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Research Article

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Ambient Cl^- Ions Modify Rat Mesangial Cell Contraction by Modulating Cell Inositol Trisphosphate and Ca^{2+} via Enhanced Prostaglandin E2

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Abstract

Our recent observation showed that angiotensin II (AII) and arginine vasopressin (AVP) stimulate Ca^{2+}-activated Cl^- conductance in mesangial cells. These data raise the possibility that mesangial cell function may be modulated by extracellular chloride concentration ($[Cl^-]_o$). The present study was undertaken to test this possibility using cultured rat mesangial cells. When the $[Cl^-]_o$ was reduced to zero, the percentage of mesangial cells showing contraction responding to AII and AVP was decreased from 72±9 to 33±10% and from 60±4 to 24±11%, respectively. Ca^{2+} transients induced by AII and AVP, measured in mesangial cells loaded with Ca^{2+}-sensitive photoprotein aequorin, were attenuated as $[Cl^-]_o$ decreased. Also, when $[Cl^-]_o$ decreased, inositol trisphosphate (IP3) levels of mesangial cells were suppressed, both in the presence and absence of AII or AVP. PGE2 production by mesangial cells increased when $[Cl^-]_o$ decreased and the effects of ambient Cl^- deprivation could be restored by addition of indomethacin to the Cl^--free medium. Moreover, PGE2 decreased mesangial cell contractility, Ca^{2+} transients, and IP3 production in response to AII and AVP. These data suggest that the decrease in $[Cl^-]_o$ attenuates mesangial cell contraction by suppressing IP3 production and thus Ca^{2+} transients in response to AII and AVP through enhanced PGE2 production.

Introduction

Angiotensin II (AII)[1] and arginine vasopressin (AVP) evoke contraction (1–6) and stimulate PG synthesis (3, 7–9) of mesangial cells. Inositol trisphosphate (IP3) production and Ca^{2+} transients are thought to play an important role in mediating the hormonal effects in mesangial cells (10–13). We have reported that AII and AVP increase Cl^- conductance of the mesangial cell plasma membrane, a response which coincides with cell contraction (1). However, the physiological significance of the Cl^- conductance remains unclear. The presence of Cl^- conductance suggests the possibility that mesangial cell function may be modified by the ambient chloride concentration ($[Cl^-]_o$). Indeed, because of the increased Cl^- conductance, the reversal potential of the depolarizing response induced by these peptides was greatly affected by changes in $[Cl^-]_o$. In the present study, we investigated the effects of changing $[Cl^-]_o$ on physiological characteristics of mesangial cells, i.e., AII- and AVP-induced cell contraction, Ca^{2+} transients, IP3 contents, and PGE2 production.

Methods

Cell culture. Rat mesangial cells were obtained by culturing isolated glomeruli in MEM containing 17% FCS under humidified air containing 5% CO_2 as reported previously (1). In this study, only mesangial cells in the primary culture and the first passage were used since the characteristics of mesangial cells might change after multiple passages (3, 5, 10).

Cell contraction. Mesangial cell contraction was evaluated using a semiquantitative analytical method. In brief, the mesangial cells were partially detached from the floor of the culture dish by incubation at 37°C for 10–15 min in Ca^{2+}-free, phosphate-buffered saline containing 0.54 mM EDTA. They were incubated again in MEM containing 17% FCS at 37°C for 30 min under humidified air containing 5% CO_2. After this procedure, the contour of the mesangial cell became round and smooth and mesangial cells were mostly, but not completely, separated from each other (Fig. 1 *a*) thus allowing the identification of individual mesangial cell contraction easier under the phase-contrast microscope. The medium was then changed to an appropriate experimental solution (see below) containing 8 mg/ml BSA. After 30 min incubation at 37°C, photographs of randomly selected two to three areas, each containing 15–40 cells, were taken before and at 50–70 s after an addition of either AII or AVP (Fig. 1, *a* and *b*). This timing of taking photographs were chosen because the cells contracted within 30 s after the addition of the agent and remained contracted for 2–5 min. Each cell was compared before and after the hormone addition for the changes in both cell contour and cytoplasmic density, morphological changes thought to represent contraction (Fig. 1 *b*) (1, 2, 6). The presence and absence of these unequivocal morphological changes was evaluated without knowledge of the experimental protocols, and the percentage of the cells showing contraction was calculated.

