Canonical Transient Receptor Potential 1 Channel Is Involved in Contractile Function of Glomerular Mesangial Cells

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Contractility of mesangial cells (MC) is tightly controlled by \([\text{Ca}^{2+}]_i\). \(\text{Ca}^{2+}\) influx across the plasma membrane constitutes a major component of mesangial responses to vasoconstrictors. Canonical transient receptor potential 1 (TRPC1) is a \(\text{Ca}^{2+}\)-permeable cation channel in a variety of cell types. This study was performed to investigate whether TRPC1 takes part in vasoconstrictor-induced mesangial contraction by mediating \(\text{Ca}^{2+}\) entry. It was found that angiotensin II (AngII) evoked remarkable contraction of the cultured MC. Downregulation of TRPC1 using RNA interference significantly attenuated the contractile response. Infusion of AngII or endothelin-1 in rats caused a decrease in GFR. The GFR decline was significantly reduced by infusion of TRPC1 antibody that targets an extracellular domain in the pore region of TRPC1 channel. However, the treatment of TRPC1 antibody did not affect the AngII-induced vasopressing effect. Electrophysiologic experiments revealed that functional or biologic inhibition of TRPC1 significantly depressed AngII-induced channel activation. Fura-2 fluorescence-indicated that \(\text{Ca}^{2+}\) entry in response to AngII stimulation was also dramatically inhibited by TRPC1 antibody and TRPC1-specific RNA interference. These results suggest that TRPC1 plays an important role in controlling contractile function of MC. Mediation of \(\text{Ca}^{2+}\) entry might be the underlying mechanism for the TRPC1-associated MC contraction.


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glomerular mesangial cells (MC) are located within glomerular capillary loops and contribute to the physiologic regulation of glomerular hemodynamics (1). Altered responsiveness of MC to hormones is one of the major causes that lead to various renal diseases. \(\text{Ca}^{2+}\) influx across the plasma membrane is a major component of MC responses to vasoconstrictors (1). Several types of \(\text{Ca}^{2+}\)-conductive channels in the plasma membrane are involved in the physiologic processes. These channels include voltage-operated \(\text{Ca}^{2+}\) channel (VOCC), receptor-operated channel (ROC), and recently found store-operated channel (SOC) (1,2). In contrast to the widely known VOCC, the molecular identity, physiologic significance, and regulatory mechanism of ROC and SOC in the glomerular contractile cells remain unknown.

Recently, the channel proteins from a new family, canonical transient receptor potential (TRPC), were found in a variety of cells (3). TRPC family includes seven related members, designated as TRPC1 through 7 (3). Pharmacologic and electrophysiologic studies in conjunction with molecular biologic tools and \(\text{Ca}^{2+}\) imagings have demonstrated that TRPC channel activity is tightly linked to the signaling cascade of G-protein–coupled receptor or receptor tyrosine kinase (4,5), supporting the current hypothesis that TRPC proteins are potential candidates for ROC and SOC. TRPC proteins have been identified in glomeruli and glomerular MC (6–8). Our previous work also demonstrated that human MC selectively express TRPC1, 3, 4, and 6 (9). However, the function, regulation, and physiologic relevance of these glomerular TRPC are unexplored at large extent. In this study, we focused on TRPC1 and investigated its contribution to mesangial contraction in vitro and in vivo. Our results indicate that TRPC1 is an important component mediating contractile responses of MC. The TRPC1-involved mesangial contraction is attributed to TRPC1-associated \(\text{Ca}^{2+}\) influx.

Materials and Methods

Animals

Two- to 3-mo-old male Sprague-Dawley rats were used in this study. All rats were purchased from Harlan (Indianapolis, IN). Care and use of all animals in this study were in strict agreement with the guidelines set forth by the University of North Texas Health Science Center.

