CALL FOR PAPERS | Cardiovascular-Kidney Interactions in Health and Disease

Posttranscriptional mechanisms contribute to osmotic regulation of ANG type 1 receptors in cultured rat renomedullary interstitial cells

Sunghou Lee, Zheng Wu, Kathryn Sandberg, S-E. Yoo, and Christine Marie

Posttranscriptional mechanisms contribute to osmotic regulation of ANG type II receptors in cultured rat renomedullary interstitial cells. Am J Physiol Regul Integr Comp Physiol 290: R44–R49, 2006. First published August 11, 2005; doi:10.1152/ajpregu.00476.2005.—Previously, we showed that ANG II receptors in cultured rat renomedullary interstitial cells (RMICs) are osmotically regulated (19). The current study examined the mechanisms underlying this osmotic regulation in RMICs cultured in isosmotnic (300 mosmol/\(kg\)\(H_2O\)) and hyperosmotic (600 mosmol/\(kg\)\(H_2O\)) conditions. Radioligand competition analysis coupled with RNase protection assays (RPA) and ligand-mediated receptor internalization studies revealed that RMICs primarily express the type I angiotensin receptor (AT\(_{1a}\)R). When cultured under hyperosmotic conditions, the density (\(B_{max}\)) of AT\(_{1a}\)R in RMIC membranes decreased by 31% [\(B_{max}\) (pmol/mg protein): 300 mosmol/\(kg\)\(H_2O\), 6.44 ± 0.46 vs. 600 mosmol/\(kg\)\(H_2O\), 4.42 ± 0.37, \(n = 8, P < 0.01\)], under conditions in which no detectable changes in AT\(_{1a}\)R mRNA expression or in the kinetics of ligand-mediated AT\(_{1a}\)R internalization were observed. RNA electromobility shift assays showed that DNA protein complex (RPC) formation between RMIC cytosolic RNA binding proteins and the 5′ leader sequence (5′LS) of the AT\(_{1a}\)R was increased 1.5-fold under hyperosmotic conditions [5′LS RPC (arbitrary units): 300 mosmol/\(kg\)\(H_2O\), 0.79 ± 0.08 vs. 600 mosmol/\(kg\)\(H_2O\), 1.17 ± 0.07, \(n = 4, P < 0.01\)]. These results suggest that the downregulation of AT\(_{1a}\)R expression in RMICs cultured under hyperosmotic conditions is regulated at the posttranscriptional level by RNA binding proteins that interact within the 5′LS of the AT\(_{1a}\)R mRNA. The downregulation of AT\(_{1a}\)R expression under hyperosmotic conditions may be an important mechanism by which the activity of ANG II is regulated in the hyperosmotic renal medulla.

angiotensin II; angiotensin receptors; osmolality; posttranscriptional regulation; RNA binding proteins

THE RENIN-ANGIOTENSIN SYSTEM (RAS) plays an important role in regulating systemic blood pressure and fluid and electrolyte balance (4, 21). Although more than one peptide has thus far been reported to mediate the physiological action of the RAS in the kidney (1), still to date, the main active peptide of the RAS is ANG II. ANG II mediates its actions through a number of receptor subtypes; however, the majority of its actions are mediated through the AT\(_1\) receptor subtype (AT\(_1\)R). In the kidney, AT\(_1\)Rs are widely distributed: they are localized to cortical mesangial cells and proximal tubules (11) and renomedullary interstitial cells (RMICs) (35). Given the localization of AT\(_1\)Rs in both the renal cortex and medulla, we do not commonly think that the mechanisms that regulate their expression and activity may be different. As a result of functional urine-concentrating mechanisms, renal medullary cells are normally exposed to very high extracellular concentrations of NaCl (6). It is thus conceivable that these constant fluctuations in extracellular osmolality will affect the activity of ANG II and will contribute to the regulation of its cell surface receptor. To date, very little is known about the osmotic regulation of AT\(_1\)Rs in the renal medulla.

