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Coordination of Mesangial Cell Contraction by Gap Junction–Mediated Intercellular Ca^{2+} Wave

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Abstract. Gap junction intercellular communication (GJIC) plays a fundamental role in mediating intercellular signals and coordinating multicellular behavior in various tissues and organs. Glomerular mesangial cells (MC) are rich in GJ, but the functional associations of these intercellular channels are still unclear. This study examines the potential role of GJ in the transmission of intercellular Ca^{2+} signals and in the coordination of MC contraction. First, the presence of GJ protein Cx43 and functional GJIC was confirmed in MC by using immunochemical staining or transfer of Lucifer yellow (LY) after a single cell injection, respectively. Second, mechanical stimulation of a single MC initiated propagation of an intercellular Ca^{2+} wave, which was preventable by the GJ inhibitor heptanol but was not altered by pretreatment of MC with ATP or addition of apyrase into the assay system. Third, the phospholipase C (PLC) inhibitor U73122 could largely eliminate the mechanically elicited propagation of intercellular Ca^{2+} waves, suggesting a possibly mediating role of inositol trisphosphate (IP_3) in the initiation and transmission of intercellular Ca^{2+} signaling. Fourth, injection of IP_3 into a single cell caused contraction, not only in the targeted cell, but also in the adjacent cells, as indicated by the reduction of cellular planar area. Fifth, addition of two structurally unrelated GJ inhibitors, heptanol and α-glycyrrhetinic acid (GA), into MC embedded in collagen gels significantly attenuated the reduction of gel areas after exposure to serum. This study provides the first functional evidence supporting the critical role of GJIC in the synchronization of MC behaviors.

Mesangial cells (MC) are considered to be specialized smooth muscle cells that play a pivotal role in the regulation of glomerular hemodynamics. In renal glomerulus, the MC form a tree-like network, branching from the hilar site to the glomerular capillary loops and connecting with each other. One of the striking features of MC is their remarkable richness in gap junctions (GJ). This finding was first reported in the rat kidney using freeze-fracture techniques (1) and was subsequently confirmed by immunohistochemistry and reverse transcription–PCR (2–4). Cultured rat and human MC were also shown to express GJ protein Connexin43 (Cx43) (5). Additionally, the presence of gap junctional intercellular communication (GJIC) among cultured rat MC, its regulation by pathophysiologic factors, and its potential role in the propagation of Ca^{2+} waves have also been reported (6,7). It has been speculated that GJ, acting as a sophisticated cellular communication system, bridges each MC between the juxtaglomerular region (extraglomerular mesangium) and the glomerular mesangium and provides the mesangium with the characteristics of a functional syncytium (1,7,8).

There is a great lack of functional studies that directly address the above hypothesis. It is still poorly understood how the functional syncytium in mesangium can be realized. More recently, the notion of propagation of intercellular Ca^{2+} waves from one cell to the next after mechanical stimulation has emerged as an attractive and physiologically relevant model for investigating the mechanisms responsible for coordinated cell and tissue reactions (9–11). Intercellular Ca^{2+} waves have been demonstrated in a variety of cell types and are presumably responsible for multicellular processes (11). As a versatile cellular signal, Ca^{2+} is critically involved in the control of many different MC functions (12–14). It is therefore highly possible that the functional syncytium in MC is generated via GJ–mediated intercellular Ca^{2+} signals. This study was designed to address this hypothesis. In this article, the propagation of a Ca^{2+} wave via GJ in MC after mechanical stimulation is demonstrated; furthermore, the potential role of this wave in the coordination of MC contraction could be established.

Materials and Methods

Materials

Inositol trisphosphate (IP_3) was purchased from Calbiochem (La Jolla, CA). Fura 2-acetoxymethyl ester (Fura-2 AM) came from Dojindo Molecular Technologies, Inc. (Bethesda, MD). All other reagents were obtained from Sigma (St. Louis, MO).

Rat MC Culture

MC isolation and culture was performed as described previously (6,15,16). MC at passages 5 to 15 were used for experiments.

Immunocytochemical Analyses

Immunocytochemical staining for Cx43 in frozen kidney sections and cultured MC was done as described previously (6,17). In brief, the kidney sections and confluently cultured MC were fixed in 2% para-

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formaldehyde in phosphate-buffered saline (PBS) for 15 min and permeabilized with 1% Triton X-100 before staining. Then, the materials were incubated overnight with anti-Cx43 antibody (diluted 1:200 in 1% fetal calf serum [FCS] in PBS; 4°C). After rinsing with PBS, the appropriate secondary antibody (diluted in 1% FCS in PBS; 37°C) was added for 2 h before final washing. The slides and sections were covered with Tris-buffered moviol (pH 8.6), and microscopy was performed with an Olympus BX50 microscope with a 40 × Planapo and 570-nm emission filter. Immuofluorescence was photographed using Fujichrome Sensia II (100 ASA) film (Fuji, Tokyo, Japan).

Measurement of GJIC

Using the method described previously (6), GJIC was assessed by transfer of the membrane-impermeant fluorescent dye, Lucifer yellow (LY), after single cell microinjection with an automated microinjection system (Zeiss, Oberkochen, Germany). Briefly, confluent MC in 35-mm dishes were starved for 2 d in 0.5% FCS-DMEM. Cells were then microinjected with a mixture of Lucifer yellow (10% dissolved in 0.33 mol/L lithium chloride) and 0.5 mg/ml ethidium bromide (for nuclear staining) using a Zeiss-Eppendorf automated microinjection system (Zeiss, Oberkochen, Germany) at pressures of 500 hectopascals applied for 0.5 s. The intracellular Lucifer yellow/ethidium bromide fluorescence was examined under a fluorescence microscope immediately thereafter. The number of cells exhibiting dye labeling was counted.

