significantly increased by hyposmotic solution after nicardipine treatment ($n = 9$; $p < 0.01$). The $NP_o$ in hyposmotic stress was 0.401 ± 0.109, which was not significantly different from the $NP_o$ without the pretreatment. This result indicates that L-type Ca channel is not involved in this pathway.

### Gadolinium blocks hyposmotic swelling-induced activation

We next examined the effect of the pretreatment of 100 nM gadolinium ($Gd^{3+}$), a stretch-activated non-selective cation (SA) channel blocker. As shown in Fig. 3d, $Gd^{3+}$ attenuated the activation of IKca channels induced by hyposmotic swelling ($n = 9$). These results suggested that the influx of extracellular $Ca^{2+}$ is through the SA channel and not the L-type calcium channel and that it is involved in the activation of IKca channels.

### PKC and F-actin are involved in the pathway

Although $Ca^{2+}$-free extracellular solution or the presence of $Gd^{3+}$ attenuated the activation, the IKca current was still enhanced in those conditions. Furthermore, in the experiments of inside-out patch configuration, membrane stretch activated the IKca channel current at constant $Ca^{2+}$ concentration. Therefore, other signal pathways may exist besides those that depend upon an increase in intracellular $Ca^{2+}$.

In order to test the involvement of PKC in hyposmotic swelling-induced IKca channel activation, the effect of GF109203X, a PKC inhibitor, was examined in the experiments using $Ca^{2+}$-free extracellular solution. Pretreatment of A10 cells with 10 μM GF109203X for 20 min abolished the effect of hyposmotic shock ($n = 6$; Fig. 4a). Subsequent application of 10 μM A23187 induced a significant increase in IKca channel current.

Another possibility involves a possible role played by the actin cytoskeleton in cell membrane stretch-dependent regulation of IKca channels. We therefore examined the effect of treatment with cytochalasin D. To disrupt the F-actin cytoskeleton in some experiments, smooth muscle cells were pre-incubated for 3 h in 3 μM cytochalasin D before being subjected to membrane stretch experiments. This treatment almost completely eliminated the response of IKca channels to cell swelling (Fig. 4b).

The GF109203X application data suggested that PKC is involved in the pathway of swelling-induced IKca channel

