Adenosine A1-receptor knockout mice have a decreased blood pressure response to low-dose ANG II infusion

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Lee DL, Bell TD, Bhupatkar J, Solis G, Welch WJ. Adenosine A1-receptor knockout mice have a decreased blood pressure response to low-dose ANG II infusion. Am J Physiol Regul Integr Comp Physiol 303: R683–R688, 2012. First published August 8, 2012; doi:10.1152/ajpregu.00116.2012.—Adenosine, acting on A1-receptors (A1-AR) in the nephron, increases sodium reabsorption, and also increases renal vascular resistance (RVR), via A1-ARs in the afferent arteriole. ANG II increases blood pressure and RVR, and it stimulates adenosine release in the kidney. We tested the hypothesis that ANG II-infused hypertension is potentiated by A1-ARs influence on Na+ reabsorption. Mean arterial pressure (MAP) was measured by radiotelemetry in A1-AR knockout mice (KO) and their wild-type (WT) controls, before and during ANG II (400 ng·kg−1·min−1) infusion. Baseline MAP was not different between groups. ANG II increased MAP in both groups, but on day 12, MAP was lower in A1-AR KO mice (KO: 128 ± 3 vs. 139 ± 3 mmHg, P < 0.01). Heart rates were significantly different during days 11–14 of ANG II. Basal sodium excretion was not different (KO: 0.15 ± 0.03 vs. WT: 0.13 ± 0.04 mmol/day, not significant) but was higher in KO mice 12 days after ANG II despite a lower MAP (KO: 0.22 ± 0.03 vs. WT: 0.11 ± 0.02 mmol/day, P < 0.05). Phosphate excretion was also higher in A1-AR KO mice on day 12. Renal expression of the sodium-dependent phosphate transporter and the Na+/glucose cotransporter were lower in the KO mice during ANG II treatment, but the expression of the sodium hydrogen exchanger isoform 3 was not different. These results indicate that the increase in blood pressure seen in A1-AR KO mice is lower than that seen in WT mice but was increased by ANG II nonetheless. The presence of A1-ARs during a low dose of ANG II-infusion limits Na+ and phosphate excretion. This study suggests that A1-AR antagonists might be an effective antihypertensive agent during ANG II and volume-dependent hypertension.

ANG II and adenosine regulate renal vasculature tone (1, 9) and renal tubular uptake of Na+, especially in the proximal tubule (4, 6, 27), by activating specific receptors. Both of these actions can alter renal vascular resistance (RVR) and impact the role of the kidney in the regulation of systemic blood pressure. Renal vasoconstriction is initiated by activation of angiotensin, type 1 receptors (AT1-R) (26) and adenosine, type 1 receptors, (A1-AR) (29, 33). Simultaneous application of ANG II and adenosine increases renal resistance in in vivo models (14). In isolated perfused afferent arterioles, ANG II and adenosine have a synergistic effect to increase resistance when adenosine is applied at low concentrations (22). During increased activity of ANG II, the renal vessel constrictor effect of adenosine is increased (13, 41). Additional studies demonstrate that ANG II is able to increase de novo renal adenosine content through a decrease of adenosine-metabolizing enzymes. The accumulation of adenosine induces downregulation of adenosine A2-AR receptor population without modifying adenosine A1-ARs, thereby enhancing the constrictive effects of ANG II in the renal vasculature (12, 13).

Conversely, ANG II activation of AT2-Rs (15) and adenosine activation of A2-ARs (23) dilate renal vessels, implying these systems have sensitive control of RVR. In addition, these systems may be linked. Blockade of AT1-Rs reduced renal vasoconstriction caused by adenosine (35, 42). ANG II infusion in A1-AR-deficient mice was less effective on reduction of renal resistance vessels and GFR (15). Much of the effect of adenosine on RVR is due to its role as a mediator of tubuloglomerular feedback (TGF), a major intrinsic regulator of renal blood flow (RBF) and glomerular filtration rate (GFR) (31). TGF is absent in A1-AR knockout mice (6, 36).