Cell Stimulation

Mechanical stimulation of a single MC was achieved by briefly distorting the apical surface of the cells with a prepulled Eppendorf micropipette (Eppendorf, Hamburg, Germany). The movement of the pipette was under the control of an automated microinjection system (Zeiss). The tip of the pipette was positioned near the apical membrane of a single cell; it was automatically deflected downward to give a rapid (approximately 1 s) mechanical stimulation of the cell when the action command was given.

Measurement of Ca^{2+}

MC were cultured to confluence in special glass-bottom microwell dishes (MatTek Corporation, Ashland, MA) and then loaded with fura-2 by incubation with 5 μmol/L Fura-2 AM in Hanks balanced salt solution (HBSS) containing 2.0 mmol/L $CaCl_2$ and 1 mmol/L $MgCl_2$ at room temperature in the dark for 45 min. Cells were then rinsed and incubated with the fura-2–free Hanks solution for an additional 15 min to allow complete de-esterification of intracellular Fura-2-AM. The dish was mounted in the chamber of an inverted epifluorescence microscope (Zeiss). Ca^{2+} was determined by the ratio method and on the basis of *in vitro* calibration (18). Fura-2 was alternatively excited at 340 and 380 nm, and fluorescence emission at 510 nm was detected with an ICCD camera (Hamamatsu, Japan). Images were captured and stored directly onto the hard disk of the attached computer and processed with special Ca^{2+} image analyzing software Argus/Hisca (Hamamatsu, Japan).

Morphologic Studies

For morphologic studies, cells were grown on a 35-mm culture dish and placed into the chamber of an inverted microscope. The images of the cells before or after treatment were captured into the computer using an ICCD camera (Hamamatsu, Japan).

Contraction of MC Embedded in Collagen Gels

To determine gel contraction, MC (1×10^5/ml) were suspended in a solution of DMEM and type I collagen (1.5 mg/ml) and 0.5-ml aliquots were placed in 24-well tissue culture dishes, which had previously been coated with 2% bovine serum albumin (BSA) and allowed to gel for 3 h at 37°C in an atmosphere of 5% CO_2 in air. DMEM containing 6% FCS was then added together or without GJIC inhibitors. The gel contraction that occurred over the subsequent 4 d was monitored and captured on the computer. The planar surface area of the gel was determined using NIH image analyzing software.

Statistical Analyses

Values are expressed as either mean ± SEM or mean ± SD. Statistical analyses were performed by unpaired, two-tailed *t* test. $P < 0.05$ was considered as statistically significant.

Results

Presence of Functional GJ Communication in Rat MC

First, we reconfirmed the presence of the major GJ protein, Cx43, in MC both *in situ* and *in vitro* by immunofluorescence staining of renal tissue section and cultured MC, respectively, with an anti-Cx43 antibody. As shown in Figure 1A, there were abundant punctate immunofluorescence characteristics of GJ along the distribution of MC in glomerulus. In cultured MC, Cx43 molecules were found in the cell membranes at the regions of cell-cell contact and around the nucleus (Figure 1B).

The existence of functional GJIC in MC was demonstrated by the transfer of the LY. Microinjection of LY into a single MC led to the diffusion of this dye to the neighboring cells (Figure 2). In a confluent MC culture, typically 5 to 10 cells were dye-coupled. Pretreatment of MC with the GJ inhibitor heptanol (0.5 mmol/L) for 30 min could largely prevent the transfer of LY (number of dye-coupled cells [control *versus* heptanol] 8.5 ± 1.9 *versus* 2.8 ± 1.8; $n = 8$; $P < 0.01$).

Participation of GJIC in the Transmission of the Mechanically Induced Ca^{2+} Wave

More recently, mechanically elicited formation and subsequent propagation of Ca^{2+} wave has been employed for studying intercellular communication in a variety of cell types (10,11,19–23). MC are considered to be subject to mechanical stress under both physiologic and pathologic conditions (24); mechanical stress therefore serves as a physiologically relevant model for studying the role of GJ channels in the transmission of intercellular signals in MC.

Mechanical stimulation of a single MC using a micropipette induced an immediate elevation of Ca^{2+} in the targeted cell, leading to a propagation of Ca^{2+} signal from that cell to surrounding cells. The mechanical stress caused a eightfold increase of Ca^{2+} concentrations in the stimulated MC, from 33 nM at the basal level to 257 nM after stimulation (Figure 3A). The propagation of mechanically elicited Ca^{2+} waves could be quantified by determining the total number of cells that increased Ca^{2+} to >80 nM (Figure 3B). It was found that 6.8 ± 2.3 MC ($n = 10$) were implicated in the Ca^{2+} wave propagation. Pretreatment of MC with the GJ inhibitor heptanol could