Measurement of intracellular Ca^{2+} concentration. Mesangial cells were loaded with Ca^{2+}-sensitive photoprotein aequorin using our modifications (14) of the method by Morgan and Morgan (15). Aequorin-loaded mesangial cells were divided into several aliquots and each batch of the cells was incubated at 37°C for 20–30 min in 1 ml of an appropriate experimental solution (see below) containing 8 mg/ml BSA. The luminescence signal was monitored using a platelet aggregometer (Chrono-Log Corp., Havertown, PA) in a cuvette under constant stirring. At the end of the experiments, the maximum luminescence of each aliquot was obtained by permeabilizing cells with 20 μM A23187 or 0.1% Triton X. The height of each agonist-induced peak was calibrated for comparison with the respective maximum luminescence thus obtained. The viability of aequorin-loaded cells was ascertained by observing that most of the cells excluded trypan blue and by the presence of AII-induced morphological changes: thus, when 100

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1. *Abbreviations used in this paper:* AII, angiotensin II; IP3, inositol trisphosphate; $[Ca^{2+}]_i$, intracellular calcium concentration; $[Cl^-]_o$, extracellular chloride concentration; TGF, tubuloglomerular feedback.

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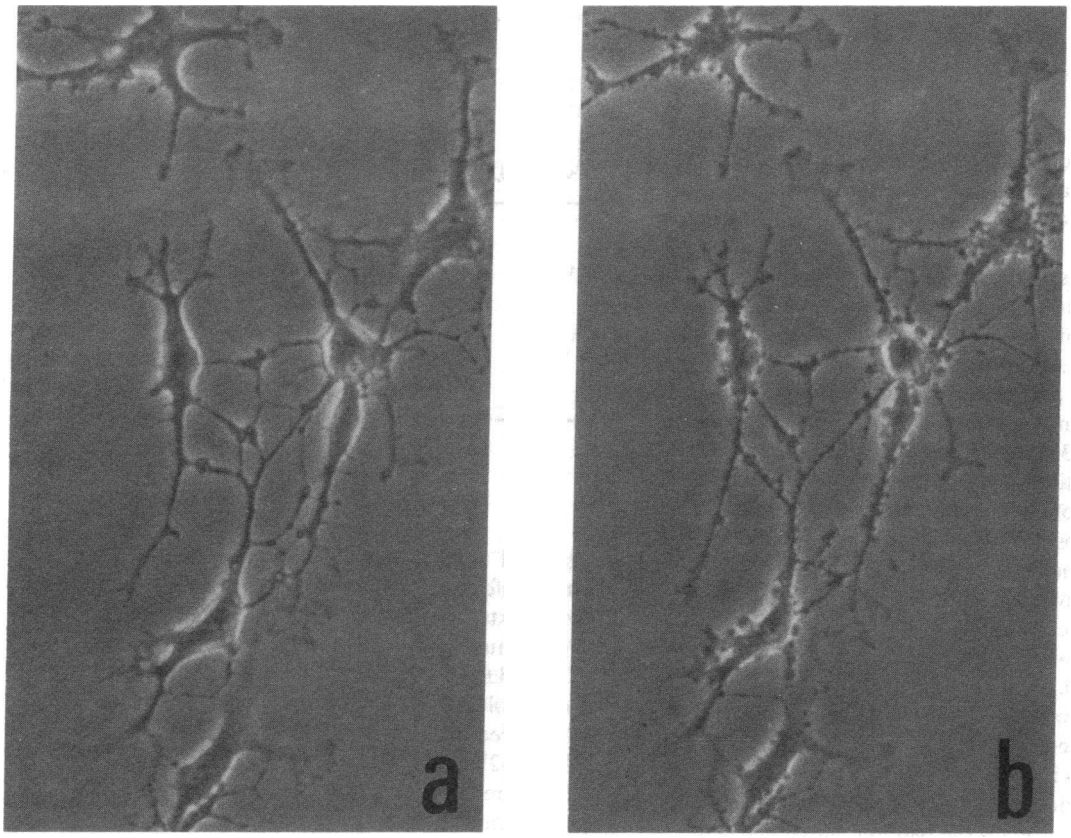

Figure 1. Mesangial cell contraction in response to vasoactive peptides. Mesangial cells, after undergoing the pretreatment described in the text, have the morphological appearances shown in *a*. In this particular field of the photograph, seven cells are presented. Within ~ 1 min after AII addition to the incubation media at a final concentration of 100 nM, all the cells in this field responded unequivocally with changes in cell contours and appearance of cytoplasmic densities (*b*). These changes are specific to AII or AVP since the addition of vehicle causes similar morphological changes only in < 5% of cells.

nM AII was added to the aequorin-loaded mesangial cell suspension, in which almost all the suspended cells had a smooth round contour, the cell contour became rough, a quite similar shape change with that observed in cells partially attached to the floor of the culture dish as shown in Fig. 1. In some experiments, aequorin-loaded cells were further incubated for ~ 12 h with cytodex glass beads. In this condition, viable cells attached onto the glass beads within several hours. Intracellular calcium concentration $[Ca^{2+}]_i$ was examined using the mesangial cells attached on cytodex, thus allowing for the experiments done with the cells under more physiological conditions.