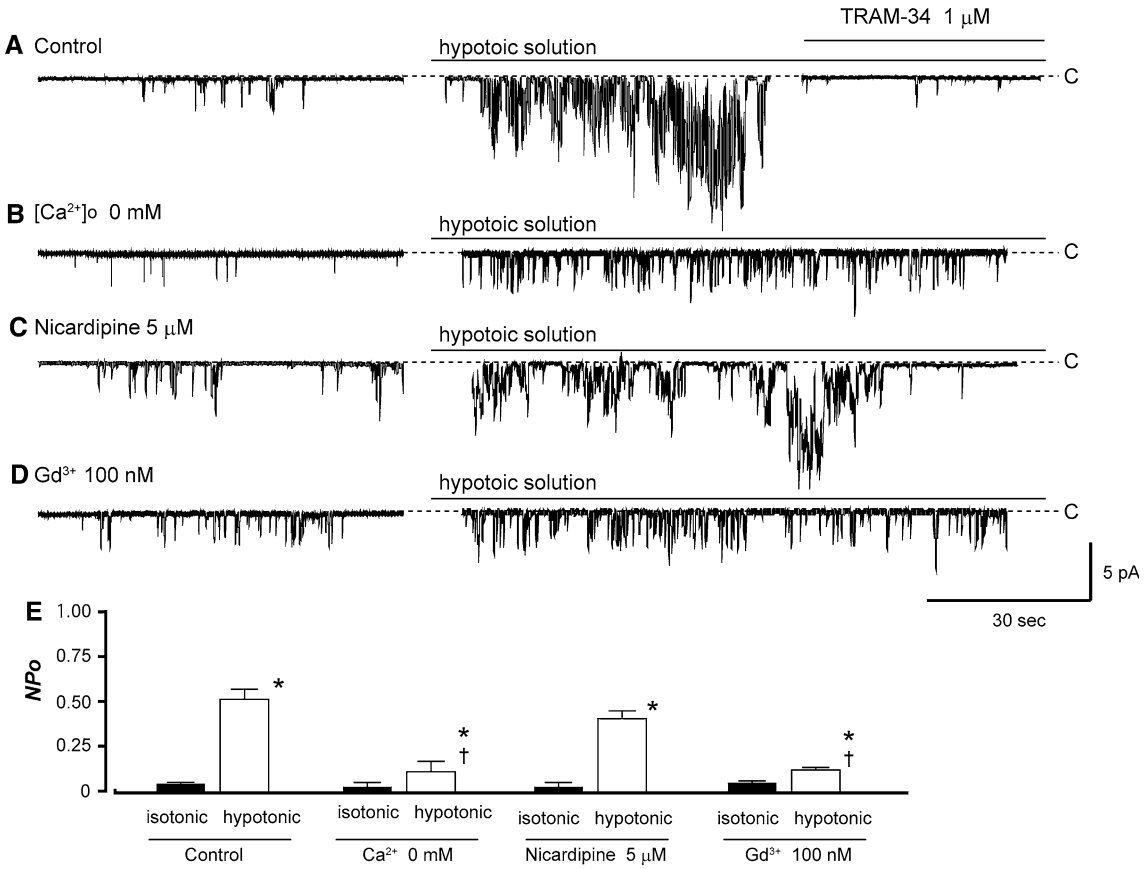


**Fig. 3** Effect of hyposmotic stretching on IKca channels. **a** Representative recordings showing the effect of hyposmotic stretching on IKca channels. IKca channel currents are recorded from cell-attached patches at a pipette voltage of +40 mV. Hyposmotic solution was added as indicated. Hyposmotic stress activated IKca current. Subsequent application of TRAM-34 inhibited the activated current. The *dashed line* shows zero current level. **b** Effect of extracellular $Ca^{2+}$ on the activation of IKca current. The *trace* shows a recording of cell-attached patch. $Ca^{2+}$-free isosmotic solution was replaced with $Ca^{2+}$-free hyposmotic solution. The activation of IKca channels was attenuated under this condition. **c** Effect of nicardipine on the activation of IKca current. The *trace* shows a recording of cell-attached patch that had been pretreated with nicardipine (5 μM). Hyposmotic stress activated IKca channels under this condition. **d** Effect of Gadolinium ($Gd^{3+}$) on the activation of IKca current. The *trace* shows a current recording pretreated with $Gd^{3+}$ (100 nM). $Gd^{3+}$ attenuated the activation of IKca channels. **e** Effect of extracellular $Ca^{2+}$, nicardipine, and $Gd^{3+}$ on IKca channel activation by hyposmotic swelling. The *bars* show mean $NP_o$ (+SEM) of IKca current before (*filled bars*) and after the hyposmotic stress (*open bars*) under these pretreatment conditions. *$p < 0.01$ versus isotonic condition of each pretreatment. †$p < 0.01$ versus hypotonic stress under the control (without the pretreatment) condition

activation. Therefore, we tested the effect of DOG, a membrane permeable analog of 1,2-diacylglycerol (DOG) on IKca channel activity in the presence of cytochalasin D. In six cells pretreated with GF109203X, DOG failed to activate the IKca current, whereas DOG increased $NP_o$ from 0.470 ± 0.101 to 1.380 ± 0.112 without the presence of cytochalasin D.

### Effect of PKC and F-actin on the experiments of negative pressure

In order to confirm the signal transduction of the mechanical stretch demonstrated in the hyposmotic stretch experiments, we tested the effect of GF109203X and cytochalasin D in the cell-attached patch configuration. These examinations were performed in $Ca^{2+}$-free extracellular solution. Pretreatment with GF109203X abolished the stretch-induced IKca channel activation (Fig. 5a). Cytochalasin D also inhibited the effect of stretch on IKca current (Fig. 5b). Although DOG activated IKca channels in the control condition, the pretreatment with cytochalasin D abolished the effect of DOG (Fig. 5c).