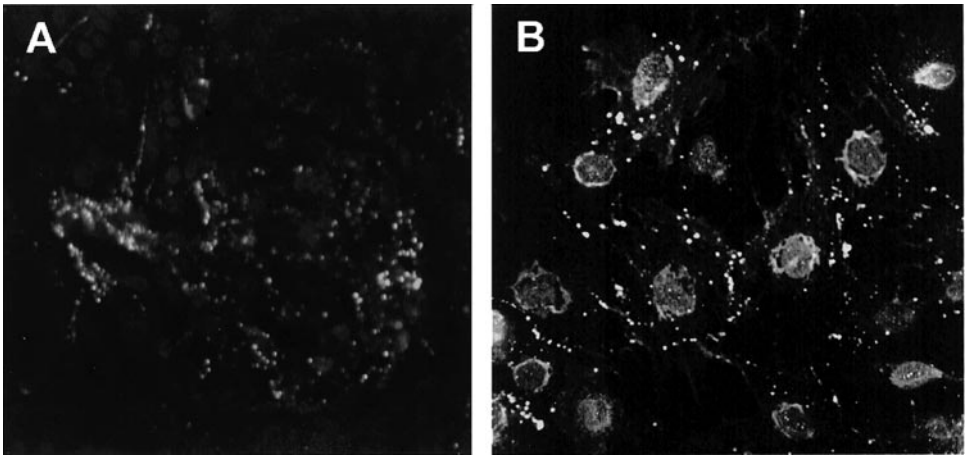

Figure 1. Presence and localization of connexin43 (Cx43) in glomerulus and cultured mesangial cell (MC) monolayers. Immunofluorescent staining of renal section and cultured MC with monoclonal anti-Cx43 antibody. Note the characteristic spotted staining of Cx43 along the distribution of MC in glomerulus as well as at the cell-cell contact and perinuclear regions in culture. Magnification, ×400.

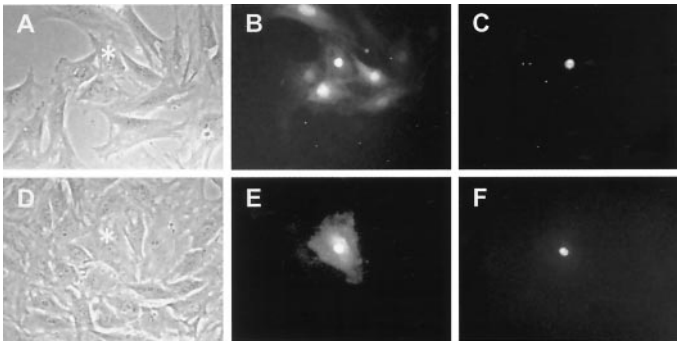

Figure 2. Diffusion of Lucifer yellow (LY) dye from microinjected MC. LY was pressure injected, together with ethidium bromide, into a single MC. LY diffusion into adjacent cells was monitored over a three-minute time period. (Upper panels) MC without pretreatment with heptanol. (Lower panels) MC pretreated with 0.5 mmol/L heptanol for 30 min before injection. (A and D) Phase-contrast micrograph. (B and E) LY diffusion. (C and F) Ethidium bromide staining of the microinjected cell. * indicates the microinjected cell. Magnification, ×320.

remarkably block the transmission of the intercellular Ca^{2+} signaling, as reflected by the obviously reduced number of cells participating in the Ca^{2+} wave (Figures 3B). Heptanol prevented the calcium wave propagation in a concentration-dependent manner. A significant inhibition could be observed at a concentration as low as 0.1 mM. Heptanol itself, however, had little influence on the Ca^{2+} levels of the stimulated cells under both basal and experimental conditions (Figure 3A). A typical sequence of Ca^{2+} ratio images obtained before and after stimulation of one single cell in the presence or absence of heptanol is depicted in Figure 3C.

Previous studies in various cell types have demonstrated that propagation of Ca^{2+} waves between cells can be mediated by the intercellular diffusion of messengers via GJIC, by the extracellular diffusion of cell-released ATP via activation of purinergic receptors, or by moth mechanisms simultaneously (9,10,25–28). MC express purinergic receptors and respond to externally applied ATP (29); we therefore investigated the potential role of ATP in the transmission of intercellular Ca^{2+} signaling. For this purpose, two widely used strategies were employed to change MC response to extracellular ATP. As shown in Figure 4, pretreatment of MC with high doses of ATP (100 μmol/L) for 15 min to desensitize purinergic receptors or addition of the ATP-degrading enzyme apyrase (50 U/ml) directly into the assay medium could largely eliminate MC Ca^{2+} responses to the subsequent challenge of extracellular ATP. When mechanical stimulation was used to evoke intercellular Ca^{2+} wave propagation under the above conditions, neither pretreatment of ATP nor addition of apyrase into the assay system could affect the mechanically elicited rise of Ca^{2+} in the stimulated cells as well as the propagation of Ca^{2+} wave into the surrounding cells (Figure 5). The failed cell response was not due to depletion of internal calcium stores by these procedures, because the subsequent addition of endothelin 1 (ET1), which activates another G-protein coupled receptor in these cells, resulted in cytosolic calcium transients. Thus changes in Ca^{2+} that occur during mechanically stimulated Ca^{2+} wave propagation in MC are mediated mainly by GJ channels, but not ATP-dependent pathway.

Requirement of Phospholipase C Activity for the Propagation of Intercellular Ca^{2+} Wave

IP_3 has been considered to be the major second messenger that diffuses through the GJ to propagate Ca^{2+} waves in various cell types after mechanical stimulation (9,11,25,30–33). It is likely that the same mechanism operates in MC. To confirm this, we have examined the MC Ca^{2+} responses after exposure to phospholipase C (PLC) inhibitor U73122. As indicated in Figure 6, U73122 treatment only slightly changed the Ca^{2+} concentrations of the stimulated cells under both basal and experimental conditions; however, it greatly blunted