Because of the technical difficulties in examining the direct physiological role of cells residing in the renal inner medulla, including RMICs, our knowledge in this regard is rather limited. Thus far, RMICs have been proposed to play an important role in regulating renal medullary structure and function under both physiological and pathophysiological conditions (17, 18, 23). RMICs express receptors for a number of vasoactive peptides, including ANG II (AT\(_1\)) (36), atrial natriuretic peptide (ANP: NPR\(_A\) and NPR\(_B\)) (8), endothelin (ET-1: ETA and ETB) (32), and bradykinin (5). The fact that RMICs express these receptors suggests that their role in the renal medulla is not merely to provide structural support to medullary nephron segments and collecting ducts but may well play an active physiological role in regulating renal medullary function.

We have previously reported that AT\(_1\)Rs in cultured RMICs are sensitive to changes in extracellular osmolality (19). When cultured under hyperosmotic conditions, binding of ANG II to AT\(_1\)Rs is reduced compared with isosmotic conditions. However, the underlying mechanisms for the osmotic sensitivity and regulation of AT\(_1\)Rs are not as yet understood. The current study investigated the molecular mechanisms underlying the osmotic regulation of AT\(_1\)Rs in cultured RMICs.

METHODS

Materials. \(^{125}\)I-laee [\(\text{Sar}^1, \text{lle}^8\)]ANG II (2,176 Ci/mmol) was purchased from Peptide Radioiodination Center (Pullman, WA). ANG II (human) and [\(\text{Sar}^1, \text{val}^3, \text{ala}^5\)]ANG II (saralasin) were purchased from Sigma (St. Louis, MO), and PDI23319 was purchased from Research Biochemical International (Natick, MA). The nonpeptide AT\(_1\)R antagonist SK1080 was obtained from the Korea Research Institute of Chemical Technology, Taejon, South Korea.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Culture of RMICs. RMICs were isolated from the kidneys of Sprague-Dawley rats and characterized according to the method described previously (18). The cells were maintained at 37°C in a humidified atmosphere of 95% air-5% CO₂ in isosmotic (300 mosmol/kgH₂O) culture medium RPMI 1640 (Biofluids, Rockville, MD) containing 25 mM HEPES and 0.27 U/ml insulin. Cells between passages 10 and 25 were used in all experiments. Cytosol viability was assessed by determining levels of lactate dehydrogenase released into the media (Roche Molecular Biochemicals, Indianapolis, IN).

Manipulation of culture media osmolality. Cells were plated at a density of 1 × 10⁵ cells/cm² in culture medium RPMI 1640 (300 mosmol/kgH₂O) and allowed to attach for 24 h. The osmolality of the media was then either kept at 300 mosmol/kgH₂O or increased to 600 mosmol/kgH₂O by addition of NaCl (80 mM) and mannitol (100 mM). Our preliminary studies determined that using either NaCl or mannitol alone as an osmotic stimulus resulted in similar cell responses (e.g., ANG II binding to AT₁ receptors) compared with the combination of NaCl and mannitol (unpublished observations). We chose to use the combined NaCl and mannitol because cell viability was better preserved using this combination stimulus. The osmolality of the culture media was verified by the freezing point depression method using a Micro-Osmometer (Advanced Instruments, Norwood, MA). After changes in media osmolality, the cells were grown for 24 h; then they were harvested or further analysis was performed, as described below.

Preparation of membrane fractions for radiolabeled ligand binding. The cells were first rinsed with phosphate-buffered saline (PBS) at room temperature. All further steps were performed on ice at 4°C. The cells were scraped, washed with phosphate-buffered saline, pelleted, and homogenized in membrane homogenization buffer [10 mM Tris (pH 7.2), containing 3 mM MgCl₂] by a Micro-Osmometer. The osmolality of the culture media was verified by the freezing point depression method using a Micro-Osmometer (Advanced Instruments, Norwood, MA). After changes in media osmolality, the cells were grown for 24 h; then they were harvested or further analysis was performed, as described below.