## Discussion

Our study demonstrated that membrane stretch and hyposmotic swelling activates IKca channels in cultured artery smooth muscle cells. In cell membrane stretch condition, extracellular $Ca^{2+}$ influx through SA channels

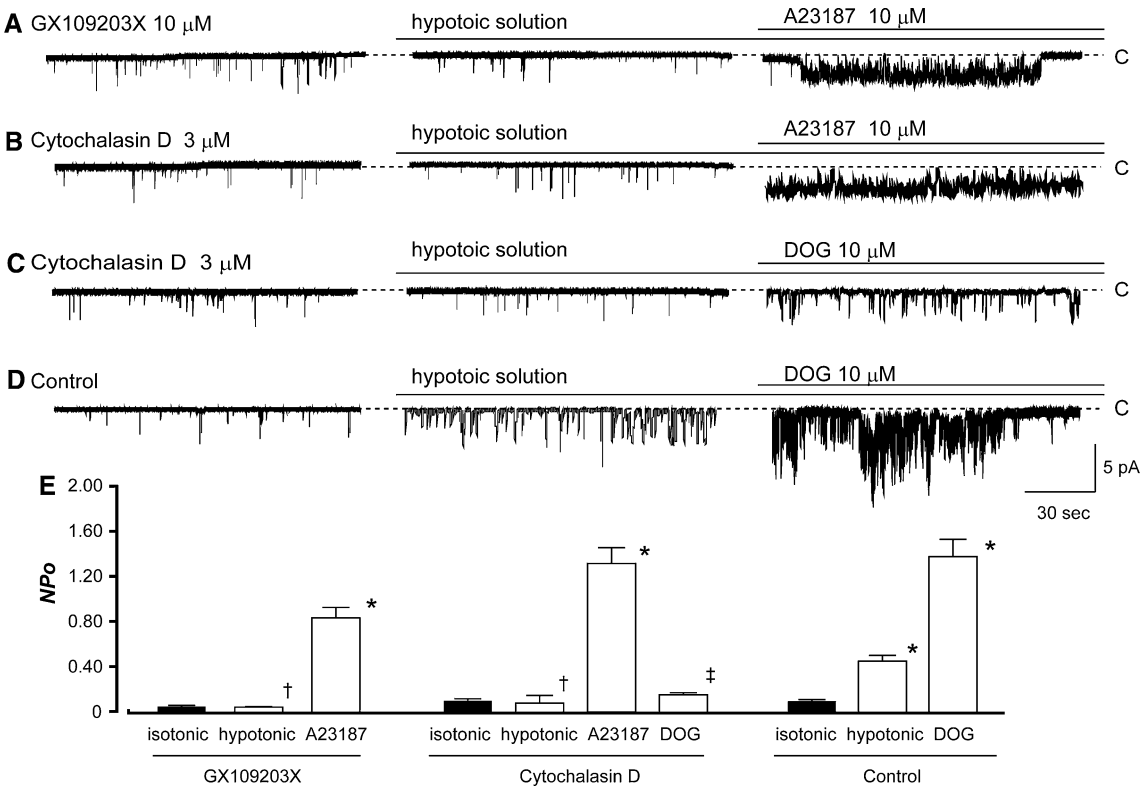


**Fig. 4** Effects of PKC and F-actin on IKca channel activation induced by hyposmotic stress. **a** Representative recordings showing the effect of GF109203X (10 μM) on the activation of IKca current induced by hyposmotic stretching. Hyposmotic solution did not activate IKca channels under this condition, while subsequent application of the calcium-ionophore A23187 (10 μM) was effective. The pipette and bath solutions were the same as in Fig. 3. The pipette voltage was +40 mV. The *dashed line* shows the zero current level. **b** Effect of cytochalasin D (3 μM) on the activation of IKca current. The *trace* shows a recording of cell-attached patch. Hyposmotic stress did not activate IKca channels under this pretreatment. Subsequent application of A23187 was effective. **c** Effect of 1,2-dioctyanoyl-*sn*-glycerol (DOG) on the activation of IKca current under the pretreatment with cytochalasin D. The trace shows a recording of cell-attached patch that had been pretreated with cytochalasin D (3 μM). Neither hyposmotic solution nor DOG (10 μM) activated IKca currents. **d** Effect of DOG on the activation of IKca current in the absence of cytochalasin D. DOG activated IKca channels under this condition. **e** Effect of GX109203X and cytochalasin D on IKca channel activation by hyposmotic swelling. The *bars* show mean $NP_o$ (+SEM) of IKca current before (*filled bars*) and after the hyposmotic stress (*open bars*). $^*p < 0.01$ versus isotonic condition of each pretreatment. $^\dagger p < 0.01$ versus hypotonic stress under the control (without the pretreatment) condition. $^\ddagger p < 0.01$ versus DOG under the control (without the pretreatment) condition

activated IKca channels. Furthermore, our results demonstrate clearly that PKC and F-actin are important factors in the cell membrane stretching-induced activation of IKca channels. Unitary conductance was not modified by suction or swelling.

