Angiotensin II and Vasopressin Stimulate Calcium-activated Chloride Conductance in Rat Mesangial Cells

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Abstract

In an attempt to clarify the mechanisms by which angiotensin II (AII) and arginine vasopressin (AVP) regulate mesangial cell function, we examined the membrane potential change of mesangial cells and found that cells contracted and membrane potential depolarized in response to AII and AVP. The depolarization was associated with decreased input resistance. Ca ionophore A23187 caused similar mesangial cell contraction and depolarization. The reversal potential (Vr) of the depolarization response to AII and AVP was −29±3 and −25±7 mV (mean±SD), respectively. Not only the Vr of the AII-induced depolarization but also Vr of the Ca ionophore–induced response was dependent upon the extracellular Cl^- concentration. Further, AII and AVP caused cell contraction and membrane depolarization in Ca^{++}-free medium containing 0.5 mM EGTA. These data suggest the presence of Ca^{++}-activated Cl^- channels in the mesangial cells and that AII and AVP increase Cl^- permeability via an elevation of $[Ca^{++}]_i$ released from the intracellular organellae.

Introduction

Mesangial cells are located in the intercapillary space in the glomerulus and are rich in contractile fibers (1, 2). These characteristics of mesangial cells suggest that the mesangial cell may regulate, through its contraction, the intraglomerular hemodynamics and thus the glomerular filtration rate (GFR)[1] (3–5). It has been reported that angiotensin II (AII) and arginine vasopressin (AVP) cause mesangial cell contraction (6–9), which suggests that mesangial cell contraction may underlie the regulation of glomerular hemodynamics and ultrafiltration by these peptide hormones (3–5). The effects of AII and AVP on mesangial cells are thought to be mediated by an increase in the concentration of intracellular Ca^{++} ions ($[Ca^{++}]_i$), since Ca^{++} ionophore mimics the hormonal effects on mesangial functions including cell contraction and prostaglandin synthesis (6, 10, 11). Nonetheless, our understanding of the mechanisms of action of AII and AVP and of the functional role of mesangial cells in regulating the glomerular functions seems hampered by the paucity of the knowledge on the physiological properties of the mesangial cell. To better understand the function of mesangial cells, we examined in the present study the hormonal responses and their mechanisms of action in the cultured mesangial cells by monitoring the electrophysiological characteristics of the cells. We found the presence of Ca^{++}-activated Cl^- channels in plasma membranes of the mesangial cells, which leads to membrane depolarization in response to AII and AVP. The presence of this unique ion channel may be of physiological significance in the hormonal regulation of mesangial cell function and of glomerular hemodynamics and ultrafiltration.

Methods

Isolation of glomeruli and culture of mesangial cells. The glomeruli were isolated from the kidney of the male Wistar rat weighing 100–200 g. Under ether anaesthesia, rats were exsanguinated, and the kidneys were immediately removed and decapsulated. The cortex was excised and minced into small pieces (~1 mm) under sterile conditions. The mince was pressed through a 250-μm sieve and suspended in isotonic saline. The suspension was successively passed through the 177-, 125-, and 53-μm sieves and glomeruli were collected on the surface of the 53-μm sieve. The methods were essentially the same as described by Venkatachalam and Kreisberg (6) and by Scharschmidt and Dunn (7). The purity of the glomeruli was >90% and almost all of them were decapsulated. Taking advantage of the differential growth characteristics of glomerular epithelial and mesangial cells, mesangial cells were obtained by culturing isolated glomeruli at 37°C for 3–5 wk in minimal essential medium containing 18% fetal calf serum and 0.006% kanamycin under humidified air containing 5% CO_2 (12, 13). It has been well established using immunohistological and morphological criteria that most of the cells obtained by this culture technique are mesangial cells (6, 7). The observation under phase-contrast microscope revealed that the cells were morphologically similar to the cultured mesangial cells reported by others (6–13), i.e., flat polygonal cells with processes showing multilayer growing. In the present study, we used more than 50 series of the primary culture of mesangial cells between 3 and 5 wk after each glomerular seeding.

