Abnormal water metabolism in mice lacking the type 1A receptor for ANG II

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Abnormal water metabolism in mice lacking the type 1A receptor for ANG II. Am. J. Physiol. 278: F75–F82, 2000.—Mice lacking AT1A receptors for ANG II have a defect in urinary concentration manifested by an inability to increase urinary osmolality to levels seen in controls after thirsting. This defect results in extreme serum hypertonicity during water deprivation. In the basal state, plasma vasopressin levels are similar in wild-type controls and Agtr1a −/− mice. Plasma vasopressin levels increase normally in the AT1A receptor-deficient mice after 24 h of water deprivation, suggesting that the defect in urine concentration is intrinsic to the kidney. Using magnetic resonance microscopy, we find that the absence of AT1A receptors is associated with a modest reduction in the distance from the kidney surface to the tip of the papilla. However, this structural abnormality seems to play little role in the urinary concentrating defect in Agtr1a −/− mice since the impairment is largely reproduced in wild-type mice by treatment with an AT1-receptor antagonist. These studies demonstrate a critical role for the AT1A receptor in maintaining inner medullary structures in the kidney and in regulating renal water excretion.

gene targeting; urinary concentration; magnetic resonance microscopy; papilla; vasopressin

THE RENIN-ANGIOTENSIN system (RAS) regulates body fluid balance. Although the effects of this system on blood pressure and renal sodium reabsorption have been most thoroughly studied, ANG II also modulates water homeostasis. In the central nervous system, ANG II stimulates the synthesis and release of vasopressin and acts as a potent dipsogen (11, 27, 28). Along with these actions in the brain, ANG II may modulate urinary concentrating mechanisms in the kidney through its effects on hemodynamics and perhaps through direct effects on renal epithelia. ANG II causes vasoconstriction of afferent and efferent arterioles and may modulate Starling forces in the peritubular capillaries of the proximal nephron to favor solute and water reabsorption (6). ANG II-mediated vasoconstriction also decreases medullary blood flow and thus may affect osmolar gradients (3, 7, 10, 19). AT1 receptors are expressed in proximal tubular cells where they act to stimulate proximal sodium reabsorption directly and therefore may also affect water handling by reducing solute delivery to the distal nephron (21). Finally, expression of AT1 receptors in thick ascending limb and collecting duct epithelia and medullary interstitial cells suggests that these receptors might have more direct effects on distal water handling (20, 38). In both the brain and the kidney, pharmacological studies suggest that the effects of ANG II on water homeostasis are mediated by type 1 (AT1) angiotensin receptors (33).

The physiological effects of the RAS to stimulate thirst and vasopressin secretion are consistent with its role in protecting the extracellular fluid volume. In addition to these direct physiological effects, recent studies using gene targeting suggest that ANG II may also play a role in the development or maintenance of structures within the kidney that determine urinary concentration. For example, Niimura et al. (22) found that angiotensinogen-deficient mice develop marked atrophy of the renal papilla. These abnormalities are similar to those found in angiotensin-converting enzyme (ACE)-deficient mice, and these anatomic alterations are associated with high urine volumes and reduced urine osmolalities (9, 24). The similarity of these defects in angiotensinogen- and ACE-deficient mice suggests a key role for ANG II in maintaining the normal anatomic configuration of the inner medulla. Although disruption of individual angiotensin receptor genes, including the AT1A receptor gene locus, does not reproduce these obvious structural abnormalities, severe atrophy of the inner medulla has been observed in mice with combined deficiency of AT1A and AT1B receptors (25, 34). It has been suggested that this abnormal inner medullary structure may result from the absence of AT1 receptor actions to promote ureteral peristalsis (18). The complete absence of AT1 signaling is essential for the pathogenesis of this abnormality. However, the
absence of either AT\textsubscript{1A} or AT\textsubscript{1B} receptors alone is not sufficient to produce this severe defect.

In our preliminary studies, we found that urine volumes were significantly increased in Agtr\textsubscript{1a} \textsubscript{−/−} mice, which lack AT\textsubscript{1A} receptors, whereas their kidney morphology appeared essentially normal. Thus we used Agtr\textsubscript{1a} \textsubscript{−/−} mice to define the contribution of the AT\textsubscript{1A} receptor to the regulation of urinary concentration. To develop a more comprehensive view of kidney structure and to assess the potential contribution of renal structural changes to abnormal water metabolism in Agtr\textsubscript{1a} \textsubscript{−/−} mice, we examined their kidneys using magnetic resonance microscopy. We find that water homeostasis is abnormal in Agtr\textsubscript{1a} \textsubscript{−/−} mice primarily due to functional changes within the kidney.