Measurement of GFR and Renal Blood Flow

GFR and renal blood flow (RBF) were estimated by measurement of inulin and para-aminohippurate (PAH) plasma clearances as described

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by Pluznick et al. (10) and by Waugh and Beall (11), respectively. All rats were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg), and the left jugular vein was cannulated with PE-10 tubing for infusion of fluids and chemicals. The bladder was exposed and implanted with PE-50 tubing for urine collection. The right and left carotid arteries were cannulated with PE-10 tubing and a Fiber Optic Blood Pressure Sensor (WPI, Sarasota, FL) for collecting blood samples and monitoring arterial BP, respectively. BP was measured by Fiber Optic Measurement System (WPI) and analyzed by MP100 system with AcqKnowledge 3.8 software (BIOPAC System, Goleta, CA). Physiological saline solution (PSS) that contained 10 mg/ml FITC-inulin or PAH was infused at a rate of 1 ml/h per 100 g body wt. After a 1-h equilibration period, a blood sample (approximately 100 µl) was taken and urine was collected during the next 30-min period. Then, angiotensin II (AngII)-containing inulin or PAH saline solution was infused into the rats and the 30-min urine sample was collected again. At the end of the period, a larger plasma sample was taken. Urinary volume was determined gravimetrically. GFR and RBF were calculated on the basis of urinary volume, urine and plasma inulin, or PAH concentrations. FITC-inulin was measured using a fluorescence microplate reader with excitation at 485 nm and emission at 538 nm in a Spectrophotometer (Victor3-1420 Multilable Counter; Perkin Elmer, Wellesley, MA), whereas PAH concentration was evaluated by measurement of absorbance in a Microplate Spectrophotometer (SpectraMax, 340 PC; Molecular Devices Corp., Sunnyvale, CA) at 450 nm. The concentrations of electrolytes in blood and urine were measured by GEM Premier 3000 (Instrumentation Laboratory, Lexington, MA), and osmolalities of the samples were measured by the Advanced Micro-Osmometer (Model 3MO Plus’ Advanced Instruments, Norwood, MA).

Cell Culture and Transient Transfection
Human MC were purchased from Cambrex Co. (East Rutherford, NJ). MC were subcultured to no more than 10 generations by standard methods (12). All plasmids were transiently transfected into MC using LipofectAmine and Plus reagent (Invitrogen-BRL, Carlsbad, CA) following the protocols provided by the manufacturer.

MC Contractility Assay
AngII-induced MC contraction was measured by changes in planar surface area. The cover slip on which the MC grew was mounted to a perfusion chamber, and MC were visualized under an inverted fluorescence microscope (Nikon TE-2000S, upgraded, Nikon, Melville, NY). The perfusates were heated through an inline heater, and the temperature was controlled by an Automatic Temperature Controller (TC-324B; WPI). Changes in the mesangial planar surface area in response to AngII were observed at 37°C with 95% O2 and 5% CO2 gassing. Using a digital camera, images of the same cells were captured serially every 5 min for 30 min. The perimeters of individual cells with clearly defined borders were outlined, and the planar surface areas were calculated using ImageJ software (National Institutes of Health, Bethesda, MD).

Patch-Clamp Procedure
The conventional cell-attached voltage clamp was used as described in our previous study (12). Single-channel analysis was made with a Warner PC-505B amplifier (Warner Instrument Corp., Hamden, CT) and pClamp 9.2 (Axon Instrument, Foster City, CA). The extracellular solution contained (in mM) 135 NaCl, 5 KCl, 10 HEPES, 2 MgCl2, 1 CaCl2, and 10 glucose. The pipette solution contained (in mM) 135 NaCl, 5 KCl, 1 CaCl2, 3 EGTA, and 10 HEPES. The calculated free Ca2+ concentration in the pipette solution was <10 nM (MTK software). At the time of the experiment, the pipette solution was supplemented with 100 µM niflumic acid, 10 mM TEA, and 100 nM iberiotoxin to block Ca2+-activated Cl− channels and K+ channels, respectively. For exclusion of the influence of fluid flow on channel activity on AngII infusion, the bathing solution continuously flowed throughout the experiments. The flow rate was adjusted by gravity and controlled by a multiple channel perfusion system (ValveLink8, Automate Scientific, San Francisco, CA). Channel activity was calculated as Open probability. Clampfit 9.2 software (Axon Instrument, Foster City, CA) was used to analyze single channel currents.

