Efferent arterioles exclusively express the subtype 1A angiotensin receptor: functional insights from genetic mouse models

Lisa M. Harrison-Bernard,1,2 Christopher J. Monjure,1 and Benjamin J. Bivona1
1Department of Physiology, Louisiana State University Health Sciences Center, and 2Department of Physiology, Tulane University Health Sciences Center, New Orleans, Louisiana

Submitted 30 June 2005; accepted in final form 1 December 2005

Harrison-Bernard, Lisa M., Christopher J. Monjure, and Benjamin J. Bivona. Efferent arterioles exclusively express the subtype 1A angiotensin receptor: functional insights from genetic mouse models. Am J Physiol Renal Physiol 290: F1177–F1186, 2006—Angiotensin (ANG) type 1A (AT1A) receptor-null (AT1A−/−) mice exhibit reduced afferent arteriolar (AA) constrictor responses to ANG II compared with wild-type (WT) mice, whereas efferent arteriolar (EA) responses are absent (Harrison-Bernard LM, Cook AK, Oliverio MI, and Coffman TM. Am J Physiol Renal Physiol 284: F538–F545, 2003). In the present study, the renal arteriolar constrictor responses to norepinephrine (NE) and/or ANG II were determined in blood-perfused juxtamedullary nephrons from kidneys of AT1A−/−, AT1B receptor-null (AT1B−/−), and WT mice. Baseline AA diameter in AT1A−/− mice was not different from that in WT mice (13.1 ± 0.9 and 12.6 ± 0.9 μm, n = 7 and 8, respectively); however, EA diameters were significantly larger (17.3 ± 1.4 vs. 11.7 ± 0.4 μm, n = 10 and 8) in AT1A−/− than in WT mice. Constriction of AA (−40 ± 8 and −51 ± 6% at 1 μM NE) and EA (−29 ± 6 and −38 ± 3% at 1 μM NE) in response to 0.1–1 μM NE was similar in AT1A−/− and WT mice. Baseline diameters of AA (13.5 ± 0.7 and 14.2 ± 0.9 μm, n = 9 and 10) and EA (15.4 ± 1.0 and 15.0 ± 0.7 μm, n = 11 and 9) and ANG II (0.1–10 nM) constriction responses of AA (−25 ± 4 and −31 ± 5% at 10 nM) and EA (−32 ± 6 and −35 ± 7% at 10 nM) were similar in AT1B−/− and WT mice, respectively. ANG II-induced constrictions were eliminated by AT1 receptor blockade with 4 μM candesartan. Taken together, our data demonstrate that AA and EA responses to NE are unaltered in the absence of AT1A receptors, and ANG II responses remain intact in the absence of AT1B receptors. Therefore, we conclude that AT1A and AT1B receptors are functionally expressed on the AA, whereas the EA exclusively expresses the AT1A receptor.

Afferent arteriole; efferent arteriole; juxtamedullary nephron; candesartan; norepinephrine

There are at least two major angiotensin II (ANG II) receptors: AT1 and AT2. In the kidney, most of the actions of ANG II on hemodynamic and tubular function, including afferent and efferent arteriolar vasoconstriction, sodium and fluid reabsorption (21), and growth and differentiation (30), are thought to be mediated via the AT1 receptor. Two subtypes of the AT1 receptor, AT1A and AT1B, have been identified in the mouse (27) and rat (12). The amino acid, open reading frame, and cDNA sequence homologies of the rat AT1A and AT1B receptors are 95, 91, and 74%, respectively (13). The mouse ANG receptor genes Agtr1a and Agtr1b map to different chromosomes: 13 and 3, respectively (18). The AT1A receptor is thought to be the predominant renal form (17); however, AT1B receptor function in the kidney has not been extensively studied. Previously, utilizing immunohistochemical approaches and an antibody that recognizes AT1A and AT1B receptor subtypes, we demonstrated the localization of the renal vascular AT1 receptor protein; these studies revealed the presence of the AT1 receptor on afferent and efferent arterioles (6) and are consistent with existing functional data (5). Similar results were obtained using an AT1A receptor-specific antibody (32) or targeted replacement of the AT1A receptor loci by the lacZ gene (19). RT-PCR analysis of AT1A and AT1B receptor mRNA in microdissected segments also indicates expression of AT1A and AT1B receptors on the afferent arteriole and glomerulus (20). However, the AT1A and AT1B receptor protein expression patterns and their differential contributions to ANG II functional responses of the efferent arteriole are not known.