Electrophysiological experiments. The membrane potential of the cultured mesangial cells was recorded by the conventional glass microelectrode technique. The electrodes containing 4.0 M potassium acetate with a resistance of 30–50 megaohms (MΩ) were used. The electrodes were made by PA-81 magnet puller (Narishige, Tokyo, Japan) and have the opening size of ~0.7 μm. The membrane resistance was monitored by applying constant current pulses of 600 ms with a bridge circuit. Cultured mesangial cells were first treated for 5–10 min with Ca^{++}-free, phosphate buffered saline containing 0.54 mM EDTA and then again incubated for 30–60 min in minimal essential medium containing 18% fetal calf serum. After this procedure, the cells became round (Fig. 1 *a*), and were thus suitable for impalement with the microelectrode. Before the electrophysiological study, the medium was changed to the experimental solution. Table I depicts the constituents of the extracellular solutions. In Cl^--deficient medium, Cl^- ions were isotonically replaced by methanesulfonate ions, which are impermeable to Cl^- channels. Relatively large cells of a diameter >50 μm were examined in the present experiment because the membrane potential of smaller cells tended to

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1. *Abbreviations used in this paper:* AII, angiotensin II; AVP, arginine vasopressin; GFR, glomerular filtration rate; i, intracellular; I–V, current–voltage; o, extracellular; TEA, tetraethylammonium; Vr, reversal potential.

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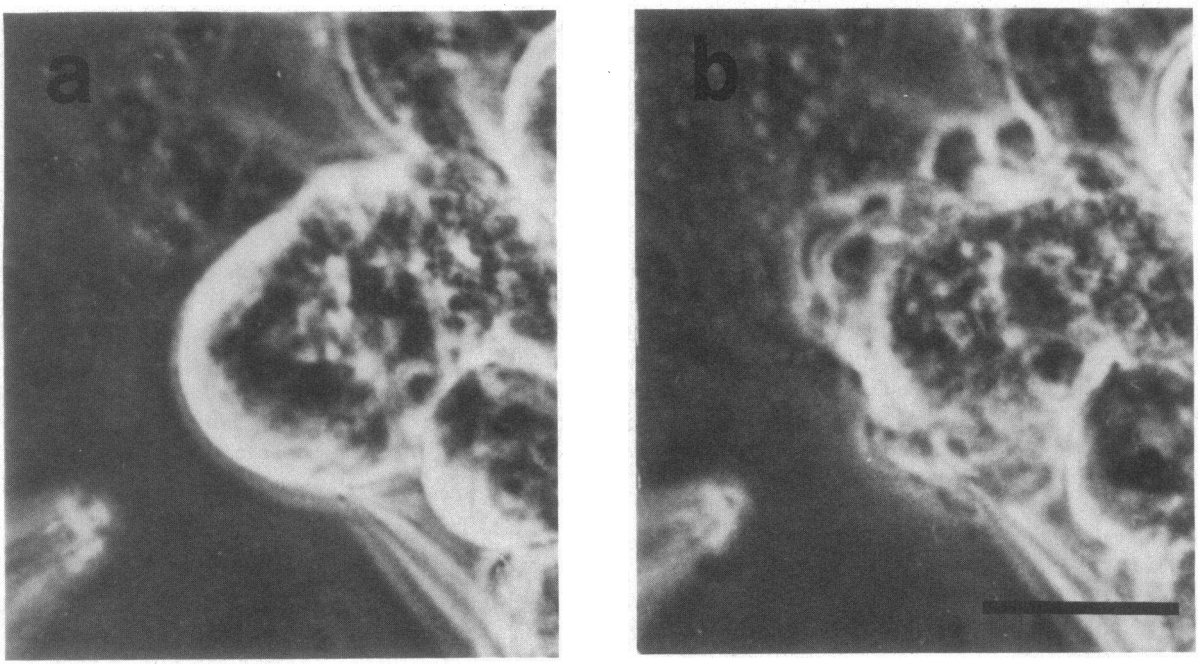

Figure 1. Mesangial cell contraction in response to AII. (*a*) Mesangial cell after 0.54 mM EDTA preparation. The cell had become rounded and the surface smoothened. The cell was impaled and the glass capillary containing the 100-nM AII was positioned near the cell (lower left). (*b*) Mesangial cell contracted in response to AII. Bar, 25 μm.

be unstable after impalement with the electrode. The temperature was kept at 30–32°C. In some experiments, the extracellular media were exchanged by superfusing the solution using a peristaltic pump.