The arterial wall is continuously exposed to mechanical stimulation such as shear stress and luminal pressure. It is well known that such mechanical strain plays a pivotal role in the development of vascular remodeling in hypertension [16, 17]. However, its exact mechanism remains unknown. Mitogen-activated protein kinases (MAPKs), members of a family of serine/threonine-specific protein kinases [18], are believed to be involved in the pathway of cell proliferation and, therefore, in vascular structural remodeling [19–21]. Kubo et al. [22] have reported that increases in perfusion pressure in isolated perfused rat aortae caused a pressure-dependent increase in the activity of MAPKs. They also demonstrated that pressure loading of the vascular wall of rat aorta can activate p42 and p44 MAPKs and that MAPK activation is mediated at least in part by the vascular angiotensin system [23]. The regulation of MAPK is dependent upon changes in intracellular $Ca^{2+}$ [24]. Furthermore, it is reported that activation of IKca channels enhances $Ca^{2+}$ influx by increasing its transmembrane electrical gradient [25, 26]. The increase in $Ca^{2+}$ influx, caused by activation of IKca, stimulates distinct cellular mechanisms associated with smooth muscle growth and proliferation [14, 15], which can be mediated via MAPK activation. These previous studies suggest that the activation of IKca channels is linked to the activation of MAPK.

We previously reported that Ang II activates IKca channels in arterial smooth muscle cells. Ang II increases the current by interacting with the AT1 receptors, a mechanism which is involved in the activation of PKC

