Am J Physiol Regul Integr Comp Physiol 285: R44–R49, 2003;
10.1152/ajpregu.00739.2002.

Attenuated renovascular constrictor responses to angiotensin II in adenosine 1 receptor knockout mice

Pernille B. Hansen, Seiji Hashimoto, Josie Briggs, and Jurgen Schnermann

National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

Submitted 4 December 2002; accepted in final form 20 February 2003

Hansen, Pernille B., Seiji Hashimoto, Josie Briggs, and Jurgen Schnermann. Attenuated renovascular constrictor responses to angiotensin II in adenosine 1 receptor knockout mice. *Am J Physiol Regul Integr Comp Physiol* 285: R44–R49, 2003; 10.1152/ajpregu.00739.2002.—In the present experiments we examined the renovascular constrictor effects of ANG II in the chronic and complete absence of A1 adenosine receptors (A1AR) using mice with targeted deletion of the A1AR gene. Glomerular filtration rate (GFR) was not different between A1AR +/+ and A1AR −/− mice under control conditions (450.5 ± 60 vs. 475.2 ± 62.5 μl/min) but fell significantly less in A1AR −/− mice during infusion of ANG II at 1.5 ng/min (A1AR +/+: 242 ± 32.5 μl/min, A1AR −/−: 371 ± 42 μl/min; $P = 0.03$). Bolus injection of 1, 10, and 100 ng of ANG II reduced renal blood flow and increased renal vascular resistance significantly more in A1AR +/+ than in A1AR −/− mice. Perfused afferent arterioles isolated from A1AR +/+ mice constricted in response to bath ANG II with an EC_{50} of $1.5 \pm 0.4 \times 10^{-10}$ mol/l, whereas a right shift in the dose-response relationship with an EC_{50} of $7.3 \pm 1.2 \times 10^{-10}$ mol/l ($P < 0.05$) was obtained in arterioles from A1AR −/− mice ($P < 0.05$). The expression of AT1A receptor mRNA was not different in kidney RNA from A1AR +/+ or A1AR −/− mice. We conclude that chronic A1AR deficiency diminishes the effectiveness of ANG II to constrict renal resistance vessels and to reduce GFR.

renal blood flow; ultrasonic flowmeter; renal vascular resistance; glomerular filtration rate; perfused arterioles

PHENOMENOLOGICAL AND MECHANISTIC aspects of the actions of ANG II and adenosine in the renal vascular bed have been explored in numerous studies. It has been a consistent conclusion from these studies that both agents cause an increase in renal vascular resistance by eliciting vasoconstriction at multiple sites along the renal vasculature. The renal vasoconstrictor response of ANG II is initiated by activation of AT1 receptors while adenosine causes vasoconstriction through the A1 adenosine receptor (A1AR). Several studies suggest that the degree of activation of AT1 or A1A receptors determines the magnitude of the constrictor effects to acute changes in the concentration of the other agonist. The majority of studies exploring such an interaction between adenosine and ANG II have investigated the modifying role of variations of ANG II on the constrictor action of adenosine. The general approach in these studies has been to modulate the renin-angiotensin system, by changing NaCl intake, by utilizing mice with a deletion of the AT1a receptor, or by acutely inhibiting ANG II formation or action with angiotensin-converting enzyme or receptor blockers. The conclusion from these studies was that a reduction in AT1 activation by reducing ANG II levels or by blocking AT1 receptors reduced the renal vasoconstrictor effect of adenosine (14, 17). The same effect was seen when plasma renin and presumably ANG II levels were changed by alterations in dietary Na intake, suggesting that both acute and chronic reductions in AT1 receptor occupation interfered with A1AR-dependent vasoconstriction (11).

The converse relationship, i.e., a dependence of ANG II vasoconstriction on simultaneous activation of A1AR, is somewhat more controversial. For example, in the split hydronephrotic kidney, acute blockade of A1AR with the A1AR antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) had no effect on the constrictor effect of ANG II (4). On the other hand, DPCPX caused an ~50% reduction in the constrictor response to ANG II in isolated and perfused afferent arterioles from rabbit kidneys (17). The availability of mice with a targeted deletion of A1AR permits the study of renal vascular responses to ANG II during chronic and complete A1AR deficiency (15). Accordingly, the present experiments were performed to determine whether the response of renal vascular resistance to ANG II can be shown to be altered in A1AR knockout compared with wild-type mice. These experiments were done as a first step to further attempts of understanding the nature of the interaction between ANG II and adenosine. Our experiments show that both the vasoconstriction and the fall in glomerular filtration rate (GFR) in response to an acute change in angiotensin plasma concentration are significantly blunted in the absence of functional A1AR and that this reflects at least in part a reduced responsiveness of afferent arterioles.