These systems may also interact in the regulation of Na+ uptake in the proximal tubule, which impacts the kidney’s ability to regulate systemic blood pressure. AT1-Rs are expressed in the proximal tubules and activation by ANG II promotes Na+ uptake in this segment. AT1-R activation increases proximal tubule reabsorption (34). Angiotensin-converting enzyme inhibitors and AT1-R blockers decrease proximal tubule Na+ and water reabsorption (16, 24). However, less is known about how ANG II and adenosine promote Na+ uptake.

A1-ARs are also expressed in the proximal tubules, as well as other segments of the nephron, and specific blockade decreases water reabsorption in the proximal tubule and elicits a powerful diuresis (43). In vitro and in vivo studies on kidney fluid and electrolyte transport indicate that endogenous adenosine by activation of A1-ARs stimulate NaCl reabsorption in the cortical proximal tubule (37). However, the interaction of adenosine and ANG II in the regulation of fluid balance and blood pressure is unknown.

The goal of this study is to determine whether the blood pressure increases caused by systemic ANG II infusion is altered in mice deficient in A1-ARs and whether the actions in the proximal tubule contribute to these effects. We tested the hypothesis that ANG II-infused hypertension is potentiated by A1-ARs influence on Na+ reabsorption.

MATERIALS AND METHODS

Animals. Mice used in this study were from a colony maintained by Georgetown University from the breeders provided by Dr. J. Schnermann. Briefly, heterozygous mice were crossed with C57BL/6 or Swiss wild-type mice for more than eight generations to generate A1-AR knockout (KO) mice in a congenic C57BL/6 or Swiss genetic background. Male homozygous (+/–) (A1-AR KO) and wild-type (+/+) (A1-AR WT) littermates, 10-wk-old (28–30 g) mice were housed in a quiet room at 25°C with a 12:12-h light-dark cycle and

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maintained on a standard chow (0.6 g/100 g Na⁺ content) with free access to food and water (36). The use of animals for this study was approved by the Georgetown University Animal Care and Use Committee and was performed according to the National Institutes of Health guidelines for the conduct of experiments in animals.

Telemetry. Mice (total = 33, n = 7–9 in each group) were anesthetized with 2% isoflurane. The tip of a PA-C20 pressure-transducing catheter (Data Sciences International, St. Paul, MN) was introduced into the aortic arch via the carotid artery. The transducer unit was placed subcutaneously in the flank. Animals were allowed to recover for 7 days, and then baseline mean arterial pressure (MAP) and heart rate (HR) data were collected for 5 days. Data were analyzed using Dataquest A.R.T. software (Data Sciences). MAP and HR were measured over a continuous 19-h period every day, and studies were begun only after normal circadian rhythm was reestablished (~4–7 days postsurgery). MAP data were collected at 500 Hz for 5 s each minute, from 3 PM to 10 AM (i.e., 19 h) every 2nd day. The 3 PM to 6 PM period and the 6 AM to 10 AM period together (7 h) were analyzed as “day” MAP, and the 6 PM to 6 AM period (12 h) was considered “night.”

ANG II osmotic minipumps. Mice were briefly anesthetized prior to subcutaneous implantation of osmotic minipumps (model no. 1002; Alzet, Palo Alto, CA). ANG II (Peninsula Laboratory, San Carlos, CA) was dissolved in 0.154 M NaCl for infusion at 0 (vehicle, V), 400 ng·kg⁻¹·min⁻¹ (ANG II 400). Animals were placed in metabolic cages for 24-h urine collections during control day 3 and day 12 of ANG II.

Renal function studies. On day 12 of ANG II, separate cohorts of WT (n = 5 or 6) and KO (n = 5 or 6), mice were anesthetized with a combination of Inactin (50 mg/kg ip) and ketamine (10 mg/kg ip), as previously described (19). Mice were placed on a servo-controlled surgical table to maintain body temperature at 37°C. A tracheostomy was performed by insertion of a short polyethylene (PE) tube (PE-90). The right jugular vein was cannulated with PE-10 for fluid infusion. The right femoral artery was cannulated with PE tubing (200–300 μm) for continuous measurement of MAP and blood sampling. The MAP was monitored with a PowerLab system (ADInstruments, Castle Hill, NSW, Australia). The bladder was catheterized via a suprapubic incision with PE-50 tubing for urine collections. During surgery, 0.154 M NaCl containing 2% BSA (Sigma, St. Louis, MO) was infused at 0.6 ml/h iv. After surgery, the intravenous infusion was changed to 0.154 M NaCl containing 1.5% albumin, [¹H]-para-aminohippurate (PAH) (New England Nuclear, Boston, MA) and [¹⁴C]-inulin (New England Nuclear) and infused at a rate of 0.35 ml/h. After a 60-min equilibration period, two consecutive 30-min urine collections and an arterial blood sample were obtained to determine whole kidney function, hematocrit, and plasma electrolytes. PAH and inulin clearances were determined in the different groups of mice.