A glass capillary with a tip diameter of a few microns containing either AII, AVP, or Ca^{++} ionophore (A23187) at concentrations of 100 nM, 100 nM, and 20 μM, respectively, was placed 20–30 μm apart from the impaled cell and the agents were delivered to the proximity of the impaled cell by applying positive pressure (0.1–0.2 kg/cm^2) to the inside of the capillary for 1–5 s if not otherwise noted. To assure that the impaled cell was a mesangial cell, the cell contraction in response to the agents was ascertained simultaneously under phase-contrast microscopy while recording the membrane potential and conductance. The data obtained from the cells that had not shown contraction in response to the agents were discarded.

AII, AVP, and A23187 are purchased from Sigma Chemical Co. (St. Louis, MO). Stock solution of 0.1 mM AII and AVP were prepared in 100 mM acetic acid and kept frozen until use. A23187 (10 mM) was dissolved in dimethylsulfoxide. These agents were diluted to the final concentration described above by the appropriate experimental solution just before the experiment. The final concentration of acetic acid and dimethylsulfoxide was 0.1 mM and 0.2%, respectively. Contraction or potential change was not observed by administrating the vehicles alone.

Results are expressed as mean±SD and analyzed by *t* test as appropriate.

Results

Resting potential. The resting potential of mesangial cells in the standard medium was −53±7 mV and the input resistance measured by applying hyperpolarizing currents was 47±24 MΩ (mean±SD: n = 30). The resting potential of the mesangial cells

Table I. Composition of the Experimental Solutions

Solution	Na	K	Cl	Ca	Ba	TEA	TMA	Choline	Methane-sulfonate
mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*
Standard	151.0	6.0	140.4	1.2	0.0	0.0	0.0	0.0	0.0
TEA (30)	121.0	6.0	140.4	1.2	0.0	30.0	0.0	0.0	0.0
Ba (5)	151.0	6.0	150.4	1.2	5.0	0.0	0.0	0.0	0.0
Na-free	0.0	6.0	140.4	1.2	0.0	0.0	20.0	131.0	0.0
Ca-free	153.4	6.0	140.4	0.0	0.0	0.0	0.0	0.0	0.0
K (30)	127.0	30.0	140.4	1.2	0.0	0.0	0.0	0.0	0.0
K (70)	87.0	70.0	140.4	1.2	0.0	0.0	0.0	0.0	0.0
Cl (10)	151.0	6.0	10.0	1.2	0.0	0.0	0.0	0.0	130.4
Cl (30)	121.0	6.0	30.0	1.2	0.0	30.0	0.0	0.0	110.4
Cl (60)	121.0	6.0	60.0	1.2	0.0	30.0	0.0	0.0	80.4
Cl (90)	121.0	6.0	90.0	1.2	0.0	30.0	0.0	0.0	50.4

All solutions contained 1.0 mM Mg, 5.5 mM glucose, and 8 mg/ml bovine serum albumin. pH was adjusted to 7.4 with 20 mM Hepes (Na salt in Na-containing solutions and TMA salt in Na-free solutions). In Ca-free solution, 0.5 mM EGTA-Na_2 was added. TEA, tetraethylammonium; TMA, tetramethylammonium.

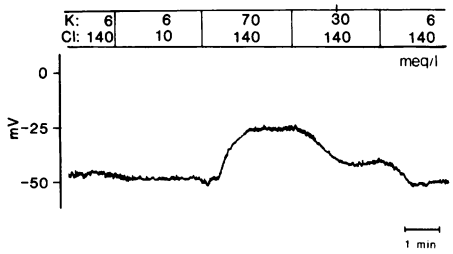

Figure 2. A representative experiment to demonstrate the changes in the resting potential in response to alterations in $[K^+]$ and $[Cl^-]$ in the incubation medium. The media were changed by continuous superfusion. The concentration of K^+ and Cl^- of the medium in meq/liter are depicted in the top panel. Resting potential did not change when $[Cl^-]_o$ was changed from 140 mM to 10 mM. When the $[K^+]_o$ was successively changed from 6 to 70, 30, then again to 6 mM, the resting potential changed in accordance with $[K^+]_o$.