Address for reprint requests and other correspondence: J. Schnermann, NIDDK, NIH, Bldg. 10, Rm. 4 D51, 10 Center Dr. MSC 1370, Bethesda, MD (E-mail: jurgens@intra.niddk.nih.gov).

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.

METHODS

GFR and renal blood flow experiments. Studies were performed in female mice of the A1AR strain generated in this laboratory (15). A1AR −/− and their littermate A1AR +/+ controls are maintained in a mixed J129/C57BL6 background. Genotyping was done on tail DNA using PCR as described previously (15). Mice were in a weight range between 17 and 33 g and were kept on standard rodent chow and tap water. Animals were anesthetized with 100 mg/kg thiobutabarbital intraperitoneally (Inactin) and 100 mg/kg ketamine intramuscularly. Body temperature was maintained at 38°C by placing the animals on an operating table with a servocontrolled heating plate. The trachea was cannulated, and a stream of 100% oxygen was blown toward the tracheal tube throughout the experiment. The femoral artery was cannulated with hand-drawn polyethylene tubing for continuous measurement of arterial blood pressure and blood withdrawal. The jugular vein was cannulated for an intravenous maintenance infusion of 2.25 g/dl bovine serum albumin (BSA) in saline at a rate of 0.5 ml/h. A catheter was placed in the bladder for urine collections.

To determine GFR, mice were infused with [^{125}I]iothalamate (Glofil, Questcor Pharmaceutical, Hayward, CA) at ~5 μCi/h. After 30–45 min of equilibration, three 10-min urine collection periods were made bracketed by two blood collections of ~4 μl each collected in heparinized 5-μl microcaps (Drummond). An ANG II infusion was then started at a rate of 1.5 ng/min (ANG II concentration 1 ng/μl) with a flow of 1.5 μl/min without interrupting the iothalamate infusion. Three additional 10-min urine collections and a terminal blood collection were made. [^{125}I]iothalamate radioactivity was measured in duplicate 0.5-μl aliquots of plasma and urine in a gamma counter.

Measurements of renal blood flow (RBF) were performed in a separate group of A1AR +/+ and A1AR −/− mice. The left renal artery was approached from a flank incision and carefully dissected free to permit placement of a 0.5 mm V-type ultrasonic flow probe (Transonic Systems, Ithaca, NY). The probe was held in place with a micromanipulator. The flow signal was digitized and analyzed using MacLab software. In each animal, RBF was determined during the intravenous injection of 1, 10, and 100 ng of ANG II. After each injection a waiting period of 10 min was allowed in which RBF and blood pressure returned to baseline.

Perfused afferent arterioles. To determine the effect of A1AR deficiency on ANG II-induced constriction at the arteriolar level, afferent arterioles from A1AR +/+ and −/− mice were isolated and microperfused. Mice (18–26 g) of either sex were used in the study. The method of isolation and perfusion was a modification of that used by Weihprecht et al. (18) and Jensen et al. (9). Afferent arterioles were microdissected at 4°C from slices of mouse kidneys. The dissection was performed in DMEM/Nutrient F12 (DMEM/F12) with 0.5% BSA under a stereomicroscope. The specimen was transferred to a thermoregulated chamber, containing oxygenated DMEM/F12 + 0.1% BSA, mounted on an inverted microscope (Olympus IMT-2), and perfused with concentric glass pipettes mounted in a moveable track system. The arteriole was aspirated into a holding pipette (tip diameter 20 μm) and cannulated with a perfusion pipette (tip diameter 5–6 μm). Once the arteriole was cannulated, the driving pressure was increased until the vessel opened, and perfusion was established. Perfusion was performed close to the minimum opening pressure because it was our impression that the perfused arterioles from the mouse are rather sensitive to pressure-induced damage. The perfusate consisting of physiological salt solution (PSS) + 1% BSA was driven from a reservoir pressurized to 60–110 mmHg. The temperature was increased to 37°C, and the vessel was allowed to recover for 25 min. In each experiment a test stimulus of high potassium (100 mmol/l K^+) or a gentle increase in pressure was applied initially to ensure viability of the vessel. Sequences of interest were recorded with a digital camera (CoolSNAP-Pro, Media Cybernetics). Images were transferred to a computer, and intraluminal vessel diameters were assessed using imaging software (Image Pro-plus, Media Cybernetics). PSS had the following composition (mmol/l): 115 NaCl, 25 $NaHCO_3$, 2.5 K_2HPO_4, 1.3 $CaCl_2$, 1.2 $MgSO_4$, 10 HEPES, and 5.5 glucose. High-potassium solution contained (mmol/l) 25 $NaHCO_3$, 20 NaCl, 95 KCl, 1.2 $MgSO_4$, 2.5 K_2HPO_4, 1.3 $CaCl_2$, 10 HEPES, and 5.5 glucose. All solutions were equilibrated with 5% CO_2 in O_2 for 30 min before use, BSA was added, and pH was adjusted to 7.35. To study the vasoconstrictor response to ANG II in arterioles from A1AR wild-type and knockout mice, ANG II was added to the bath for 3 min at increasing concentrations (10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, and 10^{-8} mol/l) using a step-up protocol. Furthermore, in a small series of experiments in afferent arterioles from A1AR +/+ mice, we tested the effect of the A1AR-selective antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (10^{-5} mol/l) on the vascular response to ANG II (10^{-10} mol/l).