Measurement of IP3 contents. The contents of IP3 of mesangial cells were measured by the conventional method reported previously (14, 16). In brief, mesangial cells grown on 35-mm culture dishes were incubated for 24 h in 1 ml of MEM containing 10 μCi/ml myo-[2-^{3}H(N)]-inositol (New England Nuclear, Boston, MA). Then the medium was changed to 1 ml of appropriate experimental solutions (see below) with or without BSA, and the cells were incubated for 30 min at 37°C. The vehicle (a final concentration of 1 μM acetic acid) or a test agent (a final concentration of 100 nM AII or AVP) was added. After 20 s, the reaction was stopped by addition of perchloric acid at a final concentration of 10% (vol/vol): 20 s incubation was chosen because it is reported that at this time-point, IP3 contents increased significantly in response to AII or AVP and did not increase very much thereafter (11, 13). Cells were scraped and homogenized by aspirating through 27-gauge needles and the homogenate was neutralized by 5 M KOH. Fractions of inositol metabolites were separated by anion exchange column chromatography (Dowex 1 − X8; 5 × 15 mm) according to the method of Berridge et al. (16).

Measurement of PGE2 production. PGE2 production by mesangial cells was determined by measuring the content of PGE2 released into the incubation media (3, 7, 8). Mesangial cells were grown in 35 mm-culture dishes. Just before the experiments, the media were aspirated and the mesangial cells were washed twice with an appropriate experimental solution (see below). Then 1 ml of experimental solution prewarmed to 37°C was added and mesangial cells were incubated at 37°C. After 15 and 30 min of incubation, 5–15-μl aliquots of the incubating media were aspirated for PGE2 determination by radioimmunoassay using a kit from New England Nuclear. At the end of incubation, the incubation medium was aspirated and the cells were lysed with 1 ml of 1 N NaOH and aliquots were assayed for protein content using a protein assay kit (Bio-Rad Laboratories, Richmond, CA). PGE2 production was expressed as ng/mg protein.

Experimental solution. The composition of the standard experimental solution was (in millimolar): Na 150, K 6, Ca 1.2, Mg 1.0, Hepes 20, Cl 140, and glucose 5.5; pH 7.4. When the Cl^- concentration was reduced, Cl^- was isoosmotically replaced by methanesulfonate, if not otherwise specified. When the effect of PGE2 was examined, ethanol, the vehicle of PGE2, was added to each experimental solution at the final concentration of 0.05%.

Statistics. The data are presented as mean±SE. Almost all the experiments were done in a paired manner, and paired *t* analysis was used for statistical analysis unless specified otherwise.

Results

Effects of extracellular chloride ion concentration

Cell contraction. Using a semiquantitative analytical method as described in Methods, the effect of $[Cl^-]_o$ on mesangial cell contraction was examined. This rough estimation of mesangial cell contraction seems to be valid because: (*a*) there exists a strong correlation ($r = 0.84$: $n = 30$) not different from the line of identity ($P > 0.1$: *t* analysis) between a pair of data taken from two different areas randomly selected in the same culture dish under a variety of experimental conditions, indicating the reproducibility of counting the number of cells contracted in the same culture dish; (*b*) the percentage of cells in different

cultures demonstrating contraction was 64±8 (n = 11: three cultures) and 67±9% (n = 12: three cultures) in response to 100 nM AII and AVP, respectively, with the coefficient of variation of 20–24%; (*c*) the percentage of the cells showing shape changes was dependent upon the concentration of AII; (*d*) when some sets of photographs were analyzed by two different investigators, there was a strong correlation between the data thus obtained independently (r = 0.93: $P < 0.05$: t analysis).