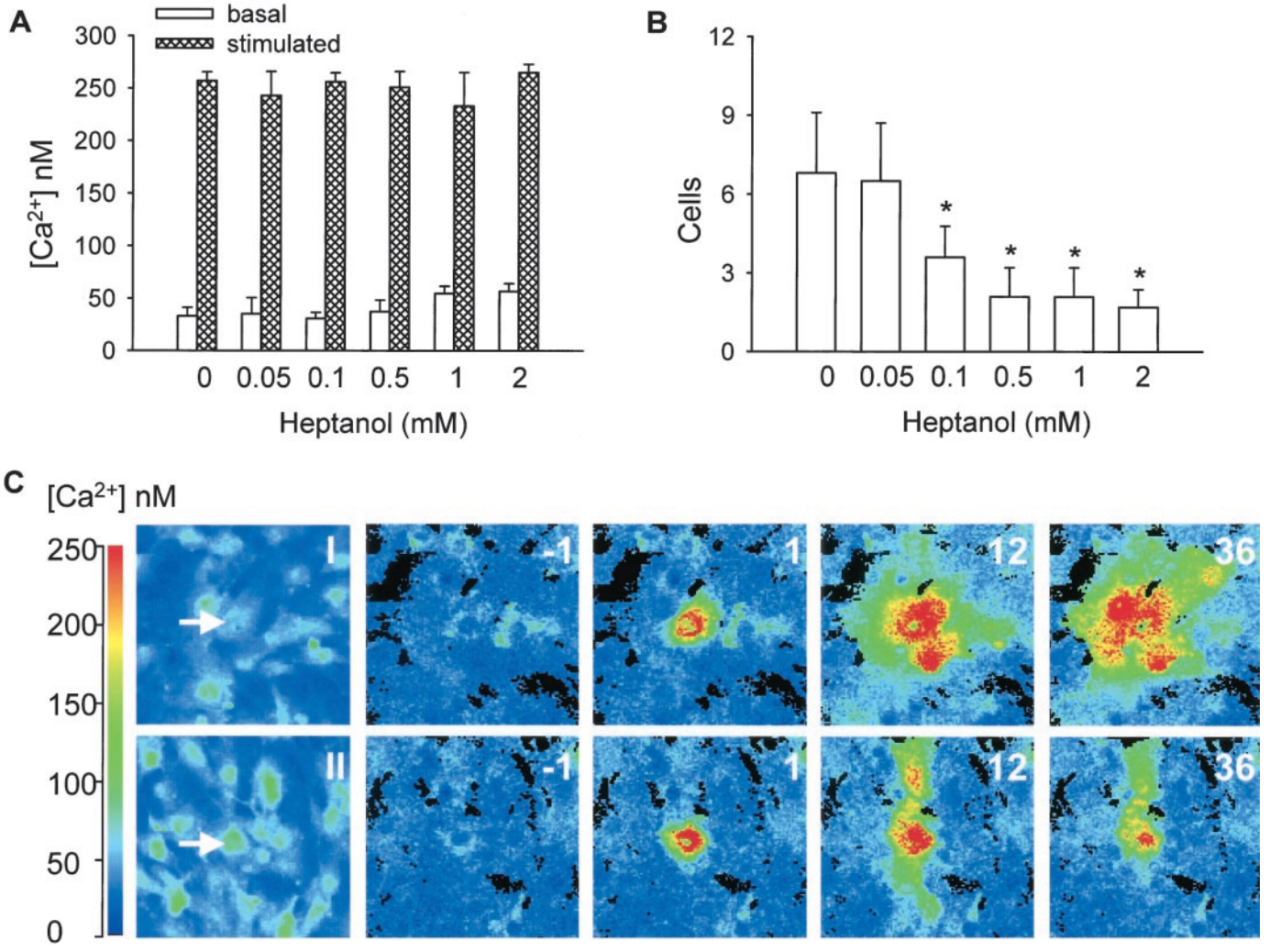

Figure 3. Intercellular Ca^{2+} wave transmission in cultured MC. (A) Ca^{2+} concentrations in the mechanically stimulated MC with or without heptanol treatments. The data were mean ± SEM from ten mechanically stimulated MC. (B) Number of cells responding to mechanical stimulation in the presence or absence of the indicated concentrations of heptanol. Cells in which Ca^{2+} increased >80 nmol/L were designated to be responding. The results were mean ± SEM from ten separate experiments. *$P < 0.01$ *versus* control. (C) A typical ratio images of Ca^{2+} obtained before and after stimulation of one single MC. Monolayers of MC were loaded with fura-2 AM, the morphology and distribution of MC could be judged by the fluorescent image of cells at 340 nm (I and II). A single MC (arrowhead) was mechanically stimulated during fluorescence ratiometric imaging. Time before or after stimulation in seconds is indicated on each panel. The pseudocolor map represents estimated Ca^{2+} concentrations. (Upper panels) MC without pretreatment with heptanol. (Lower panels) MC pretreated with heptanol 0.5 mmol/L for 30 min before stimulation.

the propagation of intercellular Ca^{2+} waves (Figure 6). The effects of U73122 were dose-dependent and a significant inhibition could be observed at the concentration as low as 1 μM. At the concentration of 10 μM, the number of cells participating in the transmission of intercellular Ca^{2+} wave decreased from 6.8 ± 2.3 ($n = 10$) in control to 1.8 ± 1.2 ($n = 10$) after U73122 treatment. In five of the total of ten separate experiments, a Ca^{2+} wave did not propagate to any adjacent cells. A representative sequence of Ca^{2+} ratio images obtained before and after stimulation of one single cell with or without U73122 pretreatment is depicted in Figure 6C. These results indicate that an increase in PLC activity with a concomitant increase in IP_3 is required in the stimulated cell for the initiation and propagation of an intercellular Ca^{2+} wave.