Quantitative real-time PCR. The expression of ANG II receptors (AT1) in the kidney cortex of 5 A1AR +/+ and 5 A1AR −/− mice was investigated using real-time PCR analysis. In addition, we determined the expression levels of A2aAR and A2bAR in A1AR −/− compared with A1AR +/+ mice. RNA was isolated using Trizol-reagent and reverse transcribed using Superscript (Invitrogen) and oligo(dT) (Pharmacia). Quantitative PCR analysis was performed using an ABI prism 7900 HT Sequence Detection System (Applied Biosystems). SyBR Green, a double-stranded DNA binding dye, was used for the fluorescent detection of DNA generated during the PCR. The PCR reaction was performed in a total volume of 20 μl with 0.4 pmol/μl of each primer, and 2× SyBR Green PCR master mix (Applied Biosystems); 1 μl cDNA corresponding to 100 ng of total RNA was used as template. Negative controls included water instead of cDNA in the PCR reaction and addition of RNA instead of cDNA. Published sequences for mouse AT1a (S37484), A2aAR (Y13346), and A2bAR (NM_007413) were used to design primers for PCR amplification (13). The AT1a primers might also amplify the AT1b isoform but not the AT2 receptor. Primer sequences were AT1 sense 5′-CCA GAT CAA GTG ATT TTG AAC AGT G-3′ and antisense 5′-GCT GTA GAG AGT AGG GAT CAT GAC AA-3′; A2aAR sense 5′-TCC TGG TCC TCA CGC AGA GT-3′ and antisense 5′-GGG TCA GGC CGA TGG C-3′; A2bAR sense 5′-TGG CTG TCG ACC GAT ATC TG-3′ and antisense 5′-GTC AAT CCA ATG CCA AAG GC-3′; β-actin sense 5′-GCT CTG GCT CCT AGC ACC AT-3′ and antisense 5′-GCC ACC GAT CCA CAC AGA GT-3′.

Statistics. Data are expressed as means ± SE. Statistical analysis was performed using the *t*-test of paired and nonpaired data. Arteriolar diameters were compared by two-way ANOVA followed by Bonferroni test. Student's *t*-test on paired data in the individual arterioles was performed when comparing EC_{50} values. $P < 0.05$ was considered significant.

RESULTS

GFR experiments. The response of GFR to an intravenous infusion of ANG II at 10 ng·min^{-1}·100 g body wt^{-1} was tested in six female A1AR +/+ and six female A1AR −/− mice. Mean arterial blood pressure increased from 99.3 ± 5.5 to 111.5 ± 7.4 mmHg in +/+

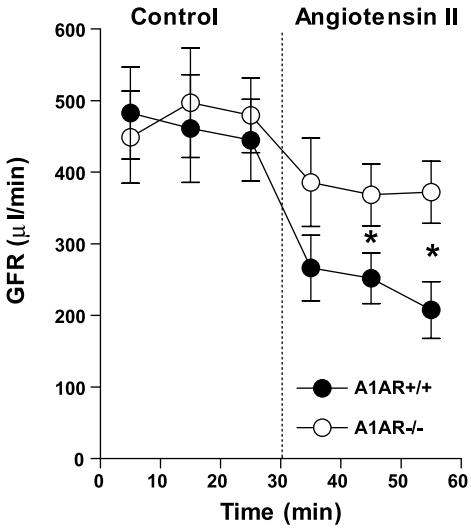

Fig. 1. Glomerular filtration rate (GFR) of wild-type and A1 adenosine receptor (A1AR) knockout mice in 3 consecutive 10-min periods during control and during intravenous infusion of ANG II at 1.5 ng/min. Values are means of 6 experiments ± SE. *Significance between A1AR +/+ and −/− for a given time period ($P < 0.05$).