was dependent on the extracellular concentration of potassium ions ($[K^+]_o$). Fig. 2 exemplifies the changes in the resting potential when the $[Cl^-]_o$ and $[K^+]_o$ were successively changed. The resting potential in the standard medium of this cell was −50 mV. It was depolarized by 24 mV in 70 mM K^+ medium, and by 10 mV in 30 mM K^+ medium, whereas there was no change in the resting potential when $[Cl^-]_o$ was reduced to 10 mM. In Fig. 3, the membrane potentials in several cells similarly obtained are plotted against $[K^+]_o$. The resting potential was essentially similar in Na^+-free medium (data not shown). The deviation of the resting potential change from the ideal K^+ electrode as Fig. 3 shows might be caused by nonselective channels or the leakage conductance. However, it is reasonable to state that the resting potential of the mesangial cells was in part determined by $[K^+]_o$ under the present experimental condition.

Steady-state current-voltage relation. The current-voltage (I-V) relation in the standard medium showed an outward-going rectification at the potential levels > −50 mV (Fig. 4, *a* and *b*). As Fig. 5 shows, the outward-going rectification was suppressed and the I-V relation became linear in the presence of either 30 mM tetraethylammonium (TEA) or 5 mM barium, suggesting that the outward-going rectification was due to K^+ channels.

Effects of AII. Fig. 6 depicts the representative membrane

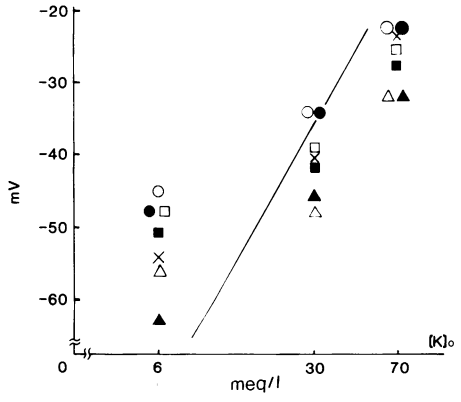

Figure 3. The relationship between the resting potential and $[K^+]_o$. The resting potential on the ordinate at varying $[K^+]_o$ on the abscissa is shown. Each symbol denotes the data in the same cell recording. The line denotes the theoretical relationship between the resting potential and $[K^+]_o$ calculated from Nernst's equation, assuming that $[K^+]_i$ is 120 mM.

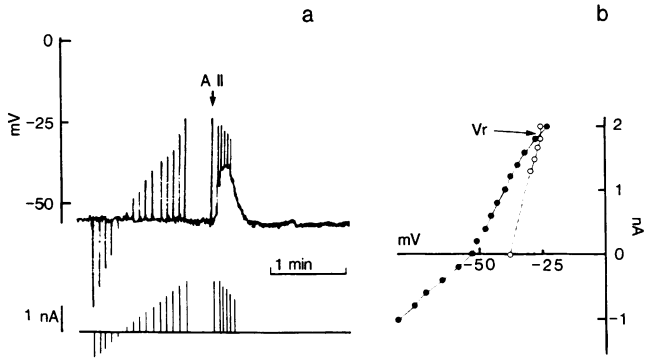

Figure 4. The I-V relationship in the standard medium before and after a 100-nM AII application. (*a*) The membrane potential response to current pulses of 600 ms with varying amplitude. (*b*) The I-V relations of mesangial cell before (solid circles) and at the peak of angiotensin II-induced depolarization (open circles) plotted from the data in *a*. The potential level where the two lines cross (−26 mV) is the Vr of membrane depolarization.

potential change in the standard medium in response to AII. In response to a brief application of AII, the membrane potential immediately depolarized by 24 mV with a decrease in input resistance. The contraction of mesangial cells, viewed under microscope, began within a minute of AII delivery. Fig. 1, *a* and *b* compares the typical morphological change induced by application of AII. The surface of the mesangial cell, which was smooth before the application of the hormone (Fig. 1 *a*), became irregular and the cell itself shrank in size (Fig. 1 *b*). This visible change of the cell shape continued for several minutes. The membrane potential changes always preceded the cell contraction viewed under microscope.