The degree to which the AT1A and AT1B receptors are involved in the renal vasoconstrictor response to ANG II or the extent of differential regulation of expression of the two receptor subtypes is unclear. Determination of the functional responses of these two subtypes is not possible using pharmacological inhibitors, because those available do not distinguish between the AT1A and AT1B receptors. In this respect, AT1A receptor-deficient (AT1A−/−) mice have provided important insights into the renal vasoconstrictor actions of ANG II (5, 25). Our previous study (5), performed in kidneys from AT1A−/− mice, suggests that the vasoconstrictor response to ANG II is mediated by AT1A and AT1B receptors in the afferent arteriole, which is consistent with the mRNA expression pattern. The conclusion was based on the finding that the vasoconstrictor response to ANG II in afferent arterioles of kidneys from AT1A−/− mice was attenuated, but not abolished, presumably because of the presence of the AT1B receptor. Surprisingly, the efferent arteriole of kidneys from AT1A−/− mice did not respond to ANG II. Therefore, these results suggested that AT1A is the only AT1 receptor expressed on the efferent arteriole. In the present study, vascular responses to norepinephrine were determined in afferent and efferent arterioles of kidneys from AT1A−/− mice to test the hypothesis that the full vasoconstrictor potential of these arterioles is not altered. Similar responsiveness to norepinephrine in wild-type (WT) and AT1A−/− mice would indicate that the renal vasoconstrictor potential of these arterioles is not altered. Similar responsiveness to norepinephrine in wild-type (WT) and AT1A−/− mice would indicate that the renal vasoconstrictor potential of these arterioles is not altered. Similar responsiveness to norepinephrine in wild-type (WT) and AT1A−/− mice would indicate that the renal vasoconstrictor potential of these arterioles is not altered.

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.
ally, studies were designed to further address the contribution of the AT1A and AT1B receptors to the vasoconstrictor responses of afferent and efferent arterioles to ANG II. Specifically, we tested the hypothesis that efferent arteriolar vasoconstrictor responses to ANG II are fully expressed in kidneys of mice lacking the AT1B receptor (AT1B<sup>−/−</sup>), whereas the afferent arteriolar response would be attenuated. In addition, because in our previous study (5) the afferent arteriolar response to 10 nM ANG II was reduced by 40% and the efferent arteriolar response was completely absent in kidneys of AT1A<sup>−/−</sup> mice, responses to a 10-fold higher concentration of ANG II were investigated to determine the maximal response of the AT1B receptor in AT1A<sup>−/−</sup> mice. Studies were carried out using the mouse in vitro blood-perfused juxtamедulillary nephron technique, which allows for direct video-microscopic visualization of afferent and efferent arteriolar diameters studied in situ in kidneys obtained from mice with gene-targeted deletion of the AT1A or AT1B receptor.