Fig. 2 shows the effects of $[Cl^-]_o$ on the mesangial cell contraction in response to 100 nM AII and AVP. In this series of experiments, 72±9% of the mesangial cells contracted within 1 min in response to AII in the standard solution ($[Cl^-]_o$ = 140 mM). However, the percentage of mesangial cells contracting in response to 100 nM AII was decreased as $[Cl^-]_o$ was reduced, thus 60±12 and 33±10% cells contracted when $[Cl^-]_o$ was 70 and 0 mM, respectively (Fig. 2, *left*). In these experiments, $[Cl^-]_o$ was isoosmotically replaced by methanesulfonate. When $[Cl^-]_o$ was isoosmotically replaced by propionate, only 42±10% of cell contracted in response to 100 nM AII ($P < 0.05$ vs. mesangial cell contraction in the standard solution: n = 4). The mesangial cell contractility in response to 100 nM AVP was also attenuated when $[Cl^-]_o$ was replaced by methane-sulfonate (Fig. 2, *right*). The appearance of mesangial cells observed under phase contrast microscopy remained unchanged when $[Cl^-]_o$ was decreased using superfusion methods (1), indicating that changes in AII- and AVP-evoked mesangial contractility induced by a decrease in $[Cl^-]_o$ may not be due to the effects of a low $[Cl^-]_o$ per se on the baseline mesangial cell morphology before adding the agents.

Calcium transient. Fig. 3 *a* compares the effect of $[Cl^-]_o$ on the Ca^{2+} transients in response to 100 nM AVP. As is clearly shown, the height of AVP-induced peak was attenuated when $[Cl^-]_o$ was decreased. With other experiments included, the heights of AII- and AVP-induced peaks obtained in Cl^--free solution were suppressed to 58±12% (n = 3) and 57±9% (n = 6) of those in the standard solution, respectively ($P < 0.05$). In contrast, the $[Ca^{2+}]_i$ change induced by the cell membrane permeabilization with Ca ionophore, A23187, was almost identical in the presence and absence of extracellular Cl^- (Fig. 3 *b*). Moreover, essentially similar results were obtained when the mesangial cells attached on cytodex were used. Fig. 4, *a* and *b* shows AVP-induced Ca^{2+} transient of cytodex-attached mesangial cells was also attenuated by extracellular Cl^- deprivation.

IP3 contents. As shown in Fig. 5, IP3 contents in the presence of vehicle or test agents, 100 nM AII or AVP, both decreased when mesangial cells were incubated in the absence of extracellular Cl^- (Cl^- was isoosmotically replaced by methanesulfonate). Moreover, IP3 contents were also reduced when extracellular Cl^- was isoosmotically replaced by propionate, thus while IP3 contents were increased from 438±95 to 493±104 dpm/dish in response to 100 nM AII in the standard solution ($P < 0.05$: n = 3), there was no significant AII-induced increase in IP3 contents in Cl^--free solution (405±88 and 427±98 dpm/dish in the absence and presence of 100 nM AII, respectively: n = 3). In these series of experiments, IP3 contents in the presence of 100 nM AII was also significantly reduced when extracellular Cl^- was replaced by propionate ($P < 0.05$: n = 3). However, reduction of $[Cl^-]_o$ to 70 mM failed to induce decrease in IP3 contents both in the absence and presence of 100 nM AII (data not shown).

PGE2 production. PGE2 synthesis by mesangial cells increased as $[Cl^-]_o$ was decreased (Fig. 6). Enhancement of PGE2 synthesis by decreased $[Cl^-]_o$ is not due to methanesulfonate, an anion used to replace Cl^-, since PGE2 production was also increased when propionate was used to replace Cl^-: thus, PGE2 production increased from 4.6±0.6 to 7.9±0.7 ng/mg protein per 30 min when $[Cl^-]_o$ was decreased from 140 to 0 mM with propionate replacement (mean±SE of six incubations from two independent cultures: $P < 0.05$, nonpaired t test analysis).

Effects of indomethacin. That extracellular Cl^- deprivation was associated with an increased production of PGE2 suggests

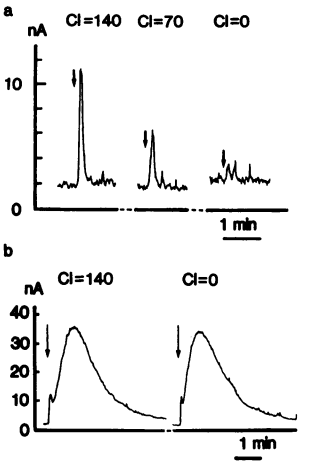

Figure 3. (*a*) The effect of $[Cl^-]_o$ on the AVP-induced Ca^{2+} transients. The cells were incubated for 30 min before the addition of 100 nM AVP (*arrow*). AVP-induced Ca^{2+} transients became smaller as $[Cl^-]_o$ was reduced from 140 to 70 and 0 mM. Separate aliquots of the same preparation of the aequorin-loaded mesangial cells were used in each experiment. (*b*) The $[Ca^{2+}]_i$ response to Ca^{2+} ionophore (20 μM A23187) was unaffected by $[Cl^-]_o$.