Preparation of membrane fractions for radiolabeled ligand binding. The cells were first rinsed with phosphate-buffered saline (PBS) at room temperature. All further steps were performed on ice at 4°C. The cells were scraped, washed with phosphate-buffered saline, pelleted, and homogenized in membrane homogenization buffer [10 mM Tris buffer (pH 7.2), containing 0.32 M sucrose, 2 mM EDTA, and 3 mM MgCl₂]. The homogenate was centrifuged at 1,000 g for 5 min, and the resulting supernatant was further centrifuged at 44,000 g for 65 min. After the final pellicel was resuspended in membrane binding buffer [10 mM Tris (pH 7.2) containing 3 mM MgCl₂], the protein content was determined using the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA).

Radiolabeled ligand binding. For the equilibrium binding analysis, membrane fractions (5 μg) were incubated with increasing concentrations of ¹²⁵I-labeled [Sar¹,Ile⁸]ANG II (0.05–4 nM) in membrane binding buffer containing 0.2% BSA for 1 h at room temperature. Specific binding of ¹²⁵I-labeled [Sar¹,Ile⁸]ANG II was defined as the total binding minus nonspecific binding. Nonspecific binding was determined experimentally in the presence of 1-²⁵I-labeled [Sar¹,Ile⁸]ANG II.

Receptor-ligand internalization assay. Subconfluent RMICs were incubated in isosmotic (300 mosmol/kgH₂O) or hyperosmotic (600 mosmol/kgH₂O) media for 24 h. The cells were then collected and plated at 2 × 10⁵ cells/well in a 24-well plate and allowed to attach for 3 h. The receptor-ligand internalization assay was then carried out as previously described (7). The cells were washed twice with serum-free DMEM, and the cells were incubated in cell binding buffer [50 mM Tris (pH 7.8), 120 mM NaCl, 4 mM KCl, 5 mM MgCl₂, 2 mM CaCl₂, 2 mg/ml β-glucose and 10 μg/ml bacitracin] with 0.5 nM of ¹²⁵I-labeled [Sar¹,Ile⁸]ANG II for 5–40 min at 37°C followed by rapid chilling on ice. At the indicated times, the cells were washed four times with cell binding buffer and incubated for 4 min with or without acetic acid solution [5 mM ice cold acetic acid in 150 mM NaCl, pH 2.5]. The samples were then solubilized with 1% SDS, 1% Triton X-100 in 10 mM Tris-HCl, pH 8.0, and radioactivity was counted in a Packard Cobra II γ-counter. After subtraction of nonspecific binding, results were expressed as the ratio [AT₁]i/[AT₁]o, based on [AT₁]i = [AT₁]i + [AT₁]o. Specific binding was determined experimentally in the presence of 1-μM unlabeled saralasin. In competition experiments, membrane fractions were incubated with increasing concentrations of specific receptor antagonists in membrane binding buffer containing 0.2% BSA and 0.3–0.5 nM ¹²⁵I-labeled [Sar¹,Ile⁸]ANG II.

Binding reactions were terminated by the addition of ice-cold membrane binding buffer and rapid filtration through glass fiber filters (GF/C Whatman, presoaked with assay buffer) using a Brandel cell harvester (Brandel M-24R, Rockville, MD). The filters were washed with ice-cold membrane binding buffer, and membrane-bound radioactivity that was trapped on the filters was measured in a Packard Cobra II γ-counter. The dissociation constant (Kd) and the maximum number of receptor-specific binding sites (Bmax) were calculated from nonlinear regression analysis using a software program PRISM (GraphPad Software, San Diego, CA). The inhibition constants (KI) for angiotensin receptor subtype-specific antagonists were determined by calculating the half-maximum inhibitory dose derived from nonlinear curve fitting.