METHODS

Animals. The procedures were approved by the Institutional Animal Care and Use Committee of Louisiana State University Health Sciences Center (LSUHSC) and Tulane University Health Sciences Center (TUHSC) and were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Afferent and efferent arteriolar diameters were assessed in kidneys obtained from 52 adult (2- to 12-mo-old) male WT (n = 20), AT1A<sup>−/−</sup> (n = 15), and AT1B<sup>−/−</sup> (n = 17) mice purchased from Jackson Laboratory (Bar Harbor, ME; WT: C57BL/6, 2.5–3.25 mo old, n = 5; AT1A<sup>−/−</sup>: B6.129P2 Agtr1a<sup>−/−</sup>, 2.75–3.25 mo old, n = 4), obtained from T. M. Coffman (Duke University and Durham Veterans Affairs Medical Center; WT: 4–11.5 mo old, n = 6; AT1A<sup>−/−</sup>: 4–12 mo old, n = 11), or bred in our colony at LSUHSC from breeder mice obtained from T. M. Coffman (WT: 1.75–4 mo old, n = 9; AT1B<sup>−/−</sup>: 3–4.3 mo old, n = 17). WT and AT1A<sup>−/−</sup> mice obtained from T. M. Coffman were generated by intercrossing heterozygous AT1A<sup>−/+;−/−</sup> mice purchased from T. M. Coffman (Duke University and Durham Veterans Affairs Medical Center). WT and AT1B<sup>−/−</sup> mice were generated by intercrossing heterozygous AT1B<sup>−/+;−/−</sup> mice purchased from T. M. Coffman (inbred 8–10 generations) and AT1A<sup>−/−</sup> C57BL/6 (inbred 6–8 generations) mice as described in detail elsewhere (16). Genotyping of mice bred in our colony was performed by PCR in duplicate on DNA obtained by tail biopsy using direct PCR (Viagen Biotech) according to the manufacturer’s instructions (200 µl total volume). PCR primer sequences for the AT1A and AT1B genotypes (Fig. 1) were kindly provided by T. M. Coffman. PCR products were subjected to electrophoresis on a 1.6% agarose gel stained with ethidium bromide (Fig. 1). Bands of PCR products were visualized using the Bio-Rad Ver-
was secured to an upright fixed-stage light microscope (model SMZ-1000, Nikon) with a high-intensity fiber-optic illuminator (model N1, Nikon) and a stereo-immersion objective in addition to a variable-focus lens. The tissue was immediately perfused with Tyrode buffer (Sigma) containing 51 g/l bovine serum albumin (98.99% albumin; Sigma) and a mixture of L-amino acids at pH 7.4 as we previously described in detail (3). A section of liver was removed from each animal that was obtained from Jackson Laboratory or T. M. Coffman, immersed into liquid nitrogen, and stored at −85°C for confirmation of genotyping by PCR (11). The kidney was placed in a perfusion chamber at room temperature for the dissection procedure: the ventral one-third of the kidney was removed, the papilla was reflected, the renal veins were cut open, 10.0 nylon suture (catalog no. DRM4, Sharp point) ties were placed on the distal segments of the large arterial branches, and connective tissue and pelvic mucosa overlying the juxtamedullary cortical surface were removed.

Rats were anesthetized with pentobarbital sodium (70 mg/kg ip) and subjected to bilateral nephrectomy. The rat was exsanguinated via a carotid arterial cannula into a heparinized syringe. Blood was processed to remove leukocytes and platelets as we previously described (5). After completion of all microdissection procedures, the Tyrode perfusate was replaced with the reconstituted rat blood (hematocrit 0.20). Renal arterial perfusion pressure was measured using a pressure transducer (model P23XL-1, Becton Dickinson) and amplified (models DA100C and TCI100, BIOPAC Systems, Goleta, CA). Pressure was maintained at 95 mmHg by continual adjustment of the perfusion apparatus (models DA100C and TCI100, BIOPAC Systems, Goleta, CA). Pressure was monitored using a pressure transducer (model P23XL-1, Becton Dickinson) and amplified (models DA100C and TCI100, BIOPAC Systems, Goleta, CA). Pressure was maintained at 95 mmHg by continual adjustment of the perfusion apparatus (models DA100C and TCI100, BIOPAC Systems, Goleta, CA).

Afferent and efferent arteriolar responses to norepinephrine in AT1A−/− and WT mice. We previously reported that afferent arteriolar constriction to ANG II were attenuated and efferent arteriolar responses were absent in kidneys from AT1A−/− compared with WT mice (5). To determine the arteriolar vasoconstrictor potential of renal arterioles from AT1A−/− and WT mice, afferent and efferent arteriolar diameters were measured during superfusion with norepinephrine. After a stabilization period, afferent or efferent arteriolar luminal diameter was monitored under baseline conditions (5 min) and during sequential exposure to increasing concentrations (0.1, 0.3, and 1.0 μM) of norepinephrine (5 min at each concentration). A 15-min recovery period followed. In some kidneys [AT1A−/− (n = 7) and WT (n = 6)], a second norepinephrine protocol was performed after a recovery period.