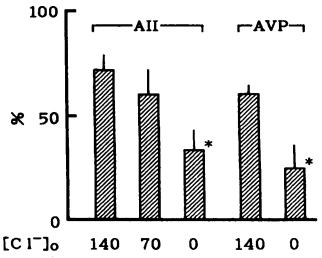

Figure 2. The effect of $[Cl^-]_o$ on mesangial cell contraction in response to AII and AVP. The data represent the mean±SE of four independent experiments examining the effect of $[Cl^-]_o$ on mesangial cell contraction in response to 100 nM AII (*left*) or AVP (*right*). *, $P < 0.05$ vs. the value obtained in the standard solution ($[Cl^-]_o$ = 140 mM).

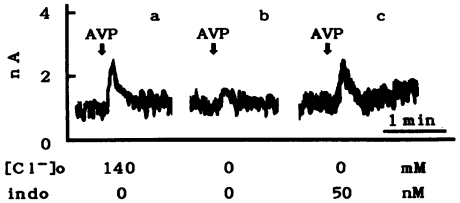

Figure 4. The effect of $[Cl^-]_o$ and indomethacin on the AVP-induced Ca^{2+} transients in mesangial cells attached on cytodex. 100 nM AVP was added at arrows. AVP-induced Ca^{2+} transient in the standard solution ($[Cl^-]_o$ = 140 mM; *a*) was attenuated in Cl^--free solution (Cl^- was isoosmotically replaced by methanesulfonate; *b*), and was restored by adding 50 nM indomethacin to Cl^--free solution (*c*). For details, see the text and the figure legend of Fig. 3.

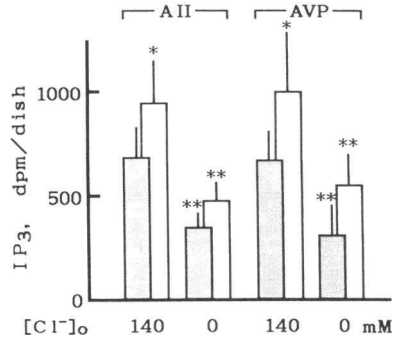

Figure 5. The effect of $[Cl^-]_o$ on IP3 contents of mesangial cells in the absence (*stippled bars*) and presence (*open bars*) of AII or AVP. Data are the means±SE of six independent experiments, in which incubations were run in duplicate. *, $P < 0.05$ vs. the value in the presence of vehicle; **, $P < 0.05$ vs. the value in the standard solution ($[Cl^-]_o$ = 140 mM).

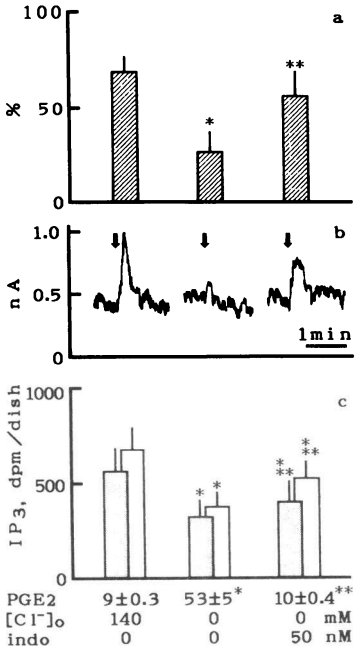

Figure 7. The effect of indomethacin on the suppression of mesangial cell responses to AII by ambient Cl^- deprivation. (*a*) The percentage of mesangial cells showing unequivocal contraction in response to 100 nM AII in the standard solution (*left*), Cl^--free solution (*center*), and Cl^--free solution containing 50 nM indomethacin (*right*). (*b*) Ca^{2+} transients in response to 100 nM AII (*arrows*) in the standard solution (*left*), Cl^--free solution (*center*), and Cl^--free solution containing 50 nM indomethacin (*right*). (*c*) IP3 contents of mesangial cells in the absence (*stippled bars*) and presence (*open bars*) of 100 nM AII incubated in the standard solution (*left*), Cl^--free solution (*center*), and Cl^--free solution containing 50 nM indomethacin (*right*). The data are the means±SE of four separate experiments, in which incubations were run in duplicate. At the bottom, PGE2 production in the corresponding experimental solution is presented (ng/mg protein per 30 min). The data are the mean±SE of four measurements. PGE2 measurements were repeated and essentially similar results were obtained in two other series of experiments. *, $P < 0.05$ vs. each standard value; **, $P < 0.05$ vs. the corresponding value in the Cl^--free solution.