Fluorescence Measurement of [Ca2+]i
Measurements of [Ca2+]i, in MC using fura-2 were performed using dual excitation wavelength fluorescence microscopy. MC, grown on a coverslip (22 × 22 mm), were loaded with fura-2 by incubation for approximately 50 min at room temperature in the dark in PSS that contained 2 µM acetoxyethyl ester of fura-2 (fura-2/AM), 0.09 g/dl DMSO, and 0.018 g/dl Pluronic F-127 (Molecular Probes, Eugene, OR) followed by washing three times. The cells were then incubated with fura-2 free PSS for an additional approximately 20 min. The coverslip was then placed in a perfusion chamber (Model RC-2OH; Warner) mounted on the stage of a Nikon Diaphot inverted microscope. Fura-2 fluorescence was monitored by a ratio technique (excitation at 340 and 380 nm, emission at 510 nm) using Metafluor software (Universal Imaging, West Chester, PA). Bath [Ca2+]i was reduced to <10 nM during the experiments by addition of EGTA according to the Ca2+ concentration program by MTK Software. [Ca2+]i, was calculated using the formula described by Grynkiewicz et al. (13). Calibrations were performed in vitro at the end of each experiment, and conditions of high [Ca2+]i, were achieved by addition of 4 µM ionomycin, whereas conditions of low [Ca2+]i, were obtained by addition of 10 mM EGTA.

Materials
TRPC1 antibody and all chemicals were purchased from Sigma-Aldrich (Sigma, St. Louis, MO). pSHAG, pSHAG-trpc1/RNA interference (RNAi) plasmids, and HA-tagged TRPC1 expression plasmids were obtained from Dr. L. Tsiokas (University of Oklahoma Health Sciences Center, Oklahoma City, OK).

Statistical Analyses
Data are reported as means ± SEM. The one-way ANOVA plus Student-Newman-Keuls test, unpaired t test, and paired t test were used to analyze the differences among multiple groups, between two groups, and before and after treatment in the same group, respectively. P < 0.05 was considered statistically significant.

Results
TRPC1 Contributed to AngII-Induced Contraction of MC
Our previous study (9) demonstrated that TRPC1 is present in human and rat MC. To determine whether TRPC1 was involved in contractile function of MC, we assessed AngII-induced contraction in cultured MC with and without knockdown of TRPC1. TRPC1 knockdown was achieved by transient transfection of short hairpin RNAi constructs (pSHAG-trpc1/RNAi) that specifically silenced human trpc1 gene. As shown in Figure 1C, TRPC1 protein was remarkably overexpressed in HEK293 cells that were transiently transfected with human TRPC1 expression plasmids. The exogenous TRPC1 protein was significantly reduced by co-transfection of the cells with RNAi constructs specific for human TRPC1 gene (pSHAG-
trpc1/RNAi). These results were consistent with our previous report (9), suggesting successful knockdown of TRPC1 protein by this tool. This construct was used for subsequent experiments in this study.