Coordination of MC Contraction by a Single Cell Injection of IP_3

The propagation of intercellular Ca^{2+} signaling through GJ may promote for the coordination of several MC, which then respond as a unit. To demonstrate this speculation, we have examined the contractile response of MC after a single cell injection of IP_3. This design was prepared on the basis of the knowledge that IP_3 is the major messenger that mediates the propagation of Ca^{2+} waves.

A single MC injection of IP_3 resulted in a simultaneous contraction of multiple cells, as demonstrated by the reduction of the cell planar area (Figure 7). The contraction began immediately after the injection and was most dramatic at 15 to 30 min. To indicate the injected cells and confirm that the

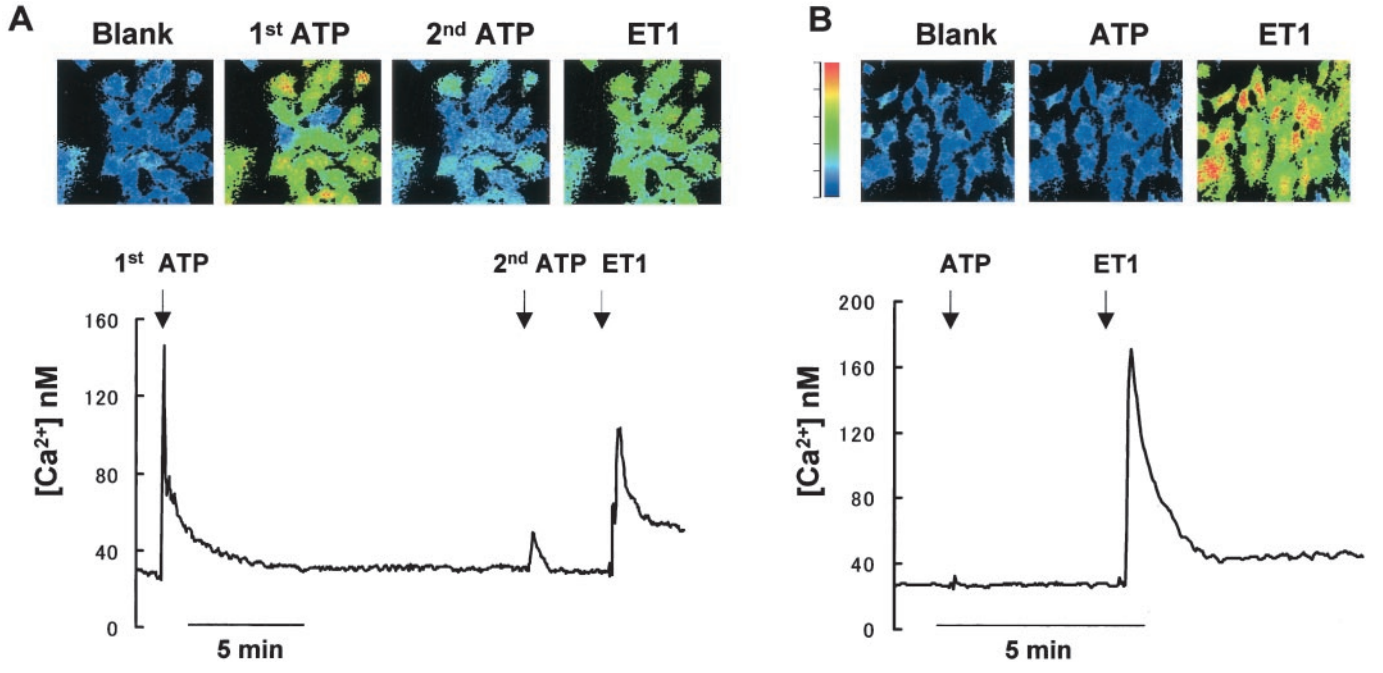

Figure 4. Modulation of MC Ca^{2+} responses to externally added ATP by pretreatment of MC with ATP or addition of apyrase into the assay system. (A) MC were preincubated with 100 μmol/L ATP for approximately 15 min before being sequentially challenged with 100 μmol/L ATP and 10^{-6} mol/L ET1. (B) MC are exposed to 100 μmol/L ATP and 10^{-6} mol/L ET1 in the presence of 50 U/ml apyrase. The results are presented as both ratiometric imaging (upper) and dynamic traces of Ca^{2+} over time (lower), representing the average level of Ca^{2+} in 12 cells in a single study. The similar results were obtained in four additional separate experiments.

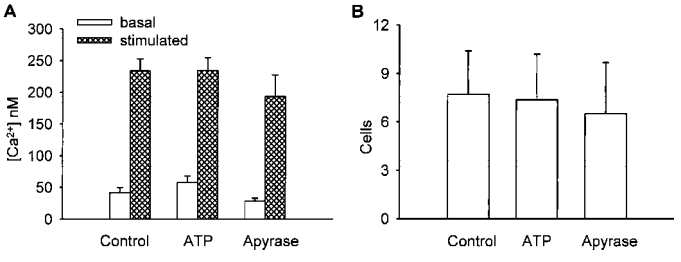

Figure 5. Summary of changes in Ca^{2+} in the mechanically stimulated MC and the numbers of MC participating in Ca^{2+} wave propagation after treatment to remove the extracellular ATP-induced Ca^{2+} response. (A) Ca^{2+} concentrations in the mechanically stimulated MC before and after mechanical strain with or without pretreatment of 100 μmol/L ATP for 15 min or addition of 50 U/ml apyrase. The data were mean ± SEM from ten mechanically stimulated MC. (B) Number of cells responding to mechanical stimulation after treatment to remove the extracellular ATP-induced Ca^{2+} response. Cells that shown an increase in Ca^{2+} >80 nmol/L were designated as responding. The results presented were mean ± SEM from ten separate experiments.

responding cells were really coupled via a GJ with that cell, the fluorescent dye was co-injected. As indicated in Figure 7, the contractile cells were limited to those that were well dye-coupled. Note that injection of the control solution alone without IP_3 did not elicit the obvious shape change in the surrounding cells.