Ribonuclease protection assay. The cells were washed with PBS at room temperature, and total RNA was isolated using Tri reagent (Molecular Research Center, Cincinnati, OH). AT₁R mRNA in RMICs was measured by ribonuclease protection assay (RPA) using RPA III (Ambion, Austin, TX) according to the manufacturer’s protocol. The specific AT₁R cRNA probe (20,000 cpm), which produces a protected fragment of 95 bases, was hybridized with 10 μg RMIC total RNA at 42°C for 16 h, as described previously. The pTRI-actin (mouse antisense control template; Ambion, Austin, TX) was used to make a 304-base cRNA probe, which produces a protected fragment of 245 bases with T7 RNA polymerase and γ-[³²P]CTP by in vitro transcription. RNase digestion with RNase T₁ was carried out at 37°C for 30 min. After the precipitation of protected fragments, the samples were separated by electrophoresis on a denaturing 5% polyacrylamide gel. After electrophoresis, the gel was transferred to filter paper, dried, exposed to Phosphor imager screen, and the total abundance of AT₁R mRNA was quantitated by Image Quant software (IQMac V1.2). The expression level of AT₁R mRNA normalized to β-actin levels in the same sample.

Receptor-ligand internalization assay. Subconfluent RMICs were incubated in isosmotic (300 mosmol/kgH₂O) or hyperosmotic (600 mosmol/kgH₂O) media for 24 h. The cells were then collected and plated at 2 × 10⁵ cells/well in a 24-well plate and allowed to attach for 3 h. The receptor-ligand internalization assay was then carried out as previously described (7). The cells were washed twice with serum-free DMEM, and the cells were incubated in cell binding buffer [50 mM Tris (pH 7.8), 120 mM NaCl, 4 mM KCl, 5 mM MgCl₂, 2 mM CaCl₂, 2 mg/ml β-glucose and 10 μg/ml bacitracin] with 0.5 nM of ¹²⁵I-labeled [Sar¹,Ile⁸]ANG II for 5–40 min at 37°C followed by rapid chilling on ice. At the indicated times, the cells were washed four times with cell binding buffer and incubated for 4 min with or without acetic acid solution [5 mM ice cold acetic acid in 150 mM NaCl, pH 2.5]. The samples were then solubilized with 1% SDS, 1% Triton X-100 in 10 mM Tris-HCl, pH 8.0, and radioactivity was counted in a Packard Cobra II γ-counter. After subtraction of nonspecific binding, results were expressed as the ratio [AT₁]i/[AT₁]o, based on [AT₁]i = [AT₁]i + [AT₁]o. Specific binding was determined experimentally in the presence of 1-μM unlabeled saralasin. In competition experiments, membrane fractions were incubated with increasing concentrations of specific receptor antagonists in membrane binding buffer containing 0.2% BSA and 0.3–0.5 nM ¹²⁵I-labeled [Sar¹,Ile⁸]ANG II.

Binding reactions were terminated by the addition of ice-cold membrane binding buffer and rapid filtration through glass fiber filters (GF/C Whatman, presoaked with assay buffer) using a Brandel cell harvester (Brandel M-24R, Rockville, MD). The filters were washed with ice-cold membrane binding buffer, and membrane-bound radioactivity that was trapped on the filters was measured in a Packard Cobra II γ-counter. The dissociation constant (Kd) and the maximum number of receptor-specific binding sites (Bmax) were calculated from nonlinear regression analysis using a software program PRISM (GraphPad Software, San Diego, CA). The inhibition constants (KI) for angiotensin receptor subtype-specific antagonists were determined by calculating the half-maximum inhibitory dose derived from nonlinear curve fitting.

Ribonuclease protection assay. The cells were washed with PBS at room temperature, and total RNA was isolated using Tri reagent (Molecular Research Center, Cincinnati, OH). AT₁R mRNA in RMICs was measured by ribonuclease protection assay (RPA) using RPA III (Ambion, Austin, TX) according to the manufacturer’s protocol. The specific AT₁R cRNA probe (20,000 cpm), which produces a protected fragment of 95 bases, was hybridized with 10 μg RMIC total RNA at 42°C for 16 h, as described previously. The pTRI-actin (mouse antisense control template; Ambion, Austin, TX) was used to make a 304-base cRNA probe, which produces a protected fragment of 245 bases with T7 RNA polymerase and γ-[³²P]CTP by in vitro transcription. RNase digestion with RNase T₁ was carried out at 37°C for 30 min. After the precipitation of protected fragments, the samples were separated by electrophoresis on a denaturing 5% polyacrylamide gel. After electrophoresis, the gel was transferred to filter paper, dried, exposed to Phosphor imager screen, and the total abundance of AT₁R mRNA was quantitated by Image Quant software (IQMac V1.2). The expression level of AT₁R mRNA normalized to β-actin levels in the same sample.
reaction and incubated for 15 min. The samples were then electrophoresed at 200 volts in a 4% polyacrylamide gel, the gel was exposed to a Phosphor imager screen, and RNA binding protein was quantitated by ImageQuant software (IQMac V1.2).