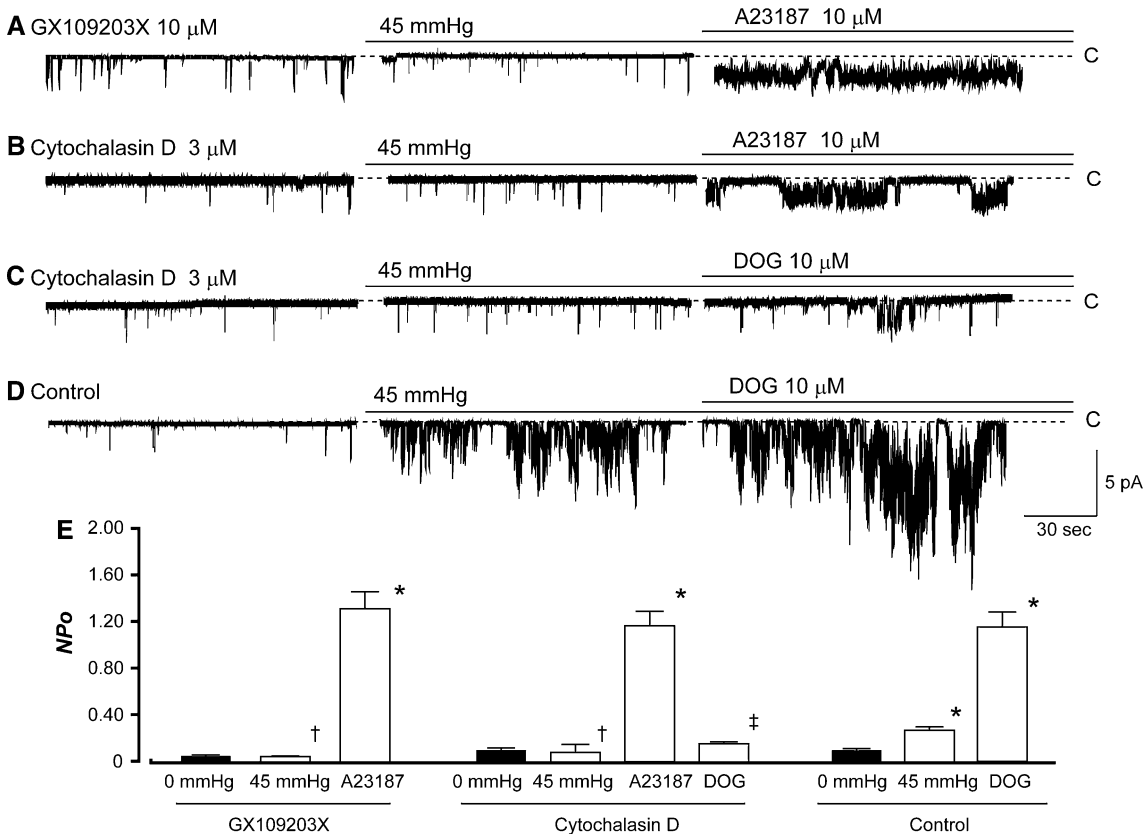


**Fig. 5** Effect of PKC and F-actin on IKca channel activation induced by negative pressure. **a** Representative recordings showing the effect of GF109203X (10 μM) on the activation of IKca current induced by membrane stretching. Negative pressure of 45 mmHg did not activate IKca channels under this condition, while subsequent application of A23187 (10 μM) was effective. The pipette and bath solutions were the same as in Fig. 2a. The pipette voltage was +40 mV. The *dashed line* shows the zero current level. **b** Effect of cytochalasin D (3 μM) on the activation of IKca current. The *trace* shows a recording of cell-attached patch. Membrane stretching did not activate IKca channels under this pretreatment. Subsequent application of A23187 was effective. **c** Effect of DOG on the activation of IKca current under the pretreatment with cytochalasin D. Negative pressure did not activate IKca current, and subsequent application of DOG was also ineffective. **d** Effect of DOG on the activation of IKca current in the absence of cytochalasin D. DOG activated IKca channels under this condition. **e** Effect of GX109203X and cytochalasin D on IKca channel activation by membrane stretching. The *bars* show mean $NP_o$ (+SEM) of IKca current before (*filled bars*) and after the addition of negative pressure (*open bars*). $^*p < 0.01$ versus 0 mmHg of each pretreatment. $^\dagger p < 0.01$ versus 45 mmHg under the control (without the pretreatment) condition. $^\ddagger p < 0.01$ versus DOG under the control (without the pretreatment) condition

[14]. IKca channel activation by Ang II is expected to contribute to $Ca^{2+}$ entry in smooth muscle cells and therefore affect migration and proliferation in some pathophysiological conditions [27].

IKca channels have an important role in cell migration and proliferation. Previous reports suggest that proliferative smooth muscle cells predominantly express IKca channels [15, 28] and that cell migration is also modulated by the activity of IKca channels [10].

Regulation of ion channel activity by changes in the organization of the F-actin cytoskeleton has been suggested for the epithelial $Na^+$ channel [29], the cystic fibrosis transmembrane regulator, CFTR [30], voltage-gated $K^+$ channels, and cardiac ATP-sensitive $K^+$ channels [31]. Changes in the structure of the F-actin cytoskeleton may also play an important role as a cell volume regulator. Generally, cell swelling is reported to cause a decrease in cellular F-actin content. Stretch has been shown to enhance vascular smooth muscle cell migration as a result of the translocation of PKC$\delta$ to the cytoskeleton [32].

The pathway of signal transduction of IKca channel activation is still unknown. Our results show that PKC and F-actin are both involved in the activation of IKca channels in cultured smooth muscle cells.

In order to study the relationship between PKC and F-actin in the activation pathway, we examined the effect of DOG together with cytochalasin D on the activation of IKca current under hyposmotic and stretched condition. The results showed that no significant current was activated as compared with the control, which was different from the activation effect of DOG alone. This suggests that activation of PKC by DOG under the condition of

depolymerization of F-actin cannot elicit IKca current any longer. This implies that the role of F-actin for regulation of IKca channel in the signal pathway would be downstream site related to the role of PKC. For stress-activated or mechanically gated channels, several studies have shown that the cytoskeleton directly interacts with the channel protein and can intrinsically sense the cell stretch [33, 34]. Stretch-activated IKca channels may act similarly. IKca channel activation by cell-membrane stretching contributes to $Ca^{2+}$ entry in smooth muscle cells and therefore affects migration and proliferation in some pathophysiologic conditions. So far there have been few functional studies of the regulation of IKca channels by mechanical stress, though the IKca channel is required for de-differentiation, proliferation, and migration [14, 15, 28]. There is a clear need for future functional studies of the role of IKca channel activation by cell membrane stretch in cardiovascular disease, especially hypertension.