that the modulation of mesangial cell function by extracellular Cl^- deprivation may be mediated by increased PGE2 production. To further evaluate the role of PG production in the effects of extracellular Cl^- deprivation, we examined the effect of cyclooxygenase inhibitor, indomethacin, on mesangial cell contraction. Indomethacin was added at a concentration of 50 nM to the Cl^--free experimental solution. This concentration of indomethacin was chosen because it is calculated to prevent an increase in PGE2 production brought about by extracellular Cl^- deprivation. Indeed, 50 nM indomethacin abolished the increase in PGE2 production induced by extracellular Cl^- deprivation (Fig. 7). Fig. 7, *a–c* clearly indicates that attenuation of AII-induced cell contraction (Fig. 7 *a*), suppression of AII-induced Ca^{2+} transient (Fig. 7 *b*), and decrease in IP3 contents both in the presence and absence of 100 nM AII (Fig. 7 *c*) are all recovered by the addition of indomethacin. Furthermore, Fig. 4 *c* shows that attenuation of AVP-induced Ca^{2+} transient could be recovered in mesangial cells attached on cytodex by adding 50 nM indomethacin to Cl^--free solution. The decrease in AVP-induced contractility and IP3 production caused by ambient Cl^- deprivation were also abolished by indomethacin (data not shown).

Effects of PGE2. We next examined the effects of PGE2, a major prostanoid produced by rat mesangial cells. As shown in Fig. 8 *a*, PGE2 decreased, in a dose-dependent manner, the number of mesangial cells showing unequivocal contraction in response to 100 nM AII. AII-induced Ca^{2+} transient was suppressed by 5 μM PGE2 (Fig. 8 *b*). Moreover, Fig. 9 shows that IP3 production was also attenuated by PGE2. IP3 contents of mesangial cells treated with PGE2 were decreased in the absence of 100 nM AII, similar to the experiments of extracellular Cl^- removal, which may imply nonspecific reduction of inositol metabolites. However, the effects of PGE2 and $[Cl^-]_o$ seem to be specific to IP3 since other inositol metabolites were not affected by PGE2 or extracellular Cl^- removal. For example, in the control group, glycerophosphoinositol content was increased by 100 nM AII from 1,170±341 to 1,466±453 dpm/dish, while glycerophosphoinositol increased from 1,130±251 to 1,431±365 dpm/dish by AII in PGE2-treated

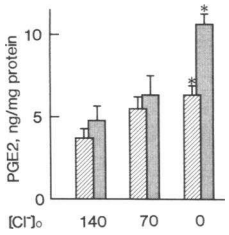

Figure 6. The effect of $[Cl^-]_o$ on PGE2 production by mesangial cells. Mesangial cells were incubated for 15 (hatched bars) and 30 min (*stippled bars*) with varying $[Cl^-]_o$. The data are means±SE of three separate experiments, each representing a mean of duplicate or triplicate incubations. *, $P < 0.05$ vs. the value in the standard solution ($[Cl^-]_o$ = 140 mM).

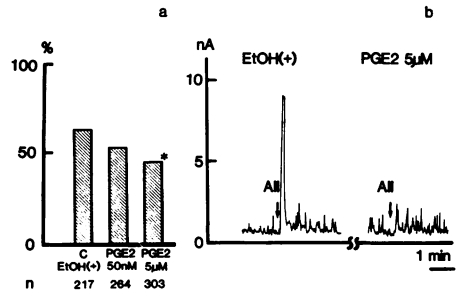

Figure 8. The effects of PGE2 on mesangial cells. (*a*) The effect of PGE2 on AII-induced mesangial cell contraction. The numbers at the bottom of the bars indicate the number of cells examined. EtOH: ethanol. *, $P < 0.05$ vs. the value in the absence of PGE2 (chi-square analysis). (*b*) The effect of PGE2 on AII-induced Ca^{2+} transients. The left and right panels show the Ca^{2+} transients in response to 100 nM AII (*arrows*) in the presence of 0.05% ethanol (vehicle of PGE2) or 5 μM PGE2, respectively. The experiments were repeated at least two times and essentially similar results were obtained.