**Statistical analysis.** Results are expressed as means ± SE. Statistical significance was evaluated by unpaired Student’s t-test except for the internalization studies, where one-way-ANOVA with Student-Neuman-Keuls multiple comparison test was used. P < 0.05 was considered statistically significant.

**RESULTS**

**AT1R binding in RMIC membranes.** Equilibrium radioligand binding studies using the dual AT1R and AT2R peptide ligand 125I-labeled [Sar1,Ile8]ANG II were performed on membranes prepared from RMICs that were cultured under isoosmotic conditions (300 mosmol/kgH2O). Specific binding of 125I-labeled [Sar1,Ile8]ANG II increased in a linear manner between 1 and 30 µg membrane protein and reached saturation by 1 h at room temperature (data not shown). All subsequent binding experiments were performed on 5 µg membrane protein with a 1-h incubation period at room temperature. A representative equilibrium binding curve is shown in Fig. 1A along with Scatchard transformation of the data (Fig. 1B). Scatchard analysis revealed that the ligand interacts with a single population of receptor sites with a KD of 0.48 ± 0.04 nM and a Bmax of 6.44 ± 0.46 pmol/mg protein.

Radioligand competition experiments were also performed. Membranes prepared from RMICs cultured under isoosmotic conditions, were incubated with 125I-labeled [Sar1,Ile8]ANG II and the AT1R specific nonpeptide antagonist, SK1080 or the AT2R-specific nonpeptide antagonist, PD123319 (Fig. 1C). PD123319 was a poor competitor of 125I-labeled [Sar1,Ile8]ANG II binding (Ki > 10 µM), whereas SK1080 completely and potently abolished the 125I-labeled [Sar1,Ile8]ANG II binding (Ki = 2.39 ± 0.13 nM). These data suggest that the vast majority of ANG II receptors expressed on RMIC membranes cultured under isoosmotic conditions are of high affinity and of the AT1R subtype.

**Effect of osmolality on AT1R binding in RMIC membranes.** To examine the effects of an osmotic challenge on ANG II receptor binding, RMICs were cultured in a hyperosmotic media at 600 mosmol/kgH2O. Scatchard transformation (Fig. 1B) of saturation binding curve data (Fig. 1A) revealed that the density of AT1Rs was decreased by 33% in RMICs cultured under hyperosmotic conditions compared with isoosmotic conditions (Bmax = 4.42 ± 0.37 pmol/mg protein, P < 0.01). No changes in ligand binding affinity (KD = 0.56 ± 0.03 nM) were observed under hyperosmotic conditions.

To determine whether changes in media osmolality altered ANG II receptor subtype expression, radioligand competition studies were performed. PD123319 remained a poor competitor of 125I-labeled [Sar1,Ile8]ANG II binding (Ki > 10 µM), whereas SK1080 potently abolished 125I-labeled [Sar1,Ile8] ANG II binding at inhibitory concentrations (Ki = 3.70 ± 1.2 nM) that were indistinguishable from concentrations observed in membranes prepared from RMICs cultured under isoosmotic conditions (Fig. 1C), suggesting that even under hyperosmotic conditions, the predominant receptor subtype expressed in RMICs is the AT1R.

No significant differences in the percentage of nonviable cells were observed between isoosmotic and hyperosmotic conditions (data not shown).