Reversal potential (Vr) of the response. Fig. 4 *b* shows the I-V curves before (solid circles) and at the peak of AII-induced depolarization (open circles) obtained from the data Fig. 4 *a*

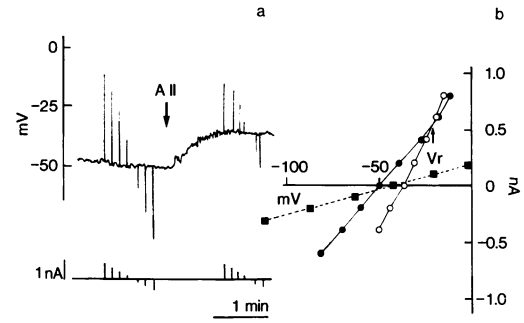

Figure 5. The I-V relationship in the presence of 30 mM TEA and 5 mM barium, and the response to AII. (*a*) The membrane potential response to current pulses of 600 ms with varying amplitude in the presence of 30 mM TEA. At the arrow, AII (100 nM) was given for 5 s and the membrane potential depolarized. (*b*) The I-V relations of mesangial cell before (solid circles) and at the peak of AII-induced depolarization (open circles) plotted from the data in Fig. 5 *a*. The potential level where the two lines cross (−22 mV) is the Vr of membrane depolarization. Solid squares with dashed line represents the I-V relation of another cell in the presence of 5 mM barium. The outward-going rectification observed in the standard medium was suppressed and the I-V relation became linear in the presence of 30 mM TEA or 5 mM barium, which suggests that the outward-going rectification was due to potassium channels.

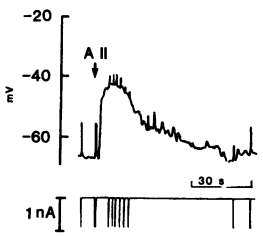

Figure 6. The membrane potential change in response to 100 nM AII in the standard medium. Mesangial cells were incubated in the standard solution and current pulses of 600 ms with a constant amplitude were applied. The membrane potential depolarized and the input resistance decreased in response to AII.

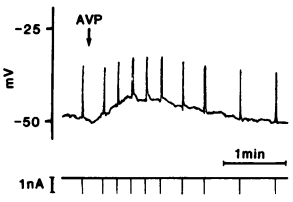

Figure 8. Membrane potential depolarization with decreased input resistance induced by 100 nM AVP in the presence of 30 mM TEA and 140 mM Cl^-.

shows. The potential level where these two I–V curves intercept (arrow in Fig. 4 *b*), indicates the Vr of the response to AII, the potential level at which the membrane potential does not change in response to AII. The average of the Vr thus obtained in the standard solution containing 140 mM Cl was −29±3 mV (n = 6, from two cultures). However, in many cells it was difficult to determine accurately the Vr because the well developed outward-going rectification prevented the recording of the I–V relation at the potential level > −30 mV. In the medium containing 30 mM TEA, the outward-going rectification was suppressed (Fig. 5, *a* and *b*, solid circles), making it feasible to accurately estimate the I–V relation at any potential. As Fig. 5 shows, the Vr of the response to AII in the presence of 30 mM TEA can be determined in the similar fashion as in the standard solution, as Fig. 4 shows. The Vr obtained in 30 mM TEA medium containing 6 mM K^+ was −27±3 mV (n = 6, from three cultures), a value almost identical to that obtained in the absence of TEA. The result indicates that TEA did not cause an appreciable change in increased ion permeability by AII. Therefore, the subsequent experiments were carried out in the medium containing 30 mM TEA.

Dependence of Vr on $[Cl^-]_o$. The presence of Cl^- channels has been reported in pancreas acinar cells and xenopus oocytes (14, 15), which demonstrate an equilibrium potential for Cl^- ions at ~ −20–−30 mV. These data suggest that the opening of Cl^- channels may account for the membrane depolarization of mesangial cells in response to AII. To test this possibility, the Vr was examined in the medium with varying $[Cl^-]_o$. Fig. 7 depicts the relation between the $[Cl^-]_o$ and Vr of the membrane depolarization by AII (solid circles). Reduction in $[Cl^-]_o$ was