Single-nephron GFR and proximal tubule reabsorption. Separate cohorts of WT and KO mice (n = 4–7) were prepared for renal micropuncture analysis and were anesthetized by spontaneous inhalation of isoflurane (1.0% in room air, delivered by a continuous pump, Univenter, Malta), as described earlier (2). The surgical preparation was similar as above with the additional placement of the left kidney in a stable Lucite holder, which was then bathed in mineral oil. Mice were infused with isotonic saline containing 1.5% BSA (Sigma Chemical, St. Louis, MO) and [³H]-jamlin (10 μCi/h) at a rate of 0.35 ml/h and for blood pressure measurements, respectively. Micropuncture studies were initiated after 45 min–1-h stabilization.

Late proximal tubular loops on the kidney surface were identified by injection of small amounts of dye-stained light mineral oil. If the bolus injection immediately disappeared from the surface, then an oil block was inserted and a 3-min timed fluid collection was initiated. Blood samples were collected immediately following micropuncture experiments and urine collection. All tubular fluid samples were transferred to 1-μl constant-bore microcaps for determination of fluid volume. Absolute proximal reabsorption (APR) was calculated as the difference between single-nephron GFR and proximal tubular flow.

Western blot analysis. Kidney protein concentration was determined by Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA). Protein lyase (50 μg) from the renal cortex of each kidney sample was denatured in boiling water for 5 min. After denaturation, the lysate was placed on ice for 5 min and loaded onto a 12.5% Tris-HCL gel (Bio-Rad). The gel was transferred to nitrocellulose membrane (Bio-Rad). The membrane was blocked with 5% BSA milk followed by overnight incubation (4°C) with polyclonal anti-Na⁺/H⁺ exchanger isoform 3 (NHE3; diluted 1:500; BD Biosciences) and polyclonal Na⁺/glucose cotransporter (SGLT) (1:500; Santa Cruz Biotechnology, Santa Cruz, CA). The antibody for sodium-dependent phosphate transporter (NaPi2) was the generous gift of Dr. Carolyn M. Ecelbarger (Georgetown University, Washington, D.C.). The secondary antibodies and [³H]-glucose were peroxidase-labeled goat anti-rabbit (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The blots were probed with β-actin (diluted 1:5,000; Sigma) for equal loading. Densitometry for Western blot analysis was performed with the ImageJ software.

Urine electrolytes. Na⁺ concentration in the urine was measured by flame photometry (model no. 2655–10; Corning, Corning, NY). PO₄⁻ excretion was measured by a colorimetric phosphate assay [Qan-tiChrom phosphate assay kit (DPI-500), BioAssay Systems Hayward, CA]. Excretion was calculated by the volume and concentration and expressed as millimoles per day. Urine glucose was measured by Siemens Multistix reagent strips for urinalysis.

Statistical analysis. Data were analyzed with a two-factor, repeated-measures ANOVA. Significant F-tests from the ANOVA at P < 0.05 were followed by post hoc comparisons using the Newman-Keuls multiple-range test.

RESULTS

MAP and HR for A₁-AR+/+ (WT) and A₁-AR−/− (KO) mice during the control period and on day 12 of ANG II infusion are shown in Table 1. Control MAP and HR were not significantly different between WT and KO mice during the control period. The mean arterial pressure circadian rhythms were not different between WT and KO during baseline and ANG II hypertension. On day 12 of ANG II hypertension, renal function studies were performed. The mean arterial pressure, heart rate, urine volume, renal blood flow, and glomerular filtration rate measurements for A₁-AR+/+ (WT) and A₁-AR−/− (KO) mice during control and day 12 of ANG II hypertension are summarized in Table 1.