**METHODS**

**Animals.** Mice lacking AT\textsubscript{1A} receptors for ANG II were generated by homologous recombination in embryonic stem cells as previously described (12). Animals were bred and maintained in the animal facility of the Durham Veterans Affairs Medical Center under National Institutes of Health guidelines. Agtr\textsubscript{1a} genotypes, designated \textquoteright+\textquoteright for the wild-type allele and \textquoteright−\textquoteright for the targeted allele, were determined by Southern blot analysis of DNA isolated from tail biopsies (12). Mice were generated from crosses of (129S-C57BL/6)\textsubscript{F2} Agtr\textsubscript{1a} \textsubscript{+/−} parents. The F2 generation Agtr\textsubscript{1a} \textsubscript{+/+} and \textsubscript{−/−} animals derived from these crosses were used in these experiments. We studied both male and female mice that were 2–4 mo old.

Measurement of serum and urine osmolalities. The effect of the Agtr\textsubscript{1a} mutation on the regulation of serum and urine osmolalities was examined in mice housed in standard cages allowed free access to 0.4% NaCl chow. Serum and urine were collected first from Agtr\textsubscript{1a} \textsubscript{+/+} (n = 12) and Agtr\textsubscript{1a} \textsubscript{−/−} (n = 10) mice that had free access to drinking water and then in separate groups of Agtr\textsubscript{1a} \textsubscript{+/+} (n = 7) and Agtr\textsubscript{1a} \textsubscript{−/−} (n = 5) mice that had been deprived of water for 48 h. After the collection of a urine sample by bladder massage, the animals were anesthetized with isoflurane, and a blood sample was obtained by cardiac puncture without heparin. All urine and serum osmolalities were measured immediately using a vapor pressure osmometer (Wescor Instruments).

Effects of altered water intake in Agtr\textsubscript{1a} \textsubscript{+/+} and Agtr\textsubscript{1a} \textsubscript{−/−} mice. To examine the effect of the Agtr\textsubscript{1a} mutation on drinking behavior, urinary flow rate, and urine concentration, Agtr\textsubscript{1a} \textsubscript{+/+} (n = 15) and Agtr\textsubscript{1a} \textsubscript{−/−} (n = 13) mice 2–4 mo of age were housed in specially constructed metabolic cages. During an initial 24-h period, water intake and urine volumes were measured while the animals had free access to water. Water bottles were then removed, and urine output and osmolality were measured during 48 h of water deprivation. Drinking and urine flow rates are expressed as milliliters per 24 hours per 20 grams of body weight. Osmolality of urine was measured in samples obtained by bladder massage just before the onset of water deprivation and at 24-h intervals thereafter. All animals were allowed free access to 0.4% NaCl chow during the entire experiment.

A separate group of Agtr\textsubscript{1a} \textsubscript{+/+} (n = 6) and Agtr\textsubscript{1a} \textsubscript{−/−} (n = 6) mice received an acute water load equivalent to 4% of their body weight, administered by gavage. Urine osmolalities and body weights were measured in this group just before the gavage and hourly thereafter until minimum urine osmolality was achieved. Changes in body weight were used to monitor delivery and excretion of the water load. Additional water and food were withheld after administration of the water load and until the completion of the experiment.

**Measurement of plasma vasopressin.** To determine whether the Agtr\textsubscript{1a} null mutation affects vasopressin levels, plasma was collected from separate groups of adult male Agtr\textsubscript{1a} \textsubscript{+/+} and \textsubscript{−/−} mice under the following two conditions: 1) with free access to drinking water (n = 7 for \textsubscript{+/+}, n = 5 for \textsubscript{−/−}) and 2) after 24 h of water deprivation (n = 7 for \textsubscript{+/+}, n = 6 for \textsubscript{−/−}). Blood was obtained by decapitation without anesthesia and collected into tubes containing EDTA. Plasma samples were assayed simultaneously for vasopressin by RIA according to the manufacturer’s instructions (Peninsula Laboratories).