mice ($P = 0.011$) and from 107.2 ± 2.9 to 119.8 ± 4.7 mmHg in A1AR −/− animals ($P = 0.02$). The difference in blood pressure between genotypes at baseline was not statistically significant ($P = 0.19$). The relative increase in mean arterial blood pressure was similar between the genotypes (12.2 ± 3.4% in +/+ and 12.6 ± 4.1% in −/− mice). Measurements of GFR in six successive clearance periods in A1AR +/+ and −/− mice are shown in Fig. 1. While GFR values were not different between genotypes in any of the control collections, the fall in GFR in the ANG II infusion period was more pronounced in the A1AR +/+ mice, reaching significance in the second ($P = 0.045$) and third collection period ($P = 0.016$). Mean GFR for the entire 30-min control period averaged 450.5 ± 60 and 475.2 ± 62.5 μl/min in wild-type and knockout mice, respectively ($P = 0.78$). During the ANG II infusion, GFR averaged 242 ± 32.5 μl/min in wild-type and 371 ± 41.7 μl/min in A1AR knockout mice ($P = 0.03$; Fig. 2). Mean urine flow rate of wild-type mice was 1.9 ± 0.45 μl/min in control and 3.03 ± 1.35 μl/min during angiotensin infusion ($P = 0.22$). In A1AR −/− animals, urine flow rate was 3.7 ± 1.2 μl/min in control and 6.6 ± 1.6 μl/min during angiotensin infusion ($P = 0.1$). Differences of urine flow rates in control and ANG II infusion periods were not significant between genotypes (control $P = 0.1$, ANG II $P = 0.08$).

RBF studies. Measurements of RBF were performed in eight wild-type and nine A1AR knockout mice. In response to intravenous bolus injections of 1, 10, and 100 ng ANG II, RBF of the left kidney fell in the wild-type mice from 930 ± 12 to 790 ± 9 μl/min, from 940 ± 15 to 490 ± 9 μl/min, and from 960 ± 18 to 220 ± 7 μl/min. In the A1AR knockout mice, the same injections caused RBF to fall from 1,030 ± 14 to 940 ± 10 μl/min, from 1,070 ± 15 to 640 ± 13 μl/min, and from 1,080 ± 20 to 400 ± 14 μl/min. All changes in both wild-type and A1AR knockout mice were significant at $P < 0.01$.

While levels of RBF were not significantly different before the ANG II injections, RBF was higher during the ANG II administration in knockout compared with wild-type mice ($P = 0.019$ at 1 ng, $P = 0.018$ at 10 ng, and $P = 0.008$ at 100 ng ANG II).

Arterial blood pressure levels were not significantly different between A1AR +/+ and A1AR −/− mice even though they tended to be lower in the latter group (103 ± 3.5 and 97 ± 2.9 mmHg, respectively; $P = 0.17$). Furthermore, blood pressure changes in response to ANG II at 1, 10, and 100 ng were nearly identical, increasing by 3.3 ± 0.2, 17.3 ± 0.4, and 47.3 ± 1 mmHg in wild-type and by 3.3 ± 0.15, 18.4 ± 0.4, and 45.5 ± 0.8 mmHg in the A1AR knockout mice. Because blood pressures tended to be lower and RBF higher in the A1AR knockout animals, renal vascular resistance under control conditions was lower in A1AR knockout than wild-type mice (112.3 ± 1.7 vs. 96.3 ± 1.5 mmHg min/ml; $P = 0.05$). Increases in renal vascular resistance caused by the injection of 1, 10, and 100 ng of ANG II are shown in Fig. 3. It can be seen that renal vascular resistance increased significantly less in the knockout mice during all three levels of angiotensin injection (paired *t*-test).

Studies in perfused afferent arterioles. The vasoconstrictor response to ANG II was studied in perfused afferent arterioles from A1AR +/+ and −/− mice. Basal diameters averaged 10.1 ± 0.4 μm in A1AR wild-type mice ($n = 6$) and 9.0 ± 0.5 μm in knockout mice ($n = 7$). Administration of 10^{-12} and 10^{-11} mol/l ANG II had no measurable effect on the inner arterio-

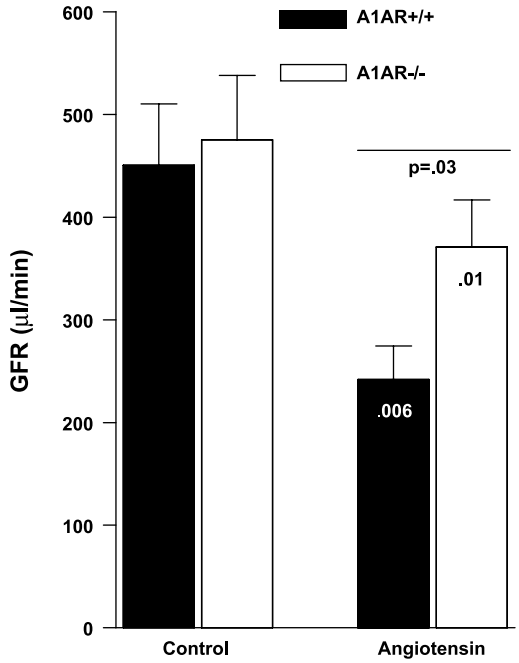

Fig. 2. Average GFR during control and angiotensin infusion periods of A1AR +/+ and A1AR −/− mice. Significance levels are given for comparisons between periods (inside columns) and between genotypes (above columns).