AngII-induced MC contraction was measured by changes in planar surface area of the cell. This contractility assay was carried out in transiently green fluorescence protein (GFP)+pSHAG (pSHAG)+ and GFP+pSHAG-trpc1/RNA interference (pSHAG-trpc1/RNAi)-transfected cells before and after AngII treatment. Arrows indicate positively transfected cells. The changes in size of the transfected cells in response to AngII are illustrated on the right (Overlap), by overlapping the images of the same cell before and after AngII treatment using Photoshop software. The cells before AngII treatment were colored as red and after treatment as green. The right panels are enlarged regions indicated by dashed rectangles in the left and middle panels. (B) Bar graph showing the AngII-induced decreases in the surface area of MC with and without canonical transient receptor potential 1 (TRPC1) knockdown, calculated as [(the surface area of MC after AngII − the original surface area of the cells)/the original surface area of the cells] × 100%. “n” indicates the number of cells counted in each group. *Significant difference between the indicated groups. (C) Western blot in HEK293 cells, showing TRPC1 protein expression in untransfected HEK293 cells (lane 1) and HEK293 cells with transient transfection of HA-tagged human TRPC1 expression plasmids (lane 2) or human TRPC1 expression plasmids plus RNAi constructs for human TRPC1 (lane 3). Actin was used as a loading control. Magnification, ×250.

**Figure 1.** Changes in the planar surface area of mesangial cells (MC) with angiotensin II (AngII) stimulation. (A) Representative morphology of MC that were used in contraction assays in green fluorescence protein (GFP)+pSHAG (pSHAG)+ and GFP+pSHAG-trpc1/RNA interference (pSHAG-trpc1/RNAi)-transfected cells before and 10 min after AngII treatment. Arrows indicate positively transfected cells. The changes in size of the transfected cells in response to AngII are illustrated on the right (Overlap), by overlapping the images of the same cell before and after AngII treatment using Photoshop software. The cells before AngII treatment were colored as red and after treatment as green. The right panels are enlarged regions indicated by dashed rectangles in the left and middle panels. (B) Bar graph showing the AngII-induced decreases in the surface area of MC with and without canonical transient receptor potential 1 (TRPC1) knockdown, calculated as [(the surface area of MC after AngII − the original surface area of the cells)/the original surface area of the cells] × 100%. “n” indicates the number of cells counted in each group. *Significant difference between the indicated groups. (C) Western blot in HEK293 cells, showing TRPC1 protein expression in untransfected HEK293 cells (lane 1) and HEK293 cells with transient transfection of HA-tagged human TRPC1 expression plasmids (lane 2) or human TRPC1 expression plasmids plus RNAi constructs for human TRPC1 (lane 3). Actin was used as a loading control. Magnification, ×250.

TRPC1 Regulated Glomerular Filtration in Rats

Because the major function of glomerular MC is to regulate glomerular filtration by regulating effective filtration surface area, we reasoned that if TRPC1 protein contributes to the contractile function of MC, then inhibition of mesangial TRPC1 function should increase GFR or compromise mesangial contraction–induced GFR decline, accordingly. Therefore, inulin clearance was measured to evaluate rats’ GFR in the presence or absence of AngII with or without TRPC1 inhibition. In
agreement with other studies [14,15], infusion of AngII (1.7 ng/min per 100 g body wt) significantly reduced GFR from 2.7 ± 0.6 of basal level to 1.4 ± 0.3 ml/min per 100 g body wt (Figure 2A, normal), representing 42 ± 10% decrease (Figure 2B, normal). Inclusion of a TRPC1 antibody (polyclonal rabbit IgG, 300 ng/ml), which is directed to an extracellular epitope (predicted pore region) of TRPC1, into perfusate did not alter the basal GFR. However, the AngII-induced decline of GFR was dramatically attenuated (from 2.3 ± 0.5 to 2.0 ± 0.7 ml/min per 100 g body wt, representing 8.9 ± 6% decrease; Figure 2, A and B, TRPC1 Ab). This inhibitory effect was specific because the same amount of rabbit IgG did not affect the AngII-induced reduction of GFR (2.3 ± 0.7 to 2.1 ± 0.4 ml/min per 100 g body wt, representing 10% decrease; Figure 2, A and B, Rb IgG). TRPC1 Channels Mediated AngII-Stimulated Membrane Currents