In summary, the present study demonstrates that cell-membrane stretch activates IKca channels, and that the activation is associated with extracellular $Ca^{2+}$ influx through SA channels and is modulated by both the F-actin cytoskeleton and the activation of PKC.

## References


1. Williams B (1998) Mechanical influences on vascular smooth muscle cell function. J Hypertens 16:1921–1926
2. Ninomiya Y, Hamasaki S, Saihara K, Ishida S, Kataoka T, Ogawa M, Orihara K, Oketani N, Fukudome T, Okui H, Ichiki T, Shinsato T, Kubozono T, Mizoguchi E, Ichiki H, Tei C (2008) Comparison of effect between nitrates and calcium channel antagonist on vascular function in patients with normal or mildly diseased coronary arteries. Heart Vessels 23:83–90
3. Fernandes-Santos C, de Souza Mendonça L, Mandarim-de-Lacerda CA (2009) Favorable cardiac and aortic remodeling in olmesartan-treated spontaneously hypertensive rats. Heart Vessels 24:219–227
4. Biselli PM, Guerzoni AR, de Godoy MF, Pavarino-Bertelli EC, Goloni-Bertollo EM (2008) Vascular endothelial growth factor genetic variability and coronary artery disease in Brazilian population. Heart Vessels 23:371–375
5. Meluzín J, Vasků A, Kincl V, Panovský R, Srámková T (2009) Association of coronary artery disease, erectile dysfunction, and endothelial nitric oxide synthase polymorphisms. Heart Vessels 24:157–163
6. Davis MJ, Hill MA (1999) Signaling mechanisms underlying the vascular myogenic response. Physiol Rev 79:387–423
7. Wu X, Davis MJ (2001) Characterization of stretch-activated cation current in coronary smooth muscle cells. Am J Physiol Heart Circ Physiol 280:H1751–H1761
8. Nelson MT, Quayle JM (1995) Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 268:C799–C822
9. Latorre R, Oberhauser A, Labarca P, Alvarez O (1989) Varieties of calcium-activated potassium channels. Annu Rev Physiol 51:385–399
10. Schwab A (2001) Function and spatial distribution of ion channels and transporters in cell migration. Am J Physiol 28:F739–F747
11. Fioretti B, Pietrangelo T, Catacuzzeno L, Franciolini F (2005) Intermediate-conductance $Ca^{2+}$-activated $K^+$ channel is expressed in C2C12 myoblasts and is downregulated during myogenesis. Am J Physiol 289:C89–C96
12. Ouadid-Ahidouch H, Roudbaraki M, Delcourt P, Ahidouch A, Joury N, Prevarskaya N (2004) Functional and molecular identification of intermediate-conductance $Ca^{2+}$-activated $K^+$ channels in breast cancer cells: association with cell cycle progression. Am J Physiol 287:C125–C134
13. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391:85–100
14. Hayabuchi Y, Nakaya Y, Yasui S, Mawatari K, Mori K, Suzuki M, Kagami S (2006) Angiotensin II activates intermediate-conductance $Ca^{2+}$-activated $K^+$ channels in arterial smooth muscle cells. J Mol Cell Cardiol 41:972–979
15. Neylon C, Lang R, Fu Y, Bobik A, Reinhart P (1999) Molecular cloning and characterization of the intermediate-conductance $Ca^{2+}$-activated $K^+$ channel in vascular smooth muscle: relationship between KCa channel diversity and smooth muscle cell function. Circ Res 85:e33–e43
16. Setoguchi M, Ohya Y, Abe I, Fujishima M (1997) Stretch-activated whole-cell currents in smooth muscle cells from mesenteric resistance artery of guinea-pig. J Physiol 501:343–353
17. Folkow B (1995) Integration of hypertension research in the era of molecular biology. J Hypertens 13:5–18
18. Kosako H, Gotoh Y, Matsuda S, Ishikawa M, Nishida E (1992) *Xenopus* MAP kinase activator is a serine/threonine/tyrosine kinase activated by threonine phosphorylation. EMBO J 11:2903–2908
19. Alvarez E, Northwood IC, Gonzalez FA, Latour DA, Seth A, Abate C, Curran T, Davis RJ (1991) Pro-Leu-Ser/Thr-Pro us consensus primary sequence for substrate protein phosphorylation. Characterization of the phosphorylation of c-myc and c-jun proteins by an epidermal growth factor receptor threonine 669 protein kinase. J Biol Chem 266:15277–15285
20. Pulverer BJ, Kyriakis JM, Avruch J, Nikolakaki E, Woodqett JR (1991) Phosphorylation of c-jun mediated by MAP kinases. Nature 353:670–674
21. Sturgill TW, Ray LB, Erikson E, Maller JL (1988) Insulin-stimulated MAP-2 kinase phosphorylates and activates ribosomal protein S6 kinase II. Nature 334:715–718
22. Kubo T, Hosokawa H, Kambe T, Fukumori R (2000) Angiotensin II mediates pressure loading-induced mitogen-activated protein kinase activation in isolated rat aorta. Eur Pharmacol 391:281–287
23. Hosokawa H, Aiuchi S, Kambe T, Hagiwara Y, Kubo T (2002) Mechanical stretch-induced mitogen-activated protein kinase activation is mediated via angiotensin and endothelin systems in vascular smooth muscle cells. Biol Pharm Bull 25:1588–1592
24. Lucchesi PA, Bell JM, Willis LS, Byron KL, Corson MA, Berk BC (1996) Ca(2+)-dependent mitogen-activated protein kinase activation in spontaneously hypertensive rat vascular smooth muscle defines a hypertensive signal transduction phenotype. Circ Res 78:962–970
25. Lepple-Wienhues A, Berweck S, Böhmig M, Leo CP, Meyling B, Garbe C, Wiederholt M (1996) $K^+$ channels and the intracellular calcium signal in human melanoma cell proliferation. J Membr Biol 151:149–157
26. Verheugen JA, Le Deist F, Devignot V, Korn H (1997) Enhancement of calcium signaling and proliferation responses in activated human T lymphocytes. Inhibitory effects of $K^+$ channel