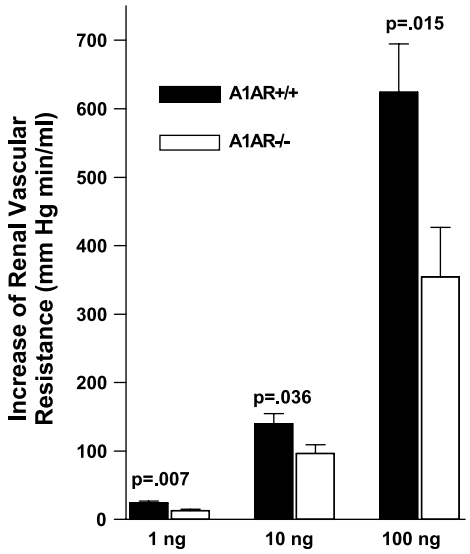

Fig. 3. Increases in renal vascular resistance in response to bolus injections of 1, 10, and 100 ng of ANG II in A1AR +/+ and A1AR −/− mice. Values are means ± SE.

lar diameter in either wild-type or knockout mice. The addition of 10^{-10} mol/l ANG II to afferent arterioles from wild-type mice induced a significant vasoconstriction ($P < 0.05$, ANOVA), whereas in knockout mice no significant vasoconstrictor effect was seen at this ANG II concentration. However, increasing the ANG II concentration to 10^{-9} mol/l was associated with significant constriction in both wild-type and knockout animals (Fig. 4*A*). The ANG II concentration causing half-maximal vasoconstriction was $1.5 \pm 0.4 \times 10^{-10}$ mol/l in wild-type mice and $7.3 \pm 1.2 \times 10^{-10}$ mol/l in A1AR knockout mice ($P < 0.05$, *t*-test, Fig. 4*B*). Figure 4*C* shows representative examples of perfused afferent arterioles from wild-type and knockout mice under control conditions and during exposure to ANG II at 10^{-10} mol/l.

In three A1AR +/+ mice, the addition of DPCPX to the bath reduced the vasoconstrictor effect of a submaximal dose of ANG II. ANG II (10^{-10} mol/l) reduced the diameter of the afferent arterioles by 4.0 ± 0.6 μm before DPCPX and by 1.5 ± 1.2 μm after DPCPX administration.

Expression of AT1 and A2AR receptors. To test if the decreased sensitivity to ANG II in A1AR −/− mice was due to a downregulation of AT1 receptors, we determined the expression of ANG II receptor mRNA in kidneys of A1AR +/+ and −/− mice. The expression levels of AT1 mRNA normalized to β-actin were found to be not significantly different in A1AR +/+ (1.0 ± 0.2) compared with A1AR −/− mice (1.1 ± 0.3). To test whether an increase in A2AR expression in the A1AR −/− mice may cause attenuation of the ANG II-induced constriction, we determined the mRNA expression levels of the A2aAR and A2bAR genes. A2aAR mRNA levels ranged from 1.1 ± 0.2 in A1AR +/+ to 1.0 ± 0.4 in −/− mice, and A2bAR ranged from 1.2 ± 0.3 in +/+ to 0.8 ± 0.2 in −/− mice ($n = 4$ in each group). Even though the level of A2bAR tended to be lower in the −/− mice, the differences were not significant.

DISCUSSION

The present experiments show that the renovascular constrictor response to ANG II is significantly reduced in mice with a genetic deficiency in the expression of A1AR. Attenuated constrictor responses at the level of