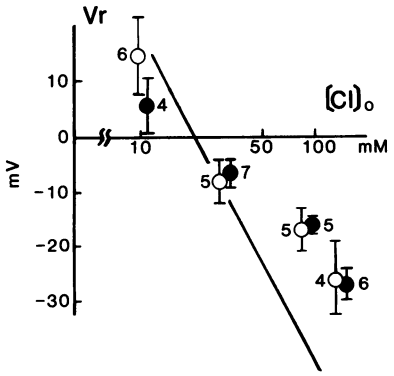

Figure 7. Dependence of the AII (solid circles) and A23187 (open circles) induced Vr on $[Cl^-]_o$. Each circle with bar denotes the mean±SD and the number by each circle is the number of the cells examined. These cells were obtained in four separate cultures for AII study and in additional four cultures for A23187 study. The solid line represents ideal Cl^- electrode potential calculated from Nernst's equation, assuming $[Cl^-]_i$ is 20 mM.

associated with less negative Vr, and in 10 mM $[Cl^-]_o$, the Vr became even positive. The data indicate that the membrane depolarization by AII is mainly caused by an increase in Cl^- conductance. Because the Vr is slightly deviated from the theoretical equilibrium potential of Cl^- ions calculated from Nernst's equation (straight line in Fig. 7), the permeability change to some other ions may also participate in the membrane depolarization.

Effects of AVP. AVP caused contraction and membrane depolarization of mesangial cells, similar to those observed with AII (Fig. 8). The Vr of AVP-induced depolarization in the presence or absence of TEA was −25±7 mV (n = 5, from two cultures). Therefore, the membrane depolarization by AVP was considered to be also caused by an increase in Cl^- conductance.

Role of Ca^{++} *ions.* Available data suggest that an increase in $[Ca^{++}]_i$ is an important mediator of the mesangial cell contraction induced by AII and AVP (6, 7). Thus, it is possible that an increase in $[Ca^{++}]_i$ may underlie the membrane potential depolarization. To test this possibility, we examined the effect of Ca ionophore (A23187) on the membrane potential of the mesangial cells. In response to 20 μM A23187, the mesangial cells contracted and the membrane potential depolarized with an increased membrane conductance (Fig. 9). The Vr in 140 mM $[Cl^-]_o$ was −26±7 mV (n = 4, from one culture), a value almost identical with those by AII and AVP. The Vr of the responses to A23187 (Fig. 7, open circles) was dependent on $[Cl^-]_o$, which was similar to the Vr with AII. These data strongly suggest that the increased Cl^- conductance by AII or AVP was mediated by an increase in $[Ca^{++}]_i$.

To determine the source of Ca^{++} ions causing a rise in $[Ca^{++}]_i$ in the response to AII or AVP, the cell contraction and the membrane potential change were examined in Ca^{++}-free medium containing 0.5 mM EGTA where Ca^{++} influx should be negligible. In this medium, the mesangial cell contracted and the membrane potential depolarized in response to AII or AVP. Fig. 10 shows the depolarization and increased membrane conductance in response to AII (100 nM) in the Ca^{++}-free medium containing 0.5 mM EGTA. The Vr of the response to AII in the Ca^{++}-free medium containing 0.5 mM EGTA and 140 mM Cl^- was −26±7 mV (n = 5, from two cultures). These data suggest that the cell contraction and the increase in Cl^- conductance

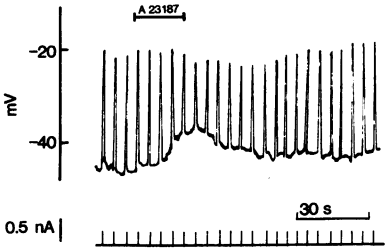

Figure 9. Membrane potential depolarization with decreased input resistance induced by applying 20 μM A23187 for 20 s.

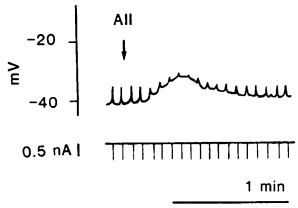

Figure 10. Membrane potential depolarization with decreased input resistance induced by 100 nM AII in Ca^{++}-free medium containing 0.5 mM EGTA and 140 mM Cl^-. AII was given by applying air pressure for 5 s.

was caused by mobilization of Ca^{++} ions from the intracellular organellae.