Table 1. Mean arterial pressure, heart rate, urine volume, renal blood flow, and glomerular filtration rate measurements for A₁-AR+/+ (WT) and A₁-AR−/− (KO) mice during control and day 12 of ANG II hypertension

<table>
<thead>
<tr>
<th></th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Heart Rate, bpm</th>
<th>Urine Volume ml/day</th>
<th>RBF, ml·min⁻¹·g⁻¹</th>
<th>GFR, μl·min⁻¹·g⁻¹</th>
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<tbody>
<tr>
<td>WT</td>
<td>120 ± 3</td>
<td>516 ± 4</td>
<td>1.3 ± 0.2</td>
<td>6.3 ± 0.5</td>
<td>472 ± 85</td>
</tr>
<tr>
<td>KO</td>
<td>117 ± 3</td>
<td>520 ± 5</td>
<td>1.4 ± 0.3</td>
<td>5.9 ± 0.6</td>
<td>684 ± 60§</td>
</tr>
<tr>
<td>Day 12 of ANG II</td>
<td>139 ± 3‡*</td>
<td>534 ± 5‡*</td>
<td>1.0 ± 0.4</td>
<td>5.4 ± 2.4</td>
<td>325 ± 50‡</td>
</tr>
<tr>
<td>KO</td>
<td>128 ± 3‡</td>
<td>502 ± 5‡</td>
<td>2.0 ± 0.4*</td>
<td>3.6 ± 0.4‡</td>
<td>305 ± 52‡</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. §P < 0.05, comparison between WT and KO baseline measurements. ‡P < 0.05, comparison between baseline period and ANG II treatment. *P < 0.05, comparison between WT + ANG II and KO + ANG II.
MAP (P < 0.05) and HR (P < 0.05) were higher in WT mice. RBF was similar between groups during the control period. ANG II infusion decreased (P < 0.05) RBF in KO mice. During the control period, GFR was significantly higher (P < 0.05) in KO compared with WT mice. GFR was reduced (P < 0.05) in both groups during ANG II treatment; however, GFR was not different between WT + ANG II and KO + ANG II (Table 1). Baseline urine flow was not different between groups, but was higher (P < 0.05) in KO mice on day 12.

Absolute proximal reabsorption (APR) was calculated by the difference in single-nephron GFR and proximal tubule flow. Previous results from our laboratory demonstrate that APR was lower in KO mice before and after acute saline loading. The control values for APRs in both groups are consistent with previously published results from our laboratory (5). In the present study, APR was also significantly lower in KO + ANG II (WT + ANG II: 7.1 ± 1.07 vs. KO + ANG II: 3.4 ± 0.97 nl/min, P < 0.05).

MAP increased in the WT mice on day 8 of ANG II treatment, which is typical of this model (19) and had a maximal increase on days 10–14. The increase in MAP in the KO mice was delayed to day 12. The increase in the HR of WT mice on day 12 was lower compared with WT mice on day 10, and the maximal increased MAP was lower compared with WT mice on days 10–14. (Fig. 1). Although significant, the increase in the HR of WT mice on day 12 of ANG II did not alter the blood pressure response.

Urinary sodium excretion during the control period was not different between WT and KO mice. During day 12 of ANG II treatment, Na⁺ excretion was higher (P < 0.05) in KO mice (Fig. 2A). Urinary phosphate (PO₄³⁻) excretion was similar between both groups during the control period and was significantly increased (P < 0.05) on day 12 of ANG II infusion in KO mice compared with WT mice (Fig. 2B). We did not observe any differences between WT and KO mice food intake during the control period or during ANG II treatment. On day 12 of ANG II treatment, food intake was lower in WT and KO compared with their respective controls (Fig. 2C).

Expression of the proximal tubule sodium phosphate cotransporter, NAPI-2, and SGLT-1, the Na⁺/glucose cotransporter, was lower in the KO mice during ANG II treatment (Fig. 3, A and B). Renal expression of NHE3 was not different between the two groups (Fig. 3C).
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DISCUSSION

The main findings in this study are 1) the increase in MAP in response to 14 days of low-dose ANG II infusion was attenuated in A1-AR KO mice; 2) urinary flow and sodium and phosphate excretion were higher in A1-AR KO mice during ANG II infusion; and 3) expression of the NaPi2 and SGLT-1, exclusive proximal tubule (PT) transporters, was lower in A1-AR KO mice. However, expression of NHE3, another PT transporter, was not different between groups. Together, these results suggest that A1-ARs in the proximal tubule enhance Na\(^+\) and PO\(_4\)\(^{3-}\) uptake during elevated ANG II, which could contribute to the higher blood pressure.