**Administration of desmopressin.** We examined the effect of desmopressin (dDAVP; Rhone-Poulenc Rorer, Collegeville, PA) on urine osmolality in Agtr\textsubscript{1a} \textsubscript{+/+} and Agtr\textsubscript{1a} \textsubscript{−/−} (n = 4) mice. dDAVP is a selective V2-receptor agonist and was selected for use in these studies because it has potent antidiuretic effects with only modest vascular actions. Before the experiments, animals were allowed free access to drinking water and 0.4% NaCl chow. After the collection of a baseline urine sample by bladder massage, mice were injected with 1.0 µg/kg dDAVP subcutaneously, and water bottles were removed. Urine samples were collected 4 h after injection, and urine osmolalities were measured immediately.

Role of abnormal water drinking in the urinary concentrating defect in Agtr\textsubscript{1a} \textsubscript{−/−} mice. In our initial experiments, we found that the daily water intakes were significantly greater in Agtr\textsubscript{1a} \textsubscript{−/−} compared with Agtr\textsubscript{1a} \textsubscript{+/+} mice. To further examine the contribution of the level of water intake to the urinary concentrating defect observed in Agtr\textsubscript{1a} \textsubscript{−/−} mice, we performed paired drinking experiments. Over a 3-day adaptation period, mice (n = 6 for each genotype) were housed in standard cages and allowed free access to drinking water and 0.4% NaCl chow. Baseline body weights, water intake, and urine osmolalities were measured, and each Agtr\textsubscript{1a} \textsubscript{−/−} mouse was paired with an Agtr\textsubscript{1a} \textsubscript{+/+} mouse based on similar body weights. Over the next 5 days, the water intake in each Agtr\textsubscript{1a} \textsubscript{−/−} mouse was restricted to the volume of water ingested by its paired \textsubscript{+/+} control during the preceding 24 h. Body weights, water intake, and urine osmolality were measured throughout the experiment.

**Magnetic resonance microscopy of mouse kidneys.** The unique strengths of magnetic resonance microscopy in defining organ structure have been demonstrated by previous work (4, 13, 31). Thus to gain a more complete visualization of the structure of the kidney, we performed magnetic resonance microscopy of kidneys in groups of male Agtr\textsubscript{1a} \textsubscript{−/−} (n = 4) and Agtr\textsubscript{1a} \textsubscript{+/+} (n = 4) mice between 2 and 3 mo of age. After anesthesia with isoflurane, the abdomen was opened, and the left renal vein was immediately ligated to prevent blood from leaving the organ so that it would act as a natural contrast agent. The excised kidney was then placed in a cylindrical 10-mm-diameter container and was immersed in Fomblin (perfluoro polyether) to limit susceptibility variation at the surface of the tissues.

Magnetic resonance microscopy was performed using a tunable custom-designed 10-mm solenoid radio frequency coil. Three-dimensional magnetic resonance images of kidney specimens were acquired at 9.4 tesla on a Bruker CSI System (Fremont, CA) equipped with actively shielded gradients. A spin-echo pulse sequence was used, with the following parameters: time of repetition = 500 ms, echo time = 10 ms, NEX (no. of excitations) 2. Spatial encoding was accomplished using a three-dimensional Fourier transform encoding, which allowed the simultaneous imaging of 128 contiguous planes, each 51-µm thick, through the kidney specimen. The field of view was 13 mm and was reconstructed on a 256 × 256
matrix, leading to a voxel size of \(51 \times 51 \times 51 \mu \text{m}\). Images were displayed and analyzed on a Silicon Graphics workstation (Reality Engine2, SGI, Mountain View, CA) using VoxelView (Vital Images, Fairfield, IA), a commercial software package developed for interactive imaging. A plane crossing through the longest axis of the inner medulla in both coronal and axial planes was used for distance and area measurements. Using clear changes in signal intensity and image pattern, distinct kidney zones were differentiated in the coronal plane as follows: cortex and outer stripe were grouped together and distinguished from the inner stripe by the appearance of radial stripes representing vascular bundles (5); the border between the innermost zone of the inner stripe and the inner medulla could not be reproducibly distinguished in these unperfused kidneys, and these zones were therefore measured as one larger combined zone (inner stripe + inner medulla). These combined kidney zones were outlined, and their areas were measured using VoxelView. The area in pixels squared was multiplied by the pixel size (51 \(\mu \text{m}\)) and was used for statistical analysis. The linear distance from the kidney surface to the tip of the papilla was measured.”