Discussion

The present study has demonstrated that AII and AVP caused the membrane potential depolarization accompanied by an increase in the membrane conductance in the mesangial cells. The membrane depolarization was caused mainly by an increase in Cl^- conductance. Ca^{++} ionophore A23187 similarly increased the membrane conductance to Cl^- ions, suggesting that the increased Cl^- conductance induced by AII or AVP is mediated by an increase in $[Ca^{++}]_i$, that is, AII and AVP increase Ca^{++}-activated Cl^- conductance. Both the membrane potential depolarization by increasing $[K^+]_o$ to 70 mM (membrane potential depolarized to -27 ± 4 mV [$n = 6$]) and continuous depolarizing currents failed to induce cell contraction and to increase Cl^- conductance. These data indicate that Ca^{++} influx through voltage-gated Ca^{++} channels may not be involved in Ca^{++}-activated Cl^- conductance in mesangial cells. This is in contrast to Ca^{++}-activated Cl^- channels reported in other cell types (15–17).

There is some controversy as to whether the effects of AII and AVP on mesangial cells are mediated by Ca^{++} influx from outside the cell or by Ca^{++} released from the intracellular organellae. Scharschmidt and Dunn have reported that enhanced prostaglandin synthesis by AII and AVP was abolished in the absence of extracellular Ca^{++} (7). However, there is a possibility that the intracellular Ca^{++} store may have been depleted in their experiments because the cells were exposed to Ca^{++}-free media as long as for 2 h. In our experiments, mesangial cells were exposed to a Ca^{++}-free environment < 30 min. Venkatachalam and Kreisberg also reported that mesangial cell contraction could be abolished in response to AVP in the Ca^{++}-free solution (6), an observation in contrast to ours. In our present study, mesangial cell contraction and the membrane depolarization in response to AII and AVP were clearly detected and reproduced in Ca^{++}-free medium containing 0.5 mM EGTA. Thus, it seems reasonable to deduce that actions of AII and AVP in mesangial cells are mediated by Ca^{++} release from the intracellular organellae, possibly endoplasmic reticulum. A recent preliminary report by Takeda and Schrier suggests that intracellular Ca^{++} mobilization initiates the effect of AII on mesangial cell contraction (18), an observation consistent with our postulate.

Results of the present study may suggest that the membrane potential change and cell contraction in response to AII, AVP, and A23187 are causally related. In most experiments, membrane depolarization and cell contraction were both observed in the impaled cells in response to three agents used in the present study. Moreover, membrane depolarization always preceded cell contraction. Therefore, it may be reasonable to deduce that the increased Cl^- conductance has some role in regulating cell contraction. However, it is equally possible that both membrane depolarization and cell contraction represent two independent cellular responses initiated by an increase in $[Ca^{++}]_i$. In this regard, it is of note that the membrane potential depolarization induced by high $[K^+]_o$ did not cause cell contraction as described above. These issues need further investigation.

Based on available information, we would like to postulate that this unique permeability to Cl^- ions of the plasma membrane may be important in the tubuloglomerular feedback system, i.e., the regulation of glomerular filtration by distal tubular fluid delivery via macula densa. It has been suggested that a change in the luminal Cl^- concentration ($[Cl^-]$) at macula densa signals the tubuloglomerular feedback mechanism (19) and that mesangial cell contraction acts as an effector mechanism of the tubuloglomerular feedback (3–5). Between macula densa and the glomerulus, there lie Goormaghtigh cells or lacis cells, interstitial cells morphologically indistinguishable from mesangial cells thus called extraglomerular mesangial cells (20). Mesangial cells are tightly coupled with each other and with Goormaghtigh cells by a gap junction, thus forming a syncytium (21). It is expected that a change in the $[Cl^-]$ in the tubular lumen will alter the $[Cl^-]$ in the absorbate. Because the area surrounding Goormaghtigh cells are neither well perfused with capillaries nor drained with lymph vessels (20, 22), it is possible that $[Cl^-]_o$ of these cells may be directly altered by a change in $[Cl^-]$ in the tubular fluid perfusing macula densa. Such a change in $[Cl^-]_o$ may affect Goormaghtigh cell function, particularly in the presence of AII, which is then transmitted to intraglomerular mesangial cells via gap junction. Further studies will support or refute such a hypothesis. In any events, based on these and other observations (22), the presence of Cl^- channels in the mesangial cells might lend support for an important role of Cl^- ions as a mediator of the tubuloglomerular feedback.

Acknowledgments

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References

1. Andrews, P. M., and A. K. Coffey. 1983. Cytoplasmic contractile elements in glomerular cells. *Fed. Proc.* 42:3046–3052.