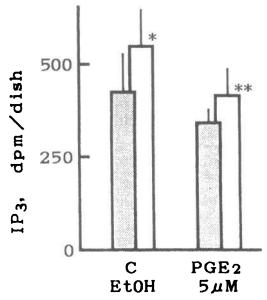

Figure 9. The effects of PGE2 on IP3 metabolism. IP3 contents in the absence (*stippled bars*) and presence (*open bars*) of 100 nM AII are shown. *, $P < 0.05$ vs. the standard value in the absence of AII and PGE2; **, $P < 0.05$ vs. the value in the presence of 100 nM AII in the standard solution containing 0.05% ethanol, vehicle of PGE2.

group ($P > 0.05$ vs. control group: $n = 4$). PGE2 similarly attenuated the mesangial cell contraction in response to 100 nM AVP: 64% cells (126/198) responded in the control solution and 44% cells (89/202) in the presence of 5 μM PGE2 ($P < 0.05$: chi-square analysis), and AVP-induced Ca^{2+} transient was suppressed by 5 μM PGE2 (data not shown).

Discussion

It has been suggested that IP3 production and Ca^{2+} transients play an important role in mediating the effects of AII and AVP in mesangial cell functions, including cell contraction and PGE2 production (1, 2, 7, 8). Several reports have documented increases in IP3 contents of mesangial cells (11, 12) and Ca^{2+} transients by AII and AVP measured using Ca^{2+} indicators such as quin-2, fura-2, aequorin (10–12, 17, 18). The present study shows that extracellular Cl^- deprivation attenuates mesangial cell contraction in response to AII and AVP. To elucidate the cellular mechanisms underlying the modulation of mesangial cell contraction by changing $[Cl^-]_o$, we examined the effects of $[Cl^-]_o$ on mesangial cell IP3 contents and Ca^{2+} transients in response to AII and AVP. Our results showed Ca^{2+} transients by AVP and AII were attenuated as the $[Cl^-]_o$ decreased. In addition, increases in IP3 contents of mesangial cells in response to AII and AVP were suppressed when $[Cl^-]_o$ decreased. Since it is thought that a rise in $[Ca^{2+}]_i$ is primarily brought about by the release of Ca^{2+} from intracellular stores triggered by IP3 (10, 12), these observations suggest that Ca^{2+} transients, in response to AII and AVP, thus the mesangial cell contraction, are subject to specific modification by $[Cl^-]_o$ via altered IP3 metabolism.

It has been reported that PGE2 may attenuate mesangial cell contraction (2, 5, 7, 19). Indeed, addition of PGE2 to the standard experimental solution containing 140 mM $[Cl^-]_o$ decreased mesangial cell contraction in response to AII and AVP. Furthermore, our present study demonstrated that the Ca^{2+} transient and IP3 production in response to AII were both suppressed in the presence of PGE2. These effects of PGE2 on mesangial cells resembled those of extracellular Cl^- deprivation. Therefore, we next tested the possibility that $[Cl^-]_o$ might modify the mesangial cell contraction by changing PGE2 production of the mesangial cells. Indeed, our data show that PGE2 synthesis by mesangial cells increased as $[Cl^-]_o$ was reduced. Enhancement of PGE2 synthesis by decreased $[Cl^-]_o$ is not due to methanesulfonate, an anion used to replace Cl^-, since PGE2 production was equally increased when propionate was used to replace Cl^-. The magnitude of enhanced PGE2 production by extracellular Cl^- deprivation does not seem to be large enough to increase the PGE2 concentration of whole incubation media up to 5 μM, the effective PGE2 concentration added exogenously. However, local concentrations of endogenous PGE2 might increase to a level high enough to exert the observed effects on mesangial cells.

Our postulate on an intermediary role of PGE2 in the effects of extracellular Cl^- deprivation is further strengthened by the experiments with indomethacin. Thus, indomethacin, at a concentration that prevented an increase in PGE2 production by ambient Cl^- deprivation, could restore at least partially the suppression of the AII- and AVP-induced cell contraction, Ca^{2+} transients, and IP3 contents (Figs. 4 and 7). However, addition of 50 nM indomethacin to the standard solution containing 140 mM Cl^- attenuated mesangial cell contraction (89±1.4% vs. 62.3±5.8%: $P < 0.05$, $n = 3$). Since indomethacin is known to decrease thromboxane production and since thromboxane is also known to contract mesangial cells (20), it might be possible that indomethacin itself decreases thromboxane production, thus decreasing mesangial cell contractility. This might explain the incomplete recovery by indomethacin from the attenuation by the extracellular Cl^- deprivation of mesangial cell contraction, Ca^{2+} transient, and IP3 production by AII. Besides, since indomethacin alters the metabolism of other prostanoids produced by mesangial cells, e.g., prostaglandin F2α, and may affect cell function per se, effects of indomethacin other than PGE2 production might explain the attenuated mesangial cell contraction by the drug in the present study. However, examinations of the effects of indomethacin per se and of thromboxane or other prostanoids on mesangial cell functions are beyond the aim of the present study.