The failure to increase MAP in the A1-AR KO to that of A1-AR WT mice in response to ANG II was partially due to the increased Na\(^+\) excretion in A1-AR KO mice, preventing Na\(^+\) retention that occurred in ANG II-infused WT mice. This was due to two possible effects related to adenosine: the decreased APR in KO mice and/or reduced Na\(^+\)-linked PO\(_4\)\(^{3-}\) reabsorption in the proximal tubule. During elevation of MAP by ANG II, proximal reabsorption is increased in WT mice, partially because of the hemodynamic effects of ANG II and partially by increased Na\(^+\) transport in the PT (8, 40). Both of these actions, therefore, enhance Na\(^+\) and volume retention in this model of hypertension. However, in A1-AR KO mice, the absence of these receptors prevents much of the effects of ANG II in the PT and, indirectly, the effect on blood pressure as well. This is demonstrated by the lower APR seen in A1-AR KO mice and the increased Na\(^+\) and fluid excretion at day 12 of ANG II infusion. Second, the expression of NaPi2, the major phosphate uptake pathway in the PT, was lower in A1-AR KO mice. This lower level of expression appears to be functionally significant since Na\(^+\) and PO\(_4\)\(^{3-}\) excretion were higher in the A1-AR KO mice. Therefore, the effect of ANG II on enhancing the PT role in Na\(^+\) retention is lost in adenosine receptor-deficient mice. This effect could be related to down-regulation of NaPi2 and facilitated excretion of Na\(^+\) and PO\(_4\)\(^{3-}\) sufficient to attenuate the blood pressure effect of ANG II.

The loss of TGF in A1-AR KO mice might contribute to increased urine flow and Na\(^+\) excretion, if not compensated in downstream segments (11). TGF is an intrinsic renal regulatory process that acts at the single-nephron level and limits the snGFR by release of vasoconstrictors that increase the vascular tone of the afferent arteriole in response to increases in solute delivery to the macula densa, a specialized group of epithelial cells. Multiple studies have indicated the role of adenosine as the mediator of TGF and a powerful locally produced vasoconstrictor (6, 15, 23, 36). In isolated afferent arterioles, attached to the glomerulus, A1-AR agonists constrict and antagonists dilate the vessel (15). Both A1-AR and A2-AR receptors are widely expressed in the afferent arteriole, and activation of these receptors has the opposite effect, consistent with the G protein-linked effects on intracellular signaling pathways of these receptors (18, 23, 37, 39). Consistent with the lack of TGF, GFR was higher in untreated A1-AR KO mice compared with WT (Table 1). However, in mice treated with ANG II, there were no differences in GFR (Table 1).

In addition to the loss of TGF, the absence of A1-ARs in the PT also contributes to increased Na\(^+\) excretion. An early report showed that adenosine stimulates sodium reabsorption in the PT and chloride reabsorption in the thick ascending limb and...
papillary collecting duct (25). These effects, especially in the PT, are linked to A1-AR, since specific blockade of this receptor increases sodium and fluid excretion in animal models (3, 20, 30, 43). Infusion of A1-AR blockers also increased Na⁺ excretion in human subjects and hypertensive patients (38). We have previously shown that proximal fluid reabsorption is reduced in rats either by acute systemic or PT infusion of A1-AR antagonists (21). PT reabsorption is also lower in A1-AR KO mice, compared with WT mice (5). The exact mechanism of A1-AR action on Na⁺ transport is not known, but we suggest that these effects are due to signaling through the Na⁺/H⁺ antiporter-3 (NHE3). In preliminary microperefusion and recollection studies in the rat PT, both of the effects of A1-AR activation and inhibition were blocked by coperfusion with a specific NHE3 blocker (D. L. Lee, personal observation). However, in the current mouse study, we show that ANG II infusion reduces expression of two other pathways for Na⁺ uptake in the PT: the SGLT-1 and the NaPi2 pathways. We had previously shown that NaPi2 in the PT was not different between A1-AR WT and KO mice during control conditions (5). This new information suggests that ANG II downregulates NaPi2 in A1-AR KO mice, by an unknown mechanism, which offsets the normal effect of ANG II to increase reabsorption in the PT (28) (8). A1-AR KO mice excreted more phosphate (Fig. 2B), suggesting the decreased NaPi2 was functionally significant and, thus, may have also contributed to the higher Na⁺ excretion. Food intake was not different between WT and KO during control and ANG II hypertension. Therefore, differences in Na⁺ and phosphate excretion are not due to a higher food intake in KO mice (Fig. 2C). We did not detect glucose in the urine, suggesting the loss of SGLT-1 in the PT was compensated by other systems and, therefore, probably did not contribute to the increased Na⁺ excretion. The A1-AR and NaPi2 deficiencies paradoxically seem to protect these mice from excessive Na⁺ retention. Conversely, these results suggest that A1-ARs, both in the afferent arterioles and in the PT, passively permit excess Na⁺ retention, especially during volume and blood pressure challenges.