2. Kreisberg, J. I. 1983. Contractile properties of the glomerular mesangium. *Fed. Proc.* 42:3053–3057.

3. Dworkin, L. D., I. Ichikawa, and B. M. Brenner. 1983. Hormonal modulation of glomerular function. *Am. J. Physiol.* 244:F95–104.

4. Ichikawa, I., and B. M. Brenner. 1984. Glomerular actions of angiotensin II. *Am. J. Med.* 76-5b:43–49.

5. Ichikawa, I. 1982. Direct analysis of the effector mechanism of the tubuloglomerular feedback system. *Am. J. Physiol.* 243:F447–455.

6. Venkatachalam, M. A., and J. I. Kreisberg. 1985. Agonist-induced isotonic contraction of cultured mesangial cells after multiple passage. *Am. J. Physiol.* 249:C48–55.

7. Scharschmidt, L. A., and M. J. Dunn. 1983. Prostaglandin synthesis by rat glomerular mesangial cells in culture: effects of angiotensin II and arginine vasopressin. *J. Clin. Invest.* 71:1756–1764.

8. Kreisberg, J. I., and M. J. Karnovsky. 1983. Glomerular cells in culture. *Kidney Int.* 23:439–447.

9. Ausiello, D. A., J. I. Kreisberg, C. Roy, and M. J. Karnovsky.

1980. Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin. *J. Clin. Invest.* 65:754–760.

10. Ardaillou, N., J. Hagege, M. P. Nivez, R. Ardaillou, and D. Schlondorff. 1985. Vasoconstrictor-evoked prostaglandin synthesis in cultured human mesangial cells. *Am. J. Physiol.* 248:F240–246.

11. Schlondorff, D., J. Perez, and J. A. Satriano. 1985. Differential stimulation of PGE_2 synthesis in mesangial cells by angiotensin and A23187. *Am. J. Physiol.* 248:C119–126.

12. Lovett, D. H., J. L. Ryan, and B. Sterzel. 1983. Stimulation of rat mesangial cell proliferation by macrophage interleukin I. *J. Immunol.* 131:2830–2836.

13. MacCarthy, E. P., A. Hsu, Y. M. Ooi, and B. S. Ooi. 1985. Evidence for a mouse mesangial cell-derived factor that stimulates lymphocyte proliferation. *J. Clin. Invest.* 76:426–430.

14. Maruyama, Y., and O. H. Petersen. 1983. Voltage clamp study of stimulant-evoked currents in mouse pancreatic acinar cells. *Pflugers Arch.* 399:54–62.

15. Barish, M. E. 1983. A transient calcium-dependent chloride current in the immature xenopus oocyte. *J. Physiol.* 342:309–325.

16. Owen, D. G., M. Segal, and J. L. Barker. 1984. A Ca-dependent Cl^- conductance in cultured mouse spinal neurones. *Nature (Lond.).* 311:567–570.

17. Mayer, M. L. 1985. A calcium-activated chloride current generates the after-depolarization of rat sensory neurones in culture. *J. Physiol.* 364:217–239.

18. Takeda, K., J. Kim, and R. Schrier. 1986. Effect of angiotensin II (AII) on Ca kinetics and cell contraction in cultured rat mesangial (Ms) cells. *Kidney Int.* 29:424. (Abstr.)

19. Schnermann, J., D. W. Ploth, and M. Hermle. 1976. Activation of tubulo-glomerular feedback by chloride transport. *Pflugers Arch.* 362: 229–240.

20. Bucher, O., and E. Reale. 1961. Zur elektronenmikroskopischen Untersuchung der juxtaglomerulaeren Spezialeinrichtungen die Niere. *Z. Zellforsch. Mikrosk. Anat.* 54:167–181.

21. Taugner, R., A. Schiller, B. Kaissling, and W. Kriz. 1978. Gap junctional coupling between the juxtaglomerular apparatus and the glomerular tuft. *Cell Tissue Res.* 186:279–285.

22. Schnermann, J., and J. Briggs. 1985. Function of the juxtaglomerular apparatus: local control of glomerular hemodynamics. *In* The Kidney: Physiology and Pathophysiology. D. W. Seldin, and G. Giebisch, editors. Raven Press, New York. 669–697.