“Results
The absence of \(\text{AT}_{1A}\) receptors alters urine and serum osmolality. To examine the role of the \(\text{AT}_{1A}\) receptor for \(\text{ANG II}\) in water homeostasis, we first measured water intake and urine flow rates in \(\text{Agtr1a}^{-/-}\) mice. \(\text{Agtr1a}^{-/-}\) mice with free access to water drank significantly more than wild-type controls (4.3 \(\pm\) 0.5 vs. 3.1 \(\pm\) 0.2 ml·day\(^{-1}\)·20 g\(^{-1}\); \(P = 0.01\)). Urine flow rates were also higher in \(\text{Agtr1a}^{-/-}\) mice than in controls (2.1 \(\pm\) 0.3 vs. 1.1 \(\pm\) 0.1 ml·day\(^{-1}\)·20 g\(^{-1}\); \(P = 0.004\)). The increased urine flow in \(\text{Agtr1a}^{-/-}\) mice is associated with a reduced urine osmolality compared with wild-type mice (1,168 \(\pm\) 168 vs. 1,766 \(\pm\) 109 mosmol/kg H\(_2\)O; \(P = 0.007\)). When they are provided free access to water, \(\text{Agtr1a}^{-/-}\) mice have a lower mean serum osmolality compared with \(\text{Agtr1a}^{+/+}\) controls (311 \(\pm\) 2 vs. 318 \(\pm\) 1 mosmol/kg H\(_2\)O; \(P = 0.01\)).

\(\text{Agtr1a}^{-/-}\) mice have a defect in their ability to concentrate urine. To further evaluate urinary concentrating capacity in \(\text{Agtr1a}^{-/-}\) mice, we examined their ability to concentrate urine during water deprivation. As shown in Fig. 1A, although urine flow rates fell significantly in both \(\text{Agtr1a}^{+/+}\) and \(\text{Agtr1a}^{-/-}\) mice after 24 h of water deprivation, they remained higher in the \(\text{Agtr1a}^{-/-}\) mice compared with controls (0.9 \(\pm\) 0.1 vs. 0.6 \(\pm\) 0.1 ml·day\(^{-1}\)·20 g\(^{-1}\); \(P = 0.02\)). After 48 h of water deprivation, urine flow was reduced to negligible levels in both groups. The antidiuresis after water deprivation was associated with significant increases in spot urine osmolality at 24 and 48 h in both groups, as seen in Fig. 1B. However, during the period of water deprivation, urine osmolality remained lower in the \(\text{Agtr1a}^{-/-}\) mice compared with controls at 24 (2,055 \(\pm\) 284 vs. 3,519 \(\pm\) 209 mosmol/kg H\(_2\)O; \(P = 0.002\)) and 48 (1,975 \(\pm\) 310 vs. 3,771 \(\pm\) 247 mosmol/kg H\(_2\)O; \(P = 0.001\)) h.

Serum osmolality increased in both \(\text{Agtr1a}^{+/+}\) and \(\text{Agtr1a}^{-/-}\) mice after water deprivation. However, after 48 h of water deprivation, serum osmolality was significantly higher in \(\text{Agtr1a}^{-/-}\) mice (378 \(\pm\) 6 mosmol/kg H\(_2\)O) than in \(\text{Agtr1a}^{+/+}\) controls (340 \(\pm\) 2 mosmol/kg H\(_2\)O; \(P < 0.0001\)). Thus, when they are deprived of water, the urinary concentrating defect in \(\text{Agtr1a}^{-/-}\) mice impairs their ability to maintain normal serum tonicity.

Responses to an acute water load. To assess the integrity of thick ascending limb sodium reabsorption and thus its contribution to both concentrating and diluting processes, as well as the ability of the mice to suppress vasopressin release, we examined the response of \(\text{Agtr1a}^{+/+}\) and \(\text{Agtr1a}^{-/-}\) mice to an acute water load. In response to the water load, both groups rapidly diluted their urine. Subsequent minimum urine osmolalities were slightly higher in \(\text{Agtr1a}^{+/+}\) mice (151 \(\pm\) 11 mosmol/kg H\(_2\)O) compared with the \(\text{Agtr1a}^{-/-}\) group (122 \(\pm\) 21 mosmol/kg H\(_2\)O; \(P = 0.2\)). Based on measurements of hourly body weights, we found that both groups excreted the volume of the water load within 2 h of its administration.