Mesangial tone is controlled by intracellular Ca2+ homeostasis that is regulated by Ca2+ intrusion and extrusion across the plasma membrane. It has been known that several TRPC1 Channels Mediated AngII-Stimulated Membrane Currents

Figure 2. GFR before and during infusion of AngII (1.7 ng/min per 100 g body wt) or endothelin-1 (ET-1; 1.3 ng/min per 100 g body wt) with and without (Untreated) TRPC1 antibody (TRPC1 Ab; 300 ng/ml) or rabbit IgG (Rb IgG; 300 ng/ml) in rats. (A) GFR was calculated on the basis of urine output, concentrations of inulin in urine, and blood samples that were collected during corresponding infusion periods. “n” indicates the number of rats in each group. (B) Percentage of decrease in GFR induced by AngII in each group shown in A, calculated by \( \frac{[\text{GFR before AngII} - \text{GFR during infusion of AngII}]}{\text{GFR before AngII}} \times 100\% \). (C) Percentage decrease in GFR in response to ET-1. *Significant difference between the indicated groups.
types of cation channels, including ROC and SOC, are involved in vasoconstrictor-induced Ca$^{2+}$ responses in MC (1,2). TRPC1 has been considered a Ca$^{2+}$-permeable cation channel linked to G protein–coupled receptor-phospholipase C pathway for activation (18). Therefore, we speculated that TRPC1 regulated mesangial contraction by mediating agonist-stimulated ionic currents. Cell-attached patch clamp was used to measure membrane currents in response to AngII. Figure 4A shows the current-voltage relation curve (I-V curve) before application of AngII. Because the resting membrane potentials of the patched MC were unknown, the membrane potentials in the analysis were expressed as negative pipette potentials ($-V_p$). In the range from $-80$ to $0$ mV of command potentials ($-V_p$), the calculated single channel conductance was 17.2 pS. The extrapolated linear I-V relation was reversed at $-45$ mV (19,20), the reversal membrane potential for the observed channels is estimated to be approximately 9 mV. These biophysical features are consistent with those of TRPC1 channel (21,22).

We then investigated the responses of the membrane currents to AngII and the contribution of TRPC1 to the responses. In the untreated group, AngII increased channel activity by approximately 2.2-fold (Figure 4, B and C). However, inclusion of TRPC1 antibody (200 ng/ml) into the pipette significantly depressed the AngII-induced channel.

Table 1. Electrolyte concentrations and osmolality of plasma and urine$^a$

<table>
<thead>
<tr>
<th>Groups</th>
<th>$B_{Na}$ (mM)</th>
<th>$B_{K}$ (mM)</th>
<th>$B_{Ca}$ (mM)</th>
<th>$B_{Osm}$ (mM)</th>
<th>$U_{Na}$ (mM)</th>
<th>$U_{Ca}$ (mM)</th>
<th>$U_{Osm}$ (mM)</th>
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<tr>
<td>Untreated</td>
<td>135.9 ± 1.2</td>
<td>5.8 ± 0.6</td>
<td>0.9 ± 0.1</td>
<td>298.0 ± 5.5</td>
<td>146.5 ± 8.4</td>
<td>0.6 ± 0.2</td>
<td>999.3 ± 9.7</td>
</tr>
<tr>
<td>Rb IgG</td>
<td>135.4 ± 1.3</td>
<td>4.7 ± 0.2</td>
<td>0.7 ± 0.03</td>
<td>280.1 ± 4.4</td>
<td>155.5 ± 14.2</td>
<td>0.5 ± 0.1</td>
<td>967.8 ± 152.9</td>
</tr>
<tr>
<td>TRPC1 Ab</td>
<td>135.1 ± 0.7</td>
<td>5.1 ± 0.3</td>
<td>0.7 ± 0.06</td>
<td>280.8 ± 4.3</td>
<td>168.8 ± 14.6</td>
<td>0.6 ± 0.2</td>
<td>905.4 ± 127.7</td>
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</table>