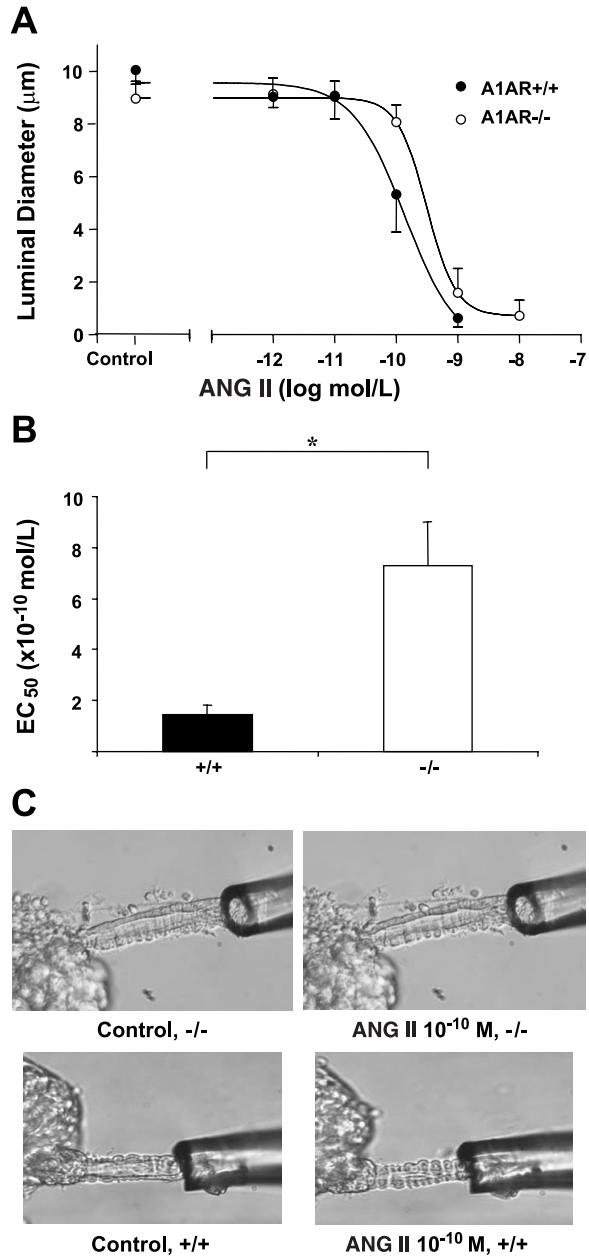

Fig. 4. *A*: dose-response relation between bath ANG II concentration and inner luminal diameter of perfused afferent arterioles of A1AR +/+ (closed symbols) and A1AR −/− mice (open symbols). Curves represent sigmoidal regression lines calculated by GraphPad Prism software. *B*: angiotensin concentrations causing half-maximum-diameter reductions (EC_{50}) of afferent arterioles from wild-type (A1AR +/+) and knockout mice (A1AR −/−). Values are means ± SE. *C*: images of perfused afferent arterioles from A1AR +/+ and A1AR −/− mice during control and during the presence of 10^{-10} mol/l ANG II in the bath. $^*P < 0.05$

the entire kidney were seen both with subpressor and with pressor doses of ANG II. Our observation that the fall in GFR caused by ANG II was also attenuated in A1AR knockout mice suggests that the reduction in ANG II constrictor potency is an expression of a reduced responsiveness in the preglomerular vasculature. The expression of A1AR in preglomerular microvessels and glomeruli supports the notion that the interaction between ANG II and adenosine suggested by the blood flow and GFR studies takes place for the most part in afferent arterioles and to a lesser extent perhaps in larger renal vessels (8, 16). Afferent arterioles as the site of interaction between angiotensin and adenosine are directly supported by the right shift in the dose-response relationship observed in isolated perfused afferent arterioles.

In view of the observation that the expression levels of ANG II receptor mRNA were comparable between A1AR +/+ and −/− mice, we consider it unlikely that differences in AT1 receptor density are responsible for the reduced angiotensin sensitivity in the A1AR knockout mice. Furthermore, the unchanged expression levels of both A2a and A2b adenosine receptors between A1AR −/− and A1AR +/+ mice do not support the possibility that an increase in A2AR abundance and activation may be responsible for the attenuation of the ANG II-induced response in the A1AR −/− animals. While there could be a number of other reasons for the lower renal vascular responsiveness to ANG II in A1AR knockout mice, it would seem most likely that it is an expression of the previously noted relation between angiotensin-induced constriction and A1AR activation. Studies from our laboratory have shown that the constrictor effect of ANG II in isolated rabbit afferent arterioles was blocked by ~50% when the A1AR inhibitor DPCPX was present in the bath (17), a finding that was reproduced in the present studies in mouse afferent arterioles. In confirmation of an earlier observation, a recent study in dogs showed that the reduction in RBF caused by ANG II was augmented by intrarenal infusion of adenosine and attenuated by the A1AR antagonist KW-3902 (1, 6). The present study is the first to demonstrate that the vasoconstricting effects of acute changes in plasma ANG II levels are attenuated in animals with a life-long absence of A1AR. Thus the fundamental mechanism by which adenosine affects angiotensin actions in renal blood vessels does not seem to adapt to chronic A1AR deficiency.