Afferent and efferent arteriolar responses to 100 nM ANG II in AT1A−/− and WT mice. Because we previously observed that afferent arteriolar constriction to 10 nM ANG II were attenuated and efferent arteriolar responses were absent in kidneys from AT1A−/− compared with WT mice (5), afferent and efferent arteriolar diameters of AT1A−/− and WT mice were measured during superfusion with a 10-fold higher concentration of ANG II to determine the maximal ANG II-induced AT1R Receptor-mediated vasoconstriction. After recovery from the norepinephrine protocol, afferent or efferent arteriolar luminal diameter was measured under baseline conditions (5 min), during exposure to 100 nM ANG II (5 min), and during a recovery period (5 min). In some kidneys [AT1A−/− (n = 5) and WT (n = 5)], a second ANG II protocol was performed after the recovery period. We previously reported that mouse afferent arteriolar responses to first and second applications of increasing concentrations of these doses of ANG II did not differ significantly (5). In one each of the AT1A−/− and WT kidneys, a third protocol was performed after the recovery period.

Afferent and efferent arteriolar responses to ANG II before and after AT1 receptor blockade in AT1B−/− mice. To determine whether the afferent and efferent arteriolar ANG II vasoconstrictions observed in kidneys of AT1B−/− mice are mediated by an AT1 receptor, responses to ANG II were observed before and after blockade of the AT1 receptor (n = 8) in the same vessel. After a stabilization period, afferent or efferent arteriolar luminal diameter was monitored under baseline conditions (5 min) and during sequential exposure to increasing concentrations (0.1, 1.0, and 10 nM) of ANG II (5 min at each concentration) in kidneys of AT1B−/− and WT mice. A 10-min recovery period followed. In some AT1B−/− (n = 6) and all WT kidneys, a second ANG II protocol was performed after the recovery period. We previously reported that mouse afferent arteriolar responses to first and second applications of increasing concentrations of these doses of ANG II did not differ significantly (5). In one each of the AT1A−/− and WT kidneys, a third protocol was performed after the recovery period.

Reagents. Norepinephrine (norepinephrine bitartrate injection, 1 mg/ml; catalog no. L-680, Levpod, Abbott Laboratories) was diluted in Tyrode solution on the day of the experiment to achieve a final concentration of 1 μM. Serial dilutions were performed to achieve 0.3 and 0.1 μM norepinephrine. Human ANG II (catalog no. 002-12, Phoenix Pharmaceuticals) was dissolved in 0.9% NaCl at 1 mM, stored at −20°C, and diluted on the day of the experiment in Tyrode solution to achieve a final concentration of 100 or 10 nM. Serial dilutions were performed to achieve 1.0 and 0.1 nM ANG II.

Data analysis. Renal arterial perfusion pressure and vessel diameters were sampled at 1 Hz and converted to digital form using a computer (Intel Pentium 4 at 2.8 GHz, Optiplex GX260, Dell Com-
RESULTS