Inhibition of MC Contraction by GJ Inhibitors

The participation of GJ-mediated signaling in MC contraction was further confirmed in an additional system in which MC were embedded in collagen gel and the contraction force of MC in response to serum was reflected by the reduction of surface areas of gels. As indicated in Figure 8, the addition of two structurally unrelated GJIC inhibitors, heptanol or α-glycyrrhetinic acid (α-GA), into MC-embedded gels significantly prevented the reduction of the gel area. The difference between inhibitor-treated group and control persisted throughout the 4-d observation period. It should be noted that treatment of MC with the GJ inhibitors at the concentrations indicated did not cause any obvious morphologic changes nor interfere with the formation of the mesh-like cellular network in gel. A representative MC morphology 4 d after culture in the presence or absence of GJ inhibitors is shown in Figure 9.

Discussion

One of the intriguing features of MC is that they are interconnected by an extensive network of relative small GJ assemblies. However, the functional roles related to these GJ channels are still largely unclear. In this study, we demonstrated the critical role of GJ in the transmission of intercellular Ca^{2+} signals and in the coordination of MC contraction, thus supporting the idea that mesangium comprises a functional syncytium.

To achieve a coordinated cellular reaction (functional syncytium) in the mesangium, the MC have to find a way to

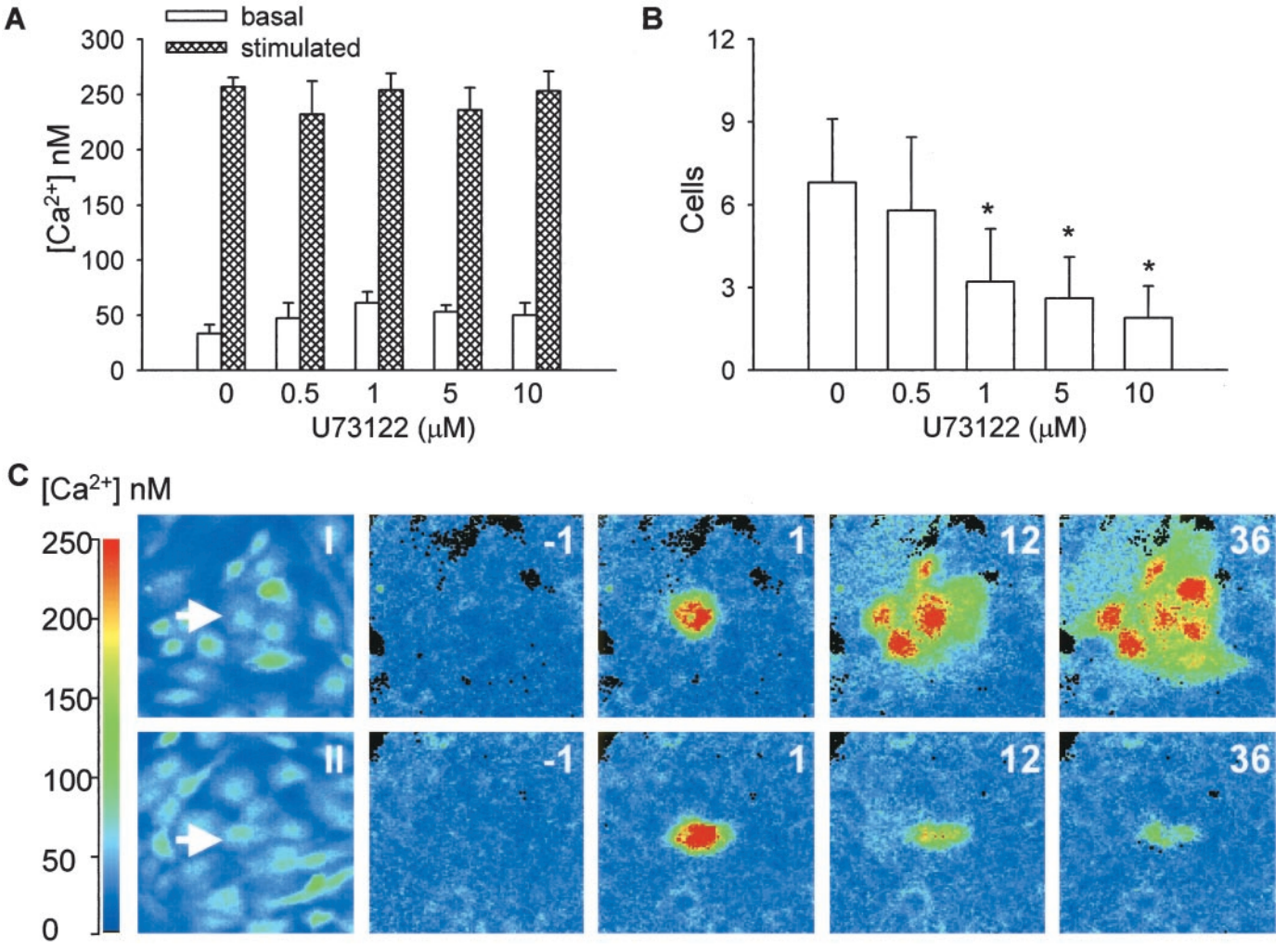

Figure 6. Prevention of mechanically elicited Ca^{2+} wave propagation by PLC inhibitor U73122. (A) Ca^{2+} concentrations in the mechanically stimulated MC with or without U73122 pretreatment (5 min). The data were mean ± SEM from ten mechanically stimulated MC. (B) Number of cells responding to mechanical stimulation with or without pretreatment of various concentrations of U73122. Cells where Ca^{2+} increased >80 nmol/L were designated to be responding. The results were mean ± SEM from ten separate experiments. *$P < 0.01$ *versus* control. (C) A typical ratio images of Ca^{2+} obtained before and after stimulation of one single MC. Monolayers of MC were loaded with fura-2 AM, the morphology and distribution of MC are indicated by the fluorescent image of cells at 340 nm (I and II). A single MC (arrowhead) was mechanically stimulated during fluorescence ratiometric imaging. Time before or after stimulation in seconds is indicated on each panel. The pseudocolor map represents estimated Ca^{2+} concentrations. (Upper panel) MC without pretreatment with U73122. (Lower panel) MC pretreated with 5 μmol/L U73122 for 5 min before stimulation.