$^a$B$_{Na}$, B$_{K}$, B$_{Ca}$, and B$_{Osm}$ represent plasma concentrations of Na$^+$, K$^+$, and Ca$^{2+}$ and plasma osmolality, respectively. U$_{Na}$, U$_{Ca}$, and U$_{Osm}$ represent urine concentrations of Na$^+$ and Ca$^{2+}$ and urine osmolality, respectively. Rb IgG, rabbit IgG. TRPC1 Ab, canonical transient receptor potential 1 antibody.
activation. Similar to in vivo experiments, the inhibitory effect of TRPC1 antibody seemed to be specific because the same concentration of rabbit IgG did not significantly affect the AngII-induced currents (approximately 2.5-fold increase; Figures 4C and 5A).

We further assessed the AngII-induced channel response in TRPC1-knocked down MC. As shown in Figure 5A, transient transfection of pSHAG-trpc1/RNAi constructs into human MC significantly inhibited AngII-stimulated channel activity compared with control transfected cells (pSHAG empty vector). The inhibition of TRPC1 channel either by its antibody or by RNAi also significantly reduced the basal activities of the AngII-responsive channels (Figure 5B). These results suggest that TRPC1 contributes not only to vasoconstrictor-induced mesangial contraction but also to the basal tone of MC.

**TRPC1 Contributed to AngII-Induced Ca\(^{2+}\) Entry in MC**

An increase in [Ca\(^{2+}\)], is a downstream event of AngII-induced channel responses and a key factor to trigger mesangial contraction. Contribution to elevation of [Ca\(^{2+}\)], might be an underlying mechanism for TRPC1-associated MC contraction. To test this speculation, we assessed fura-2 fluorescence–indicated [Ca\(^{2+}\)] in response to AngII in cultured MC with and without TRPC1 inhibition. Consistent with our previous studies (23,24), AngII evoked a rapid and striking cytosolic Ca\(^{2+}\) transient followed by a steady state of lower elevation of [Ca\(^{2+}\)] in the presence of 1 mM Ca\(^{2+}\) extracellular solution. Removal of extracellular Ca\(^{2+}\) immediately reduced the [Ca\(^{2+}\)] to a level lower than baseline. Re-addition of Ca\(^{2+}\) resulted in an increase in [Ca\(^{2+}\)], that is attributed to Ca\(^{2+}\) influx (12) (Figure 6A, untreated). Bath application of rabbit IgG or TRPC1 antibody (200 ng/ml) did not alter the profile of the AngII-induced Ca\(^{2+}\) responses. However, the AngII-stimulated Ca\(^{2+}\) entry in response to Ca\(^{2+}\) re-addition was significantly inhibited by TRPC1 antibody but not by rabbit IgG treatment (Figure 6A and C). The inhibition of Ca\(^{2+}\) influx by TRPC1 antibody was not due to changes in AngII-induced Ca\(^{2+}\) release from