It seems reasonable to predict that the effect of adenosine blockade on the ANG II-induced vasoconstriction may depend on the level of expression of A1AR in a given vascular bed. Thus in a vascular bed with low levels of A1AR expression, the effect of A1AR inhibition on angiotensin responses should be low. We believe that this notion may be the explanation for some of the apparently discrepant results that can be found in the literature. For example, blockade of A1AR did not measurably affect ANG II-induced vasoconstriction in afferent arterioles of juxtamedullary nephrons (3). Functional evidence mainly based on the vasoconstrictor efficacy of adenosine would indicate that the nucleoside causes no more than a 10% reduction in afferent arteriolar diameter and that even when A2AR receptors are blocked, the maximum vasoconstriction is only 15% (3, 10). In contrast, A1AR activation has previously been observed to cause a 45–50% diameter reduction in more superficial afferent arterioles of the rabbit and in the hydronephrotic kidney preparation (7, 19). We believe that this dataset is most likely a reflection of a markedly lower abundance of A1AR in deep nephrons. Thus any interaction between AT1 and A1AR activation would be expected to be less obvious in juxtamedullary afferent arterioles, an expectation directly supported by the observation that converting enzyme blockade reduced the vasoconstriction caused by N^6-cyclohexyladenosine in superficial arterioles but enhanced it in juxtamedullary arterioles (5). A second important variable may be the rate of production of adenosine and its appearance in and around the A1AR expressing vessels. In the hydronephrotic kidney preparation, the selective A1AR antagonist DPCPX did not alter the vasoconstrictor effect of ANG II (4). If one assumes that the source of adenosine or its precursor ATP is predominantly the renal epithelial cells, one would not expect A1AR to be highly activated in a preparation that is devoid of epithelial structures.

The mechanisms responsible for the effect of A1AR activation on ANG II responsiveness have not been addressed in this study. However, there is substantial evidence for a synergistic interaction between Gq- and Gi-coupled receptors using a variety of different agonists in a number of different tissues. Overall, these studies show that activation of Gi-coupled receptors such as A1AR often synergistically enhances the accumulation of inositol trisphosphate and subsequent increases in cytosolic calcium caused by activation of Gq-coupled receptors. The underlying reason appears to be an enhanced activation of phospholipase C, resulting from simultaneous exposure to Gαq and to the Gβγ dimers dissociated from Gαi (21). There is also evidence that this synergy is especially effective in cells with a high level of A1AR expression, and this may explain why afferent arterioles are a site where the angiotensin-adenosine interactions are detectable most clearly (2). Further studies are needed to test the validity of this concept in the renal vasculature.

ANG II has been found in the present experiments to constrict isolated afferent arterioles from wild-type mice with an EC_{50} of $\sim 10^{-10}$ M and to cause maximum constriction consisting of essentially complete vessel closure at $\sim 10^{-9}$ M. In previous studies in perfused afferent arterioles from rabbits and rats, EC_{50} values for ANG II vasoconstriction of 10^{-9} M and maximum effects at 10^{-7} M have been reported, suggesting that mouse arterioles may be somewhat more sensitive to ANG II (18, 20). This conclusion is slightly at variance with a study in which vasoconstriction of isolated perfused afferent arterioles from the mouse required higher threshold concentrations of ANG II and in which the maximum effect consisted of an about 30–

40% reduction in diameter (12). Effects comparable to those seen in our study were only observed when vessels were treated with the NOS blocker L-NAME, suggesting that the effect of ANG II was counteracted by the release of nitric oxide. Whether differences in endothelial function are responsible for the enhanced response to ANG II in our studies remains to be determined. Compared with vessels isolated from wild-type mice, arterioles originating from A1AR knockout mice had a significantly lower response to ANG II in the threshold concentration range around 10^{-10} M, resulting in a significant right shift of the dose-response relationship and a significantly higher EC_{50} concentration. This observation indicates that vessels from wild-type mice generate adenosine that via activation of A1AR enhances the effect of ANG II.

In conclusion, the reduction in GFR and the increase in renal vascular resistance caused by acute ANG II administration is significantly reduced in A1AR knockout mice. Furthermore, the relationship between bath ANG II and vasoconstriction of isolated perfused afferent arterioles is shifted to the right in vessels from A1AR knockout compared with vessels from wild-type mice. Thus chronic absence of A1AR diminishes the effectiveness of ANG II to constrict renal resistance vessels.

This work was supported by intramural funds from the Institute of Diabetes and Digestive and Kidney Diseases at the National Institutes of Health.