**Effect of osmolality on AT1R mRNA expression in RMICs.** The levels of AT1R mRNA expression in RMICs were determined by an RPA that we previously developed, which differentiates between the AT1a and AT1b receptor mRNA subtypes (33). To test the dose response of extracted total RNA, the specific AT1A cRNA probe (20,000 cpm) was hybridized with total RNA (2–30 µg) from RMICs grown under isoosmotic (300 mosmol/kgH2O) (○) and hyperosmotic (600 mosmol/kgH2O) (●) conditions. To test the dose response of extracted total RNA, the specific AT1A cRNA probe (20,000 cpm) was hybridized with total RNA (2–30 µg) from RMICs grown under isoosmotic (○, ●) and hyperosmotic conditions (●, ●). Data are expressed as the means ± SE from three experiments each performed in duplicate.

No significant differences in the percentage of nonviable cells were observed between isoosmotic and hyperosmotic conditions (data not shown).
levels of AT₁aR mRNA expression when normalized to β-actin were not significantly different in RMICs cultured under isoosmotic and hyperosmotic conditions (AT₁aR/β-actin (arbitrary units): 300 mosmol/kgH₂O, 0.17 ± 0.01 vs. 600 mosmol/kgH₂O, 0.13 ± 0.01, n = 4) (Fig. 2). No AT₁bR mRNA was detected in cultured RMICs by RPA (data not shown).

Effect of osmolality on AT₁R internalization in RMICs. To determine whether osmolality alters the degree of ligand-mediated AT₁R endocytosis, receptor-ligand internalization studies were performed in RMICs cultured under isoosmotic and hyperosmotic conditions (Fig. 3). Total specific bound radioligand typically accounted for 85–95% of total surface bound radioligand (acid-sensitive fraction), indicating that the majority of AT₁R were internalized by 40 min. The sum of specific membrane-bound or internalized radioligand did not exceed 10% of the total radioligand added per well, so the amount of available radioligand was not limiting. Under these experimental conditions, there were no significant differences in the degree of receptor internalization in RMICs cultured under isoosmotic or hyperosmotic conditions (endocytosis rate constant (min⁻¹): 300 mosmol/kgH₂O, 0.076 ± 0.008 vs. 600 mosmol/kgH₂O, 0.043 ± 0.012).

Effect of osmolality on AT₁R RNA-protein complex formation in RMICs. We have previously shown that alterations in the levels of cytosolic proteins, which bind to the 5'LS of the AT₁aR are associated with inverse changes in AT₁aR protein expression (15, 16, 22). To determine whether osmolality alters the levels of RNA-protein complex (RPC) formation in the 5'LS in RMICs, we performed RNA EMSAs with radiolabeled 5'LS on cytosolic extracts prepared from RMICs cultured under isoosmotic and hyperosmotic conditions (Fig. 4A). The degree of 5'LS RPC formation was increased by 1.5-fold in RMICs cultured under hyperosmotic conditions compared with cells cultured under isoosmotic conditions. [5'LS RPC (AU): 300 mosmol/kgH₂O, 0.79 ± 0.08 vs. 600 mosmol/kgH₂O, 1.17 ± 0.07; n = 4, P < 0.05].

DISCUSSION

The present study demonstrates that under hyperosmotic conditions, the density of AT₁Rs on cell membranes of cultured RMICs is decreased, under conditions in which AT₁aR mRNA expression and agonist-mediated AT₁R internalization are not detectably altered. Additionally, we provide evidence suggesting that high osmolality regulates AT₁aR expression, in part, by a posttranscriptional mechanism involving cytosolic protein binding within the 5'LS of the AT₁aR mRNA.

Consistent with our previous report, this study shows that the AT₁R is the predominant ANG II receptor subtype expressed in cultured rat RMICs (18). The present study also shows that the density of AT₁Rs is reduced in RMICs cultured under hyperos-
It will be of interest in future studies to identify the protein components of this RNA-protein complex and to determine how they regulate AT_1R mRNA translation. These findings are consistent with our previous studies that showed RNA-protein complex formation in the AT_1R 5′/3′UTR is regulated in a manner that inversely correlates with changes in AT_1R densities under a variety of conditions, including dietary sodium manipulation (16), estrogen deficiency (33), and renal mass ablation (22).