Plasma vasopressin response to dehydration. As shown in Fig. 2, during periods of free access to drinking water, plasma vasopressin levels tend to be lower in \(\text{Agtr1a}^{-/-}\) (53 \(\pm\) 8 pg/ml) mice than in \(\text{Agtr1a}^{+/+}\) (37 \(\pm\) 9 pg/ml) controls, although this difference does not achieve statistical significance. After water deprivation, there is an approximately fourfold increase in plasma vasopressin levels in \(\text{Agtr1a}^{+/+}\) mice (126 \(\pm\) 31 pg/ml; \(P = 0.008\) vs. baseline). Similarly, plasma vasopressin levels also increase significantly in thirsted mice. Data analysis. The values for each parameter within a group are expressed as means \(\pm\) SE. For comparisons between \(\text{Agtr1a}^{+/+}\) and \(\text{Agtr1a}^{-/-}\) groups, statistical significance was assessed using an unpaired t-test. A paired t-test was used for comparisons within groups. ANOVA and Bonferroni’s test were used for multiple comparisons.
Agtr1a^{−/−} mice (159 \pm 28 pg/ml; P = 0.006 vs. baseline), and the enhanced levels of plasma vasopressin that are observed after 24 h of water deprivation are not different between the groups.

Agtr1a^{−/−} mice respond to dDAVP. To further investigate urinary concentrating mechanisms in Agtr1a^{−/−} mice, we evaluated their responses to the V2-specific vasopressin analog dDAVP. As we had observed previously, pretreatment urine osmolalities were significantly lower in Agtr1a^{−/−} mice (1,038 \pm 184 mosmol/kgH₂O) than controls (1,974 \pm 107 mosmol/kgH₂O; P = 0.004). In response to dDAVP, urine osmolalities increased significantly in both groups, as shown in Fig. 3. Urine osmolality increased from 1,038 \pm 184 to 2,167 \pm 374 mosmol/kgH₂O in Agtr1a^{−/−} mice (P = 0.03 vs. baseline) and from 1,975 \pm 107 to 3,539 \pm 395 mosmol/kgH₂O in controls (P < 0.001 vs. baseline).

Fig. 1. Effects of water deprivation on urine flow (A) and urine osmolality (Uosm; B) in Agtr1a^{−/−} and +/+ mice. ○, Agtr1a^{−/−} mice (n = 13); ■, +/+ mice (n = 15). Time indicates hours of water deprivation. Agtr1a^{−/−} mice have higher urine flows after 24 h of thirsting. By 48 h, urine flow is negligible in both groups. Urine osmolality was measured on spot urine samples collected at the given time points. Both Agtr1a^{−/−} and +/+ mice are able to increase their urine osmolality significantly. However, Agtr1a^{−/−} mice are unable to concentrate their urine to the degree seen in wild-type mice in response to water deprivation. Data are presented as means \pm SE (*P < 0.05 vs. +/+; †P < 0.02 compared with time 0).

Fig. 2. Plasma vasopressin levels in Agtr1a^{+/+} and −/− mice. Plasma vasopressin levels were measured in groups of Agtr1a^{+/+} and Agtr1a^{−/−} mice allowed free access to water or after 24 h of water deprivation. Filled bars, Agtr1a^{+/+} mice; open bars, Agtr1a^{−/−} mice. Plasma vasopressin levels are similar in Agtr1a^{+/+} and Agtr1a^{−/−} mice allowed free access to drinking water, and vasopressin levels are similarly elevated in Agtr1a^{+/+} and −/− mice in the setting of 24 h of water deprivation. Data are presented as means \pm SE [∗P = 0.008 vs. +/+ water ad libitum (ad lib); †P = 0.001 vs. −/− water ad lib, ANOVA].