REFERENCES

1. **Aki Y, Nishiyama A, Miyatake A, Kimura S, Kohno M, and Abe Y.** Role of adenosine A(1) receptor in angiotensin II- and norepinephrine-induced renal vasoconstriction. *J Pharmacol Exp Ther* 303: 117–123, 2002.
2. **Biber K, Klotz KN, Berger M, Gebicke-Harter PJ, and van Calker D.** Adenosine A1 receptor-mediated activation of phospholipase C in cultured astrocytes depends on the level of receptor expression. *J Neurosci* 17: 4956–4964, 1997.
3. **Carmines PK and Inscho EW.** Renal arteriolar angiotensin responses during varied adenosine receptor activation. *Hypertension* 23: I114–I119, 1994.
4. **Dietrich MS, Endlich K, Parekh N, and Steinhausen M.** Interaction between adenosine and angiotensin II in renal microcirculation. *Microvasc Res* 41: 275–288, 1991.
5. **Dietrich MS and Steinhausen M.** Differential reactivity of cortical and juxtamedullary glomeruli to adenosine-1 and adenosine-2 receptor stimulation and angiotensin-converting enzyme inhibition. *Microvasc Res* 45: 122–133, 1993.
6. **Hall JE and Granger JP.** Adenosine alters glomerular filtration control by angiotensin II. *Am J Physiol Renal Fluid Electrolyte Physiol* 250: F917–F923, 1986.
7. **Holz FG and Steinhausen M.** Renovascular effects of adenosine receptor agonists. *Renal Physiol* 10: 272–282, 1987.
8. **Jackson EK, Zhu C, and Tofovic SP.** Expression of adenosine receptors in the preglomerular microcirculation. *Am J Physiol Renal Physiol* 283: F41–F51, 2002.
9. **Jensen BL, Ellekvist P, and Skott O.** Chloride is essential for contraction of afferent arterioles after agonists and potassium. *Am J Physiol Renal Physiol* 272: F389–F396, 1997.
10. **Nishiyama A, Inscho EW, and Navar LG.** Interactions of adenosine A_1 and A_{2a} receptors on renal microvascular reactivity. *Am J Physiol Renal Physiol* 280: F406–F414, 2001.
11. **Osswald H, Schmitz HJ, and Heidenreich O.** Adenosine response of the rat kidney after saline loading, sodium restriction and hemorrhagia. *Pflügers Arch* 357: 323–333, 1975.
12. **Patzak A, Mrowka R, Storch E, Hocher B, and Persson PB.** Interaction of angiotensin II and nitric oxide in isolated perfused afferent arterioles of mice. *J Am Soc Nephrol* 12: 1122–1127, 2001.
13. **Sasamura H, Hein L, Krieger JE, Pratt RE, Kobilka BK, and Dzau VJ.** Cloning, characterization, and expression of two angiotensin receptor (AT-1) isoforms from the mouse genome. *Biochem Biophys Res Commun* 185: 253–259, 1992.
14. **Spielman WS and Osswald H.** Blockade of postocclusive renal vasoconstriction by an angiotensin II antagonists: evidence for an angiotensin-adenosine interaction. *Am J Physiol Renal Fluid Electrolyte Physiol* 237: F463–F467, 1979.
15. **Sun D, Samuelson LC, Yang T, Huang Y, Paliege A, Saunders T, Briggs J, and Schnermann J.** Mediation of tubuloglomerular feedback by adenosine: evidence from mice lacking adenosine 1 receptors. *Proc Natl Acad Sci USA* 98: 9983–9988, 2001.
16. **Weaver DR and Reppert SM.** Adenosine receptor gene expression in rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 263: F991–F995, 1992.
17. **Weihprecht H, Lorenz JN, Briggs JP, and Schnermann J.** Synergistic effects of angiotensin and adenosine in the renal microvasculature. *Am J Physiol Renal Fluid Electrolyte Physiol* 266: F227–F239, 1994.
18. **Weihprecht H, Lorenz JN, Briggs JP, and Schnermann J.** Vasoconstrictor effect of angiotensin and vasopressin in isolated rabbit afferent arterioles. *Am J Physiol Renal Fluid Electrolyte Physiol* 261: F273–F282, 1991.
19. **Weihprecht H, Lorenz JN, Briggs JP, and Schnermann J.** Vasomotor effects of purinergic agonists in isolated rabbit afferent arterioles. *Am J Physiol Renal Fluid Electrolyte Physiol* 263: F1026–F1033, 1992.
20. **Yuan BH, Robinette JB, and Conger JD.** Effect of angiotensin II and norepinephrine on isolated rat afferent and efferent arterioles. *Am J Physiol Renal Fluid Electrolyte Physiol* 258: F741–F750, 1990.
21. **Zhu X and Birnbaumer L.** G protein subunits and the stimulation of phospholipase C by Gs-and Gi-coupled receptors: lack of receptor selectivity of Gα(16) and evidence for a synergic interaction between Gβγ and the α subunit of a receptor activated G protein. *Proc Natl Acad Sci USA* 93: 2827–2831, 1996.