\(\text{AT1A}^{-/-}, \text{AT1B}^{-/-}, \text{and WT animals.} \) Age (7.6 ± 1.0 and 5.5 ± 1.2 mo) and body weight (39.8 ± 2.8 and 36.0 ± 3.5 g) were not different between all AT1A\(^{-/-}\) (n = 15) and WT (n = 11) mice, respectively. Because the laboratory was relocated during this portion of the study (from TUHSC to LSUHSC), AT1A\(^{-/-}\) and WT mice were studied at a younger and an older age. Age and body weight were similar in younger AT1A\(^{-/-}\) (4.0 ± 0.4 mo, 31.6 ± 1.9 g, n = 8) and younger WT (3.3 ± 0.3 mo, 29.8 ± 1.7 g, n = 8) mice, as well as in older AT1A\(^{-/-}\) (11.3 ± 0.2 mo, 49.1 ± 2.4 g, n = 7) and older WT (11.3 ± 0.3 mo, 52.3 ± 3.5 g, n = 3) mice. Because norepinephrine responses of the efferent arterioles of kidneys from AT1A\(^{-/-}\) mice and afferent and efferent arterioles of WT mice were not statistically different as a function of age, the data were combined. However, afferent arteriolar responses to norepinephrine were significantly attenuated in the old compared with the young AT1A\(^{-/-}\) mice as discussed in detail below. Age (3.7 ± 0.1 vs. 2.8 ± 0.3 mo) and body weight (29.2 ± 0.7 vs. 26.7 ± 0.7 g) were slightly but significantly greater in male AT1B\(^{-/-}\) (n = 17) than WT (n = 9) mice.

Afferent and efferent arteriolar responses to norepinephrine in AT1A\(^{-/-}\) and WT mice. Afferent arteriolar baseline diameters were not significantly different between AT1A\(^{-/-}\) (n = 7) and WT (n = 8) mice, averaging 13.1 ± 0.9 and 12.9 ± 1.4 \(\mu\)m, respectively, as reported previously by us (5) (Fig. 2A) and others (9). Norepinephrine produced a rapid and significant constriction in afferent arterioles of AT1A\(^{-/-}\) and WT mice (Fig. 2A). The norepinephrine-induced afferent arteriolar constriction was not different between AT1A\(^{-/-}\) and WT mice \((P = 0.12)\). Afferent arteriolar diameter of AT1A\(^{-/-}\) mice decreased 10 ± 2, 24 ± 6, and 40 ± 8% in response to 0.1, 0.3, and 1.0 \(\mu\)M norepinephrine, respectively \((P < 0.05; \text{Fig. } 2B)\). In WT mice, afferent arteriolar diameter decreased 12 ± 3, 29 ± 4, and 51 ± 6%, respectively, in response to the same concentrations of norepinephrine \((P < 0.05; \text{Fig. } 2B)\). Maximal afferent arteriolar contractions to 1,000 nM norepinephrine were 22% less in AT1A\(^{-/-}\) than WT mice, although this was not statistically significant \((P = 0.23)\). The vasoconstrictor responses of afferent arterioles of AT1A\(^{-/-}\) and WT mice to 0.1–1.0 \(\mu\)M norepinephrine were not significantly different, indicating that the vasoconstrictor potential of the preglomerular vascular smooth muscle cells is not altered by the absence of AT1A expression.

The norepinephrine vasoconstrictor response of afferent arterioles of AT1A\(^{-/-}\) mice was reduced with increasing age. Afferent arterioles of older AT1A\(^{-/-}\) mice \((n = 5, 11.5 ± 0.2\) mo) responded to 0.1, 0.3, and 1.0 \(\mu\)M norepinephrine with a reduction in vessel diameter of only 4 ± 1, 9 ± 2, and 27 ± 5%, respectively, which was significantly less than the response to norepinephrine in the younger AT1A\(^{-/-}\) mice \((4.1 ±\)
Efferent arteriolar baseline diameters were significantly decreased in AT1A
mice and by 6% in AT1A
mice does not reflect an inability of vascular smooth muscle cell contraction. Diameters of afferent and efferent arterioles of AT1A
mice and WT mice returned to values not different from baseline on removal of norepinephrine. Afferent and efferent arteriolar responses to 100 nM ANG II in AT1A
and WT mice. To determine the full ANG II constrictor potential of the AT1B receptor, arteriolar responses to 100 nM ANG II were examined in kidneys from AT1A
and WT mice. ANG II produced a significant decrease in diameter of afferent (n = 8), but not efferent (n = 8) arterioles of AT1A
mice (Fig. 4). Afferent (n = 5) and efferent (n = 7) arteriolar diameters of WT mice decreased significantly in response to 100 nM ANG II (Fig. 4). However, the ANG II responses were significantly attenuated in the absence of the AT1A receptor. Afferent arteriolar diameter decreased 1.2 ± 0.4 and 3.9 ± 1.2 μm, whereas efferent diameter decreased 0.1 ± 0.1 and 3.1 ± 0.8 μm, in AT1A
and WT mice, respectively. In WT mice, ANG II (100 nM) reduced afferent and efferent arteriolar diameter 29 ± 7 and 28 ± 7%, respectively, indicating that the ANG II-induced vasoconstriction is nearly identical in pre- and postglomerular arterioles of the normal mouse kidney. The afferent arteriolar vasoconstrictor response to 100 nM ANG II in the AT1A
mice was only 25% of the response in the WT mice, indicating the contribution of the AT1B receptor. These data show that the efferent arteriole of AT1A
mice does not respond to ANG II, even at very high concentrations, indicating that the efferent arteriole expresses only the AT1A receptor. Diameters of afferent and