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**Figure 4.** Single-channel currents in response to 1 μM AngII stimulation in MC with and without (Untreated) Rb IgG (200 ng/ml) or TRPC1 Ab (200 ng/ml). (A) Current-voltage (I-V) relation curves, showing development of single-channel currents with changes in membrane potentials in untreated MC. Membrane potentials are expressed as negative pipette potentials (−Vp). The calculated single-channel conductance was 17.2 pS. The reversal potential was estimated as 54 mV (−Vp). (B) Open probability (NPO) of single channels before and after application of AngII in untreated MC. Holding potential was 80 mV (pipette). “n” indicates the number of cells analyzed. *Significant difference before and after application of AngII. (C) Representative traces, showing channel activity before (Before AngII) and after (AngII) AngII stimulation in untreated, Rb IgG–treated, and TRPC1 Ab–treated MC, respectively. Arrows indicate closed state of channels. Downward deflects indicate inward currents. Holding potential was 80 mV (pipette). Bottom trace on the right (inside a dashed rectangle) is the time-expanded portion of the trace indicated by a small dashed rectangle (indicated by a dashed arrow).
sarcoplasmic reticulum, because the initial Ca\(^{2+}\) transients were comparable among untreated, rabbit IgG–treated, and TRPC1 antibody–treated MC (Figure 6A). Normalization of Ca\(^{2+}\) entry to Ca\(^{2+}\) release revealed a significantly lower ratio in TRPC1-treated group than that in the other two groups (Figure 6D). Similarly, inhibition of TRPC1 by transfection of pSHAG-trpc1/RNAi constructs into MC also significantly suppressed AngII-stimulated Ca\(^{2+}\) entry without affecting Ca\(^{2+}\) release from sarcoplasmic reticulum (Figure 6B through D). It should be mentioned that nifedipine (1 \(\mu M\)) was present in the bath during re-addition of Ca\(^{2+}\); therefore, involvement of VOCC in the Ca\(^{2+}\) entry can be excluded or neglected.

**Discussion**

MC contribute to the physiologic regulation of glomerular hemodynamics by responding to locally produced or circulating vasoactive peptides (1). Like smooth muscle cells, the contractility of MC is tightly controlled by [Ca\(^{2+}\)]\(\text{cyt}\), Ca\(^{2+}\) influx across the plasma membrane constitutes a major component of the responses of MC to vasoconstrictors. The results from this study suggest that TRPC1 channel protein is an indispensable contributor to contractile function of MC. This conclusion is based on two lines of findings from both *in vitro* and *in vivo* experiments. In cultured human MC, AngII- or ET-1–induced mesangial contraction was significantly attenuated by down-regulation of TRPC1 protein. In rats, infusion of TRPC1 antibody significantly reduced the AngII-induced GFR decline.

In addition to mesangial tone, glomerular capillary pressure and RBF are the other two major determinants of glomerular filtration and might also contribute to the AngII-induced GFR decline. The involvement of these factors could be indicated by increases in MAP and decreases in RBF during infusion of AngII (Figure 3). However, inhibition of AngII-induced vasopressing effect might not be the underlying mechanism for TRPC1 antibody treatment because the changes in resistance of renal vasculature (ratio of MAP to RBF) were comparable between control and antibody-treated rats. The TRPC1 antibody that was used in this study is directed to an extracellular epitope that is located at the predicted pore region of TRPC1 and therefore might act as a channel blocker. Indeed, using specific TRPC antibodies selectively to antagonize function of individual TRPC has become a powerful tool for studying specific TRPC functions (25,26). The same TRPC1 antibody has been reported to depress significantly TRPC1 channel-mediated currents in platelets, neurons, and endothelial cells (27–29). In addition, we repeated the *in vivo* experiments using another TRPC1 antibody (T1E3; from Dr. David Beech, University of Leeds, Leeds, UK) that also targets on the outer vestibule of TRPC1 channel (25) in four rats. Like the commercial TRPC1 antibody, the T1E3 antibody attenuated the AngII-induced GFR decline at an equivalent degree (data not shown). Therefore, we reasoned that the infused TRPC1 antibody filtered out from glomerular capillaries and then bound to the extracellular domains of TRPC1 channel protein located on the plasma membrane of MC and further blocked the AngII-stimulated Ca\(^{2+}\) entry through TRPC1 channel. As a support for this inference, glomerular deposit of an Ig from systemic circulation and further reaction to its antigen protein on the surface of MC has been well established in a disease model of glomerulonephritis (30).