communicate with each other. The propagation of intercellular Ca^{2+} wave via GJ might provide a pathway for this purpose. Ca^{2+} waves have been observed in a variety of cells (9,11,25,30–33). In this study, we presented evidence showing the presence of Ca^{2+} wave in the cultured MC, and the propagation of this wave in MC requires active GJ communication. Disruption of GJIC using a GJ inhibitor could largely prevent the spreading of the wave, as reflected by the obviously reduced number of cells involved. There are also studies showing that intercellular Ca^{2+} waves may be alternatively propagated via activation of purinergic receptors by cell-released ATP (27,28,33–35). However, this pathway is less likely to operate in MC, because the transmission of Ca^{2+} signaling persisted even after desensitization of purinergic receptor by preincubation with ATP, or in the presence of an ATP degrading enzyme, apyrase. Thus the GJ is the major pathway for the transmission of mechanical stress-elicited intercellular Ca^{2+} signals in MC.

The mechanisms underlying the mechanically elicited Ca^{2+} wave propagation in MC are probably due to the formation and diffusion of IP_3 via GJ. This is indirectly supported by our observation of the critical role of PLC activity in the initiation and propagation of the intercellular Ca^{2+} wave. Although U73122 abrogated the propagation of mechanically elicited Ca^{2+} waves, it did not affect the rise of Ca^{2+} in the stimulated MC (Figure 6, A and C). Thus, the IP_3-dependent release of Ca^{2+} from internal stores is not mainly responsible for the elevation of Ca^{2+} in the stimulated MC. A similar observation

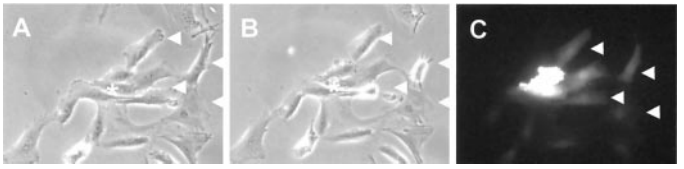

Figure 7. MC contraction induced by a single cell injection of IP_3. MC in DMEM containing 25 mmol/L HEPES (pH 7.4) and 0.1% bovine serum albumin (BSA) were injected with IP_3 (1 mmol/L dissolved in 10 mmol/L KCl) together with LY (1 mg/ml). (A) micrography of MC before injection. (B) Phase-contrast image of MC 30 min after IP_3 injection. (C) LY diffusion after single cell injection. * indicates the impaled cell. White arrows note diminution of cell planar area and the shortening of cytoplasmic extensions. The similar results were obtained in seven additional experiments. Magnification, ×320.

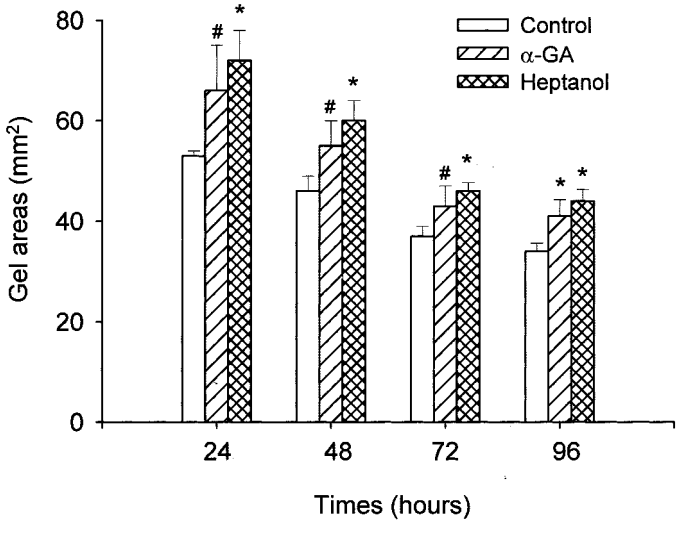

Figure 8. Inhibition of MC-embedded gel contraction by GJ inhibitors. MC embedded in collagen gel were treated with or without heptanol 0.5 mmol/L or α-GA 25 μmol/L. The degree of gel contraction was examined at the indicated time intervals, as described in the Materials and Methods section. Values are means ± SD ($n = 4$). #$P < 0.05$ *versus* control; *$P < 0.01$ *versus* control. The data shown are from one representative experiments from a series of five with similar results.

has been described in cultured airway epithelial cells by Hansen *et al.* (32). Furthermore, Boitano *et al.* (25) reported that the mechanical stimulation increased Ca^{2+} even when IP_3 receptors were blocked with heparin. It has been suggested that the increase of Ca^{2+} after mechanical strain could be also due to an influx of Ca^{2+} through Ca^{2+}-conducting channels. Indeed, the mechanically elicited elevation of Ca^{2+} in the stimulated MC was significantly blunted when the experiments were conducted in the absence of extracellular Ca^{2+}, and there was basically no propagation of mechanically elicited intercellular Ca^{2+} waves under Ca^{2+}-free circumstance (data not shown).