ANG II increases blood pressure, primarily by activation of AT₁ receptors, which constricts resistance vessels in multiple vascular beds and, therefore, increases total peripheral resistance (TPR). A secondary role is the action of ANG II to increase Na⁺ uptake in the kidney and increase Na⁺ and fluid volume in the cardiovascular system. The so-called slowpressor dose of ANG II (400 ng·min⁻¹·kg⁻¹) used in this study appears to target the kidney’s role more than higher doses, which clearly activates TPR (10, 17, 19). Therefore, ANG II infusion in this model enhances the kidney’s role in fluid retention and targets the adenosine system. This is confirmed in this study by the lower Na⁺ and fluid excretion during ANG II infusion in WT mice, suggesting that the kidney is stimulated by ANG II to retain Na⁺ and fluid, thus contributing to hypertension. The A1-AR KO mice excreted more Na⁺ during this time and escaped much of the ANG II effects both on Na⁺ reabsorption and retention and subsequently on blood pressure. Acute ANG II infusion increases PO₄³⁻ excretion in the rat (44). This effect appears to be associated with the elevated MAP, since clamping the kidney to control renal perfusion pressure during ANG II infusion prevented this effect (44). Similarly, long-term ANG II treatment, which raised BP over 14 days, also increased PO₄³⁻ excretion in rats (45). These authors concluded that NaPi2 expression was unchanged at day 4 when PO₄³⁻ excretion was maximal, suggesting that posttranscriptional regulation of location or activity of the transporter, rather than gene expression, may be involved. Our study provides additional observations that adenosine and ANG II may interact to regulate NaPi2 and PO₄³⁻ excretion, during elevated MAP associated with ANG II. Na⁺ and PO₄³⁻ excretion were actually greater in KO animals with lower MAP, suggesting that this regulation escaped the effect of blood pressure in this model. Indeed, these effects on NaPi2 appear to have protected A1-AR KO mice from more severe hypertension. This is the first observation that adenosine participates in phosphate regulation, especially during high levels of ANG II.

Blood pressure regulation by A1-ARs using KO mice has yielded conflicting results (6, 36), which may be related to the mouse strains used (7). However, plasma renin levels and A1-AR regulation of renin release may account for these differences. If renin levels were higher in A1-AR KO mice, then the effects of ANG II infusion would be enhanced; instead, we show the ANG II effect in KO mice was attenuated in this study.

In summary, our results suggest that the increase in blood pressure seen in A1-AR KO mice is lower than that seen in WT mice but was increased by ANG II, nonetheless. We suggest this is due to actions of A1 receptors in the proximal tubule, which allows excess Na⁺ reabsorption and ultimately Na⁺ retention that contributes to hypertension. We have previously shown that the effect of adenosine to promote Na⁺ uptake in the PT is partially due to NHE3 in normotensive animals (32). However, these new observations suggest that adenosine also affects Na⁺ and PO₄³⁻ uptake by its actions on NaPi2 expression and activity during ANG II-induced hypertension. In the absence of A1-ARs, ANG increases Na⁺ and PO₄³⁻ excretion due to reduced NHE3 activity and suppressed NaPi2 expression and activity in the proximal tubule. Therefore, A1-AR antagonists might be an effective antihypertensive agent in ANG II and volume-dependent hypertension.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