Fig. 3. Changes in urine osmolality after desmopressin (dDAVP) administration in Agtr1a^{+/+} and −/− mice. Urine osmolality was measured in Agtr1a^{+/+} (n = 4) and Agtr1a^{−/−} (n = 4) mice just before (PRE-dDAVP) and then 4 h after (POST-dDAVP) the administration of 1.0 µg/kg dDAVP sc. ○, Agtr1a^{+/+} mice; ■, Agtr1a^{−/−} mice. Both Agtr1a^{+/+} and Agtr1a^{−/−} mice show significant responses to acute treatment with dDAVP. Urine osmolality is lower in Agtr1a^{−/−} mice at both time points. Data are presented as the means \pm SE (*P < 0.02 vs. Agtr1a^{+/+}; †P < 0.02 vs. 0 h).
to 3,696 ± 285 mosmol/kgH₂O in Agtr1a +/+ (P = 0.01 vs. baseline). Four hours after dDAVP administration, urine osmolalities remained significantly lower in Agtr1a −/− mice (2,167 ± 374 mosmol/kgH₂O) than controls (3,696 ± 285 mosmol/kgH₂O; P < 0.02). In both wild-type and Agtr1a −/− mice, the degree of urinary concentration achieved after dDAVP administration was similar to the urine osmolalities observed after 24 h of water deprivation (see Fig. 1B).

Chronic restriction of water intake does not correct the urinary concentrating defect in Agtr1a −/− mice. Chronically increased water consumption may produce an inability to concentrate urine through washout of medullary interstitial hypertonicity (8, 15). To directly examine the contribution of water intake to the impaired urinary concentration seen in Agtr1a −/− mice, we chronically restricted their water intake to levels of wild-type controls. As shown in Fig. 4, when water consumption was restricted in Agtr1a −/− mice over a period of 5 days, urine osmolalities remained substantially lower in Agtr1a −/− mice than in their paired Agtr1a +/+ controls. Urine osmolalities in the Agtr1a −/− group did not increase appreciably during the period of water restriction, and the Agtr1a −/− mice progressively lost weight from 27 ± 1 to 22 ± 1 g (P = 0.002). After 5 days of this regimen, the AT1A Receptor-deficient animals uniformly demonstrated signs of dehydration such as lethargy and tenting of the skin.

Magnetic resonance microscopy of kidneys. Mice with targeted disruptions of the angiotensinogen and ACE genes and those with combined disruption of both Agtr1a and Agtr1b genes exhibit severe papillary atresia. These abnormalities in the renal medulla can be seen easily using routine light microscopy (9, 22, 25, 34). Although such methods have not demonstrated structural abnormalities in Agtr1a −/− mice (17, 26), subtle changes in the size and morphology of the inner medulla may not be detected using this approach because of the nonlinear shape of the murine inner medulla as it projects into the renal pelvis. To determine whether the urinary concentrating defect in Agtr1a −/− mice might be accompanied by subtle changes in the structure of the renal medulla, we performed magnetic resonance microscopy on kidneys from Agtr1a −/− mice and Agtr1a +/+ controls. Body weights and kidney weights tended to be lower in age- and sex-matched Agtr1a −/− compared with Agtr1a +/+ mice, but these differences did not achieve statistical significance (Table 1). As shown in Fig. 5 and Table 1, the areas of the renal cortex plus outer stripe are similar in the two groups. The areas of inner stripe plus inner medulla tended to be smaller in Agtr1a −/− mice compared with controls, but this difference did not achieve statistical significance. The linear distance from the surface of the kidney to the tip of the renal papilla was modestly but significantly shorter in Agtr1a −/− mice than in wild-type controls (4.6 ± 0.2 vs. 5.8 ± 0.2 mm; P = 0.008).

Pharmacological blockade of AT1 receptor signaling in kidneys from Agtr1a −/− mice. We compared two treatments: antihypertensive drugs with a diuretic effect (prazosin, captopril), and drugs selecting for reabsorption in the proximal tubules (benzothiazide, lithium). The long-term effect of prazosin in Agtr1a −/− mice is a decrease in urinary concentrating defect (Table 2). However, the effect of prazosin in Agtr1a −/− mice was less than in wild-type controls, indicating that pharmacological blockade of AT1 receptors produces a defect in urine concentration. We estimated the relative importance of structural versus functional effects of the absence of AT1A receptor signaling on urinary concentrating mechanisms by examining the effects of pharmacological blockade of AT1 receptors with losartan in Agtr1a +/+ and −/− mice. As shown in Table 2, urine osmolalities of water-deprived Agtr1a