block by charybdotoxin depend on the T cell activation state. Cell Calcium 21:1–17
27. Omae K, Ogawa T, Nitta K (2009) Influence of T-calcium channel blocker treatment on deterioration of renal function in chronic kidney disease. Heart Vessels 24:301–307
28. Köhler R, Wulff H, Eichler I, Kneifel M, Neumann D, Knorr A, Grgic I, Kämpfe D, Si H, Wibawa J, Real R, Borner K, Brakemeier S, Orzechowski HD, Reusch HP, Paul M, Chandy KG, Hoyer J (2003) Blockade of the intermediate-conductance calcium-activated potassium channel as a new therapeutic strategy for restenosis. Circulation 108:1119–1125
29. Berdiev BK, Prat AG, Cantiello HF, Ausiello DA, Fuller CM, Jovov B, Benos DJ, Ismailov II (1996) Regulation of epithelial sodium channels by short actin filaments. J Biol Chem 271: 17704–17710
30. Cantiello HF (1996) Role of the actin cytoskeleton in the regulation of the cystic fibrosis transmembrane conductance regulator. Exp Physiol 81:505–514
31. Furukawa T, Yamane T, Terai T, Katayama Y, Hirakoka M (1996) Functional linkage of the cardiac ATP-sensitive $K^+$ channel to the actin cytoskeleton. Pflügers Arch 431:504–512
32. Li C, Wernig F, Leitges M, Hu Y, Xu Q (2003) Mechanical stress-activated PKCdelta regulates smooth muscle cell migration. FASEB J 17:2106–2108
33. Hamill OP, Martinac B (2001) Molecular basis of mechanotransduction in living cells. Physiol Rev 81:685–740
34. Ingber DE (1997) Tensegrity: the architectural basis of cellular mechanotransduction. Annu Rev Physiol 59:575–599