Based on these observations, it is strongly suggested that when $[Cl^-]_o$ was decreased, the mesangial cell contraction was reduced through enhanced PGE2 production: Enhanced PGE2 production modifies the IP3 metabolism, thus affecting the Ca^{2+} transients induced by AII and AVP. In our data, the attenuation of Ca^{2+} transients by PGE2 or $[Cl^-]_o$ reduction seems inappropriately large compared with attenuation of cell contraction or IP3 content. These discrepant observations may be due, at least in part, to the nonlinear relationship between aequorin luminescence and Ca^{2+}; i.e., at relatively low Ca^{2+} concentrations (several hundred nanomolar or less) the sensitivity of aequorin is low and does not reflect changes in Ca^{2+} very well (21). Thus, even without a clear-cut increase in aequorin luminescence, an actual $[Ca^{2+}]_i$ increment may be sufficient to evoke contraction.

It is not clear at present how $[Cl^-]_o$ affects PGE2 production. The effect of $[Cl^-]_o$ may be through changes in the intracellular $[Cl^-]$ or in cell pH, as there are, in addition to Cl^- conductance, Na-dependent and Na-independent $Cl^-/HCO3^-$ exchangers in mesangial cells (22). Mechanisms other than enhanced PGE2 production may also be involved in the modification of mesangial cell function by $[Cl^-]_o$. Because Cl^- modulates the function of guanine nucleotide regulatory G proteins (23), it is possible that changes in $[Cl^-]_o$ may affect G proteins that play a critical role in the signal transduction of the AII and AVP action in mesangial cells (11). It is of note that $[Cl^-]_o$ may modify the functions of several other cell types, including smooth muscle cells (24), cells similar in many respects to mesangial cells, and pancreas acinar cells (25), cells with Cl^- conductance in plasma membranes (26). In any event, although the exact mechanisms by which a change in $[Cl^-]_o$ affects signal transduction system and prostanoid me-

tabolism are not clear, the present study clearly suggests that $[Cl^-]_o$ may be an important modulator of mesangial cell response to the vasoactive peptides.

The modification of mesangial cell contraction by $[Cl^-]_o$ may be of physiological importance. It has been suggested that mesangial cell contraction may act, at least in part, as an effector mechanism of the tubuloglomerular feedback (TGF), i.e., the regulation of glomerular filtration by distal tubule fluid via macula densa (27, 28), and that a change in the luminal $[Cl^-]$ at macula densa signals the TGF (27, 29). Between macula densa and the glomerulus, there lie Goormaghtigh cells or lacis cells, which are interstitial cells morphologically indistinguishable from mesangial cells and thus are called extraglomerular mesangial cells (30). Goormaghtigh cells, mesangial cells, and juxtaglomerular cells are coupled with each other by gap junctions, thus forming a syncytium (31). The area surrounding Goormaghtigh cells are neither well-perfused with capillaries nor drained with lymph vessels (27, 30). Therefore, it is highly possible that $[Cl^-]_o$ of these cells may be directly altered by a change in $[Cl^-]$ of the absorbate of macula densa, which may reflect $[Cl^-]$ in the tubule fluid perfusing macula densa (32). Thus a change in $[Cl^-]$ in the tubule fluid might directly affect mesangial cell function by changing $[Cl^-]_o$ surrounding Goormaghtigh cells, since these cells are coupled by gap junctions. Considering the close link between $[Cl^-]$ in the tubule fluid and mesangial cells, the reduced contraction of the mesangial cells by low $[Cl^-]_o$ could well explain the observation that the TGF may be attenuated when the tubular fluid $[Cl^-]$ at macula densa was reduced to zero (27, 29). Although these possibilities are only speculative, the presence of Ca^{2+}-activated Cl^- conductance in mesangial cells lends support to the notion that the ionic mechanisms may play a critical role in TGF.

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