A Ca^{2+} wave, propagated via GJ, is considered to coordinate multicellular processes, such as ciliary beating in tracheal

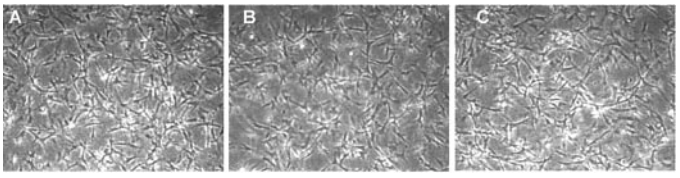

Figure 9. MC morphology in gel in the presence or absence of GJ inhibitors. MC embedded in collagen gel were left untreated (A) or treated with either α-GA 25 μmol/L (B) or heptanol 0.5 mmol/L (C) for 4 days. The images of the cells were captured into the computer using an ICCD camera. Magnification, ×100.

epithelial cells (23,36), bile expulsion in the liver (37), hormone secretion in the pancreas (38,39), and information processing in neural cells (40). We therefore tried to elucidate the physiologic relevance of this wave in MC. For this purpose, we have chosen the contraction of MC as a parameter for investigation on the basis of the following considerations: (*1*) the coordinated contraction of MC is critical for the regulation of glomerular hemodynamics (41); (*2*) the critical role of Ca^{2+} in the regulation of MC contraction is well established (13,41); (*3*) it is well known that synchronized contraction of heart myocytes depends on the GJ communication (42); and (*4*) the importance of GJ in the regulation of integrated responses of smooth muscle cells in vascular contraction and relaxation are now being recognized (43–45). To elicit a Ca^{2+} wave in MC, we also employed injection of IP_3 as an alternative to mechanical stimulation. The advantage of using IP_3 is that we can control the intensity of stimulation by adjusting the volume of IP_3 injected, and it is possible to achieve a Ca^{2+} signal strong enough to cause MC contraction. IP_3 is considered to be the major messenger moving through the GJ to transmit the Ca^{2+} wave (9,11,25,30–33); the cellular response resulting from IP_3 injection should therefore be equal in magnitude to that of a Ca^{2+} signal. Our results demonstrate that the injection of IP_3 into a single MC could lead to the synchronized contraction of multiple surrounding cells. This evidence supports the view that GJ-mediated Ca^{2+} signals play a critical role in the synchronization of MC contraction. This involvement of GJ in MC contraction is further demonstrated by the observation that the addition of GJ inhibitor into MC-embedded gels could significantly prevent FCS-induced gel contraction. The reasons why uncoupling of GJ could result in the reduced contractile forces of MC in collagen-gel is unclear. One explanation could be that the exchange of the intercellular signal via GJ might permit amplification and integration of signals, while inhibition of GJ coupling would lead to desynchronization of the Ca^{2+} signals of the coupled cells, thus causing less coordinated contraction. It is worth noting that the inhibitory action of the GJ inhibitors on the contractile response of other cell types in gels (fibroblasts and osteoblasts) has been previously reported (46,47).

The findings in this study may have significant pathophysiologic implications. In the glomerulus, the GJ may at least play a role in physiologically relevant processes detailed in the following. (*1*) MC are subjected to multiple forms of mechanical strain (fluid shear, hydrostatic pressure, and triaxial

stretch) as a result of forces exerted by the vasculature (24). From their location at the center of the glomerular lobule, MC extend cytoplasmic projections, which attach to the peripheral basement membrane. During pressure-induced glomerular expansion, the outward displacement of these anchoring points, caused by distending capillaries and mesangium, results in an intense MC stretch. This situation is somewhat similar to the mechanical stimulation used in our system, in which the mechanical strain was made via the deformation of the cell by an external device. It is conceivable that displacement of the anchoring points of MC may evoke a similar intercellular Ca^{2+} response, this might provide an important pathway to transmit the intraglomerular signals to extraglomerular MC or other effector cells, thus producing synchronizing responses to a mechanical strain. (*2*) MC also reside in the GJ interstitium, surrounded by afferent and efferent arterioles and the macula densa. Morphological documentation and histochemical studies have revealed the presence of abundant GJ among intraglomerular and extraglomerular MC and afferent arteriolar cells, and this structure makes these cells very tightly coupled and unites them as a syncytium (1,2,7,8). The functional data on transmission of Ca^{2+} signaling via GJ in this study provides important evidence, supporting the idea of a mediating role of extraglomerular MC in TG-feedback mechanisms. The GJ in MC may allow signal transduction from the macula densa to the glomeruli and affect intraglomerular hemodynamics. (3) The GJ might provide cooperation to the agonist-induced contraction and relaxation processes, thus regulating glomerular hemodynamics. Under pathologic conditions, disruption of GJ communication could be an important mechanism contributing to disordered glomerular function. A number of inflammatory mediators and growth factors have already been shown to be able to interfere with GJ communication in MC (6,7). It can be envisaged that persistent interruption of GJ communication by these agents would finally cause loss or dysfunction of coordinated glomerular processes, such as contraction, thus leading to the continuing progression of renal diseases.

In summary, we have demonstrated a critical role of GJIC in coordinating mesangial behavior. GJIC might play an important role in multiple renal physiologic and pathologic processes.

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