![Fig. 4. Effect of restricted water drinking on urine osmolalities (A) and body weights (B) in Agtr1a −/− mice, Agtr1a +/+ controls (n = 6); O, Agtr1a −/− mice (n = 6). Urine osmolalities were measured during 3-day adaptation period with free access to water, followed by a 5-day period during which Agtr1a −/− mice were restricted in their water intake, in a paired fashion, to that consumed by Agtr1a +/+ mice. Urine osmolality was lower in Agtr1a −/− mice compared with Agtr1a +/+ controls during the adaptation period. Urine osmolality remained significantly lower in Agtr1a −/− mice compared with Agtr1a +/+ controls throughout the period of pair-restricted water intake, and urine osmolality was similar in Agtr1a −/− mice at the beginning of the adaptation period and at the end of the experiment. Agtr1a −/− mice progressively lost weight during the 5-day period of restricted water consumption. Data are presented as means ± SE (⁎ P < 0.04 vs. Agtr1a +/+; **P < 0.0006 vs. Agtr1a +/+).

### Table 1. Magnetic resonance microscopy results

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>Left Kidney Wt, g</th>
<th>C + OS, mm²</th>
<th>IS + IM, mm²</th>
<th>Distance, mm</th>
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<tr>
<td>Agtr1a +/+</td>
<td>24.2 ± 2.7</td>
<td>0.198 ± 0.031</td>
<td>560 ± 64</td>
<td>338 ± 28</td>
<td>58 ± 0.2</td>
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<tr>
<td>Agtr1a −/−</td>
<td>22.3 ± 2.7</td>
<td>0.175 ± 0.028</td>
<td>522 ± 70</td>
<td>276 ± 29</td>
<td>46 ± 0.2*</td>
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</table>

Values are means ± SE. C + OS, area of cortex plus outer stripe; IS + IM, area of inner stripe plus inner medulla; Distance, linear distance from kidney surface to tip of papilla. *P = 0.008 vs. Agtr1a +/+.
Our studies using Agtr1a −/− mice suggest a key role for the AT1A receptor in the regulation of water balance by the RAS. Water homeostasis is abnormal in these animals and is most strikingly demonstrated by the extreme hypertonicity that develops when Agtr1a −/− mice are deprived of water. Our studies indicate an essential role for the AT1A receptor in maintaining water balance.

There is substantial evidence that links the functions of AT1 receptors in the central nervous system to the control of vasopressin secretion. For example, AT1 receptors are present in regions of the hypothalamus that synthesize vasopressin (35). In addition, ANG II activates vasopressin-producing neurons (16), and central administration of AT1-receptor antagonists alters the release of vasopressin induced by osmotic stimuli (28). Although both AT1A and AT1B receptors are expressed in the central nervous system, their relative effects on vasopressin secretion cannot be distinguished pharmacologically and therefore have not been defined previously. Our studies demonstrate that plasma vasopressin levels increase appropriately in Agtr1a −/− mice after 24 h of thirsting and that a failure to augment vasopressin levels does not explain the urinary concentrating defect seen in these animals. Although these values for plasma vasopressin levels are somewhat higher than those of other species such as rats and dogs, our values are within the range of those reported previously by other investigators in mice (32, 39). The preservation of this response in the absence of AT1A receptors suggests that AT1B receptors may mediate the interactions between the RAS and vasopressin release. Alternatively, the role of AT1 receptors in regulating vasopressin responses in vivo may have been overestimated.

To examine the possibility that the absence of AT1A receptors might affect renal responses to vasopressin in collecting duct epithelium, we administered a V2 receptor vasopressin analog to Agtr1a +/+ and Agtr1a −/− mice. After dDAVP administration, we found that Agtr1a −/− mice rapidly and significantly increased their urine osmolality. Within both groups of mice, the maximal osmolality achieved after this pharmacological dose of dDAVP is similar to that observed after 24 h of water deprivation. However, the maximal urine osmolality achieved by Agtr1a −/− mice after dDAVP remained substantially less than that of controls. Thus the absence of AT1A receptors does not eliminate the actions of vasopressin to augment water permeability in the distal nephron at the collecting duct level. Instead, the defect appears to be related to the generation of a maximal osmolar gradient.