In our previous report, we showed that changes in osmolality do not affect the expression of all vasoactive peptide receptors in RMICs. No changes in binding of bradykinin to its B2 receptor were observed under the same hyperosmotic conditions in which reduced binding of ANG II to AT_1Rs was observed (19). Others have also noted differences in the expression of vasoactive peptide receptors by osmolality. Vernace et al. (30) have reported a reduction in endothelin binding to its ETA receptor in cultured RMICs. In contrast, in cultured inner medullary collecting duct cells, which like RMICs, are commonly exposed to high extracellular tonicity in the renal medulla, high osmolality stimulates atrial natriuretic peptide A receptor activity and gene expression (3). Although it is tempting to suggest that high osmolality decreases the activity and expression of receptors for vasoconstrictors (ANG II and endothelin), while it increases the activity and expression of receptors for vasodilators (bradykinin and atrial natriuretic peptide), this “rule” does not seem to hold up when it comes to nonreceptor-dependent vasoactive mediators. In cultured inner medullary collecting duct cells, the expression of endothelial and inducible nitric oxide synthase isoforms are downregulated under hyperosmotic conditions (2). These studies thus suggest that the regulatory mechanisms by which extracellular tonicity regulates gene expression is far more complex.

Alterations in the expression and activity of various components of the RAS have previously been reported. Salt restriction augments the activity of circulatory RAS, while high-salt intake suppresses it (28). In contrast to the circulatory RAS, salt intake has been shown to have different effects with respect to intrarenal RAS. In the kidney, high-salt-intake has been shown to both increase (26) and decrease AT_1R mRNA expression (27). In the brain and adrenal gland, high-salt increases AT_1R mRNA expression (31, 34). Alterations in water balance have also been reported to modulate the expression of AT_1Rs. AT_1Rs are upregulated in the inner medulla during dehydration (13), while washing out the medullary gradient with furosemide, is associated with a downregulation of AT_1Rs (10). These studies support our hypothesis that AT_1Rs are differentially regulated depending on the underlying state of hydration and salt intake.

AT_1R knockout animals exhibit impairment in their urine-concentrating ability and are characterized by atrophic medullae, the severity of which is even more pronounced in the AT_1a and AT_1b double-knockout animals (25). This observation strongly supports the notion that AT_1Rs are important for the functional urine-concentrating mechanisms by maintaining the structural integrity of the renal medulla. Angiotensin-converting enzyme inhibition in early postnatal development in the rat is characterized by papillary atrophy and decreased urine-concentrating ability (24). Because the major function of RMICs in vivo is to provide the structural framework for the renal medulla (17, 20), it is conceivable that RMICs via AT_1Rs play an important role in maintaining the structural integrity of the renal medulla. Furthermore, the osmotic regulation of AT_1Rs in RMICs may be an important regulatory mechanism.
REFERENCES


ACKNOWLEDGMENTS

This research was supported by National Institutes of Health Grant HL-57502 to K. Sandberg and a National Kidney Foundation (Australia) grant to C. Maric.

Present address for Sunghoo Lee: Department of Biotechnology and Informatics, Sungmyung University, San 98-20, Anseo-Dong, Cheonan Republic of Korea, 330-720; Tel.: 82-41-550-5388; Fax: 82-41-550-518; e-mail: lees@smu.ac.kr.

by which the activity of ANG II is fine-tuned depending on the underlying hydration and salt intake.

In conclusion, the present data suggest that AT1R membrane density in RMICs is reduced under hyperosmotic conditions through a posttranscriptional regulatory mechanism that involves proteins that bind within the 5’LS of the AT1R mRNA. Therefore, posttranscriptional regulation of AT1R expression by RNA binding proteins in RMICs may contribute to regulation of renal function under both physiological and pathophysiological conditions.

R49

OSMOTIC REGULATION OF AT1 RECEPTORS