0.5 mo). Additionally, baseline diameter of afferent arterioles (18.7 ± 1.2 μm) was significantly larger in the older AT1A
than in the younger AT1A
mice.

Efferent arteriolar baseline diameters were significantly larger in kidneys from AT1A
(n = 10) than WT mice (n = 8), averaging 17.3 ± 1.4 and 11.7 ± 0.4 μm, respectively (Fig. 3A). The larger baseline efferent arteriolar diameter of AT1A
mice is in agreement with our previous findings (5). Efferent arteriolar diameters were significantly decreased in response to increasing concentrations of norepinephrine in AT1A
and WT mice. The norepinephrine-induced efferent arteriolar constriction was not different between AT1A
and WT mice (P = 0.26). Efferent arteriolar diameter decreased by 6 ± 1, 12 ± 3, and 29 ± 6% in AT1A
mice and by 6 ± 1, 17 ± 2, and 38 ± 3% in WT mice during exposure to 0.1, 0.3, and 1 μM norepinephrine, respectively (Fig. 3B). Maximal efferent arteriolar contractions to 1,000 nM norepinephrine were 24% less in AT1A
than WT mice, but this difference did not reach statistical significance (P = 0.23). Therefore, the lack of an effect of ANG II on efferent arteriolar diameter of AT1A
mice does not reflect an inability of vascular smooth muscle cell contraction. Diameters of afferent and efferent arterioles of AT1A
and WT mice returned to values not different from baseline on removal of norepinephrine.

Afferent and efferent arteriolar responses to 100 nM ANG II in AT1A
and WT mice. ANG II produced a significant vasoconstriction in both groups. Baseline efferent arteriolar diameters were significantly larger in AT1A
than WT vessels. *P < 0.05 vs. baseline. †P < 0.05 vs. WT.

Fig. 3. Efferent arteriolar diameter responses to 0.1, 0.3, and 1.0 μM norepinephrine in AT1A
(●, n = 10) and WT (○, n = 8) mice. Norepinephrine produced a significant vasoconstriction in both groups. Baseline efferent arteriolar diameters were significantly larger in AT1A
than WT vessels. *P < 0.05 vs. baseline. †P < 0.05 vs. WT.

Fig. 4. Afferent (■, n = 7 AT1A
and □, 5 WT) and efferent (■, n = 9 AT1A
and □, 7 WT) arteriolar diameter responses to 100 nM ANG II in AT1A
and WT mice. ANG II produced a significant vasoconstriction in efferent arterioles of both groups; response was reduced in AT1A
compared with WT mice. Efferent arterioles of WT mice constricted in response to ANG II; vessels from AT1A
mice did not. *P < 0.05 vs. baseline. †P < 0.05 vs. WT.
efferent arterioles of \( \text{AT}_{1A}^{-/-} \) and WT mice returned to values not different from baseline on removal of ANG II.