One potential explanation for a reduced capacity to generate maximally concentrated urine would be a disruption of the medullary gradient due to high urine flows caused by increased water intake. Such a circumstance has been described in humans drinking copious amounts of water and in water-loaded rats (8, 15). To test this possibility, we restricted the water intake of Agtr1a −/− mice to the level of wild-type controls with similar body weights. Over a period of 5 days, this

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**DISCUSSION**

Among its many physiological effects, the RAS modulates water homeostasis. This is accomplished through actions in the central nervous system and in the kidney.

**Table 2. Effect of an AT1-receptor antagonist (losartan) on water-deprived urine osmolalities of Agtr1a +/+ and −/− mice**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Vehicle</th>
<th>Losartan</th>
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<tbody>
<tr>
<td>Agtr1a +/+</td>
<td>17</td>
<td>3,674±129</td>
<td>2,884±147†</td>
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<td>Agtr1a −/−</td>
<td>6</td>
<td>2,895±185*</td>
<td>3,174±115</td>
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Values are means ± SE; n, no. of mice. Units are mosmol/kgH2O. *P < 0.005 vs. Agtr1a +/+ (vehicle); †P < 0.001 vs. Agtr1a +/+ (vehicle).
regimen had no effect on urine volume or osmolality, suggesting that medullary washout, secondary to polydipsia, does not play a significant role in impairing urinary concentrating ability in these animals.

The consequences of AT1A receptor deficiency on renal hemodynamics might also contribute to abnormal urinary concentration. For example, acute treatment of animals with an ACE inhibitor increases blood flow in the renal inner medulla (3), and an inverse relationship between papillary blood flow and inner medullary solute accumulation has been reported (19). Similarly, acute AT1 receptor blockade with losartan also increases papillary blood flow and urine flow (2, 23). Inner medullary blood flow might be similarly dysregulated in Agtr1a−/− mice, limiting the accumulation of osmotically active solute in the medullary interstitium. However, chronic ACE inhibition in rats causes a consistent increase in papillary plasma flow, but this does not impair their ability to concentrate urine in response to thirsting (7).

Interruption of the direct epithelial actions of ANG II could potentially explain the defective urine concentration of Agtr1a−/− mice. Proximal tubular AT1 receptors regulate solute and fluid reabsorption at this site and thus could also affect urine concentration by altering distal delivery of solutes (21). AT1 receptors are also expressed in thick ascending limb, collecting duct, and in medullary interstitial cells (20, 38). Because AT1 receptors in these more distal sites also seem to medulate sodium and bicarbonate flux (29, 36, 37), interruption of AT1 receptor functions in these locations might also contribute to the defect in urinary concentration.

With the use of standard histological methods, we and others have previously reported that, except for occasional slight dilatation of the renal pelvis and associated mild compression of the papilla, the kidneys of Agtr1a−/− mice appear normal (17, 26). However, the length and the nonlinear shape of the murine inner medulla hinder its complete visualization by standard methods of histological sectioning. In the current study, we have used magnetic resonance microscopy to provide a detailed characterization of the size and shape of the kidney in Agtr1a−/− mice. Although the areas of the renal cortex plus outer stripe were similar between the groups, the areas of inner stripe plus the inner medulla were smaller in Agtr1a−/− mice compared with controls. Although the linear distance from the kidney surface to the tip of the papilla is significantly, albeit modestly, shorter in Agtr1a−/− mice than in controls. In the wild-type controls, this distance determined by magnetic resonance microscopy is in general agreement with previous histomorphological measurements in mouse kidneys (14).

Although the size and length of the inner medulla in mammals generally correlate with urinary concentrating capacity (1, 30), the contribution of the modest structural abnormality to the urinary concentrating defect in Agtr1a−/− mice is difficult to quantify directly. Our findings would suggest that, if this structural abnormality plays any role in this concentrating defect, it is minor. First, the magnitude of the defect in Agtr1a−/− mice is greater than would be expected given the modest structural change. This is in contrast to mice with combined AT1A-AT1B receptor deficiency in which the renal papilla is markedly atrophic and the animals have a profound inability to concentrate their urine that is much more severe than animals lacking only AT1A receptors (25). Furthermore, pharmacological blockade of AT1 receptors in wild-type mice produces a urinary concentrating defect that is similar in magnitude to that seen in Agtr1a−/− mice. Because losartan causes no additional impairment of urinary concentration in Agtr1a−/− mice, AT1B receptors appear to play essentially no role in the physiology of this process.

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