TRPC1 has been known to function as a Ca\(^{2+}\)-permeable cation channel linked to the G protein–coupled signaling pathway for activation (4,5). However, whether the channel is activated by IP\(_3\)-induced internal Ca\(^{2+}\) store depletion (SOC) or by a second messenger between the receptor activation and Ca\(^{2+}\) store depletion (ROC) is debatable (31,32). Recently, stromal interaction molecule 1 (STIM1) was proposed to be a key activator of SOC by functioning as a Ca\(^{2+}\) sensor of internal stores (33,34). It is interesting that STIM1 was found to interact with endogenous or transfected TRPC1, and this interaction is required for SOC activation (35,36), strongly suggesting a store-operated mechanism of TRPC1. We are not able to identify the nature of the TRPC1 channel that mediates the AngII-stimulated ionic responses (SOC or ROC) from this study. We recently found that TRPC1 was also involved in thapsigargin-induced Ca\(^{2+}\) influx, a classical mechanism of store-operated Ca\(^{2+}\) entry, and STIM1 was strongly expressed in MC (data not shown), indicating that TRPC1 might be an important subunit of SOC in the glomerular contractile cells.

*Figure 5. (A) Percent changes in channel activity (NPO\(_{\text{a}}\)) induced by AngII stimulation in Rab IgG– or TRPC1 Ab–treated MC and in GFP+ pSHAG– (pSHAG) or GFP+ pSHAG-trpc1/RNAi– (pSHAG-trpc1/RNAi) transiently transfected MC. (B) Basal activities of the observed channels in MC of Rab IgG, TRPC1 Ab, pSHAG, and pSHAG-trpc1/RNAi groups. In both A and B, “n” indicates the number of cells analyzed in corresponding groups. *Significant difference between the indicated groups.*
Apparently, further study is required to define the precise mechanism of TRPC1 in AngII-evoked channel responses.

It has been documented that TRPC proteins can assemble one another as well as with auxiliary proteins to form channel complexes (3,37). The heteromultimerization and auxiliary proteins play important roles in determining the biophysical properties and activation mechanism of the TRPC channels (37). We have demonstrated that TRPC1 selectively interacts with TRPC4 and 6 in human MC (9). It is conceivable that in MC, the channel that mediates AngII-stimulated currents might be a complex that contains multiple subtypes of TRPC proteins and unknown auxiliary proteins. TRPC1 might be only one component of the channel complex.

Conclusion

The findings from the in vitro and in vivo studies provide evidence that TRPC1 participates in contractile function of MC by mediating vasoconstrictor-stimulated Ca2+ responses. Because contractility of MC has important physiologic significance in regulation of glomerular function and an impairment of mesangial tone is involved in progression of various renal diseases, such as diabetic hyperfiltration at an early stage of diabetic nephropathy, the data from this study have important physiologic and clinical relevance.

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We thank Dr. David J. Beech (School of Biomedical Sciences, University of Leeds, Leeds, UK) for providing the anti-TRPC1 antibody (T1E3).

Disclosures

None.

References


Figure 6. Fura-2 fluorescence radiometry measurement of [Ca2+]i in response to AngII stimulation. (A and B) Representative experiments, showing AngII-stimulated Ca2+ responses in untreated, Rb IgG–treated, and TRPC1 Ab–treated MC (A) and in yellow fluorescent protein (YFP) + pSHAG- (control) and YFP+pSHAG-trpc1/RNAi-transiently transfected MC (B). Application of AngII (1 μM) is indicated by the middle horizontal bars at the top of A and B. For exclusion of the involvement of voltage-operated Ca2+ channel in the AngII-induced Ca2+ responses, 1 μM nifedipine was included into the extracellular solution as indicated. “[Ca2+]B” represents Ca2+ concentration in the bathing solution, indicated by the lower horizontal bars at the top of the panels. (C) Summary data, showing the difference in the AngII-induced Ca2+ entry in response to Ca2+ re-addition in MC of all groups described in A and B. (D) Ratios of AngII-stimulated Ca2+ influx to Ca2+ release in MC of the five groups described in A and B. In both C and D, the numbers at the bottom of bars indicate the number of cells analyzed in corresponding groups. *Significant difference between the indicated groups.