**Afferent and efferent arteriolar responses to ANG II in \( \text{AT}_{1B}^{-/-} \) and WT mice.** Afferent arteriolar baseline diameters were not significantly different between \( \text{AT}_{1B}^{-/-} (n = 13) \) and WT \( (n = 10) \) mice, averaging \( 13.5 \pm 0.7 \) and \( 14.2 \pm 0.9 \mu m \), respectively (Fig. 5A). Similarly, efferent arteriolar baseline diameters were not significantly different between \( \text{AT}_{1B}^{-/-} (n = 11) \) and WT \( (n = 9) \) mice, averaging \( 15.4 \pm 1.0 \) and \( 15.0 \pm 0.7 \mu m \), respectively (Fig. 6A). ANG II produced a significant constriction in afferent and efferent arterioles of \( \text{AT}_{1B}^{-/-} \) and WT mice. Afferent arteriolar diameter of \( \text{AT}_{1B}^{-/-} \) mice decreased \( 10 \pm 2 \) and \( 25 \pm 4\% \) in response to 1 and 10 nM ANG II, respectively \((P < 0.05; \text{Fig. 5B})\). In WT mice, afferent arteriolar diameter decreased \( 10 \pm 2 \) and \( 31 \pm 5\% \) in response to 1 and 10 nM ANG II, respectively \((P < 0.05; \text{Fig. 5B})\). The vasoconstrictor responses of afferent arterioles of \( \text{AT}_{1B}^{-/-} \) and WT mice were not significantly different, indicating that, in the absence of the \( \text{AT}_{1B} \) receptor, the \( \text{AT}_{1A} \) receptor mediates ANG II constriction similar to that in WT mice. Efferent arteriolar diameter decreased \( 11 \pm 4 \) and \( 32 \pm 6\% \) in \( \text{AT}_{1B}^{-/-} \) mice and \( 11 \pm 3 \) and \( 35 \pm 7\% \) in WT mice. Candesartan blocked the vasoconstrictor response to ANG II in \( \text{AT}_{1B}^{-/-} \) mice. Baseline diameters were not significantly different. \( *P < 0.05 \) vs. baseline. †\( P < 0.05, \text{AT}_{1B}^{-/-} \) vs. \( \text{AT}_{1B}^{-/-} + \text{candesartan} \).
mice in response to 1 and 10 nM ANG II, respectively ($P < 0.05$; Fig. 6B). The ANG II vasoconstrictor responses of efferent arterioles of AT1B$^{-/-}$ and WT mice were not significantly different, indicating that the efferent arteriolar vasoconstrictor response is mediated by the AT1A receptor only.

Because ANG II responses in AT1B$^{-/-}$ and WT mice were not different, the data were pooled for afferent and efferent arteriolar responses to ANG II. For the pooled data, 1 nM ANG II produced a $10 \pm 2$ and $11 \pm 2\%$ decrease in diameter, whereas 10 nM ANG II produced a $27 \pm 3$ and $34 \pm 4\%$ decrease in diameter, in afferent ($n = 23$) and efferent ($n = 20$) arterioles, respectively. The combined afferent and efferent arteriolar responses to ANG II were not significantly different, indicating that, in the mouse kidney, ANG II produces an equal effect on pre- and postglomerular arterioles. Diameters of afferent arterioles of AT1B$^{-/-}$ mice and efferent arterioles of WT mice returned to values different from baseline on removal of ANG II. However, the 10-min recovery period may not have been sufficient for a complete return of vessel diameter of efferent arterioles of AT1B$^{-/-}$ mice and afferent arterioles of WT mice. The recovery diameters were within $4 \pm 2$ and $6 \pm 2\%$ of baseline values for efferent arterioles of AT1B$^{-/-}$ mice and afferent arterioles of WT mice, respectively.

**Afferent and efferent arteriolar responses to ANG II before and after AT1 receptor blockade in AT1B$^{-/-}$ mice.** The AT1 receptor antagonist candesartan alone did not alter afferent ($n = 4$) or efferent ($n = 4$) arteriolar diameters (102 and 100% of control) of AT1B$^{-/-}$ mice. Blockade of the AT1 receptor with candesartan completely prevented the afferent and efferent arteriolar vasoconstrictor responses to ANG II in kidneys from AT1B$^{-/-}$ mice (Figs. 5B and 6B). These data indicate that the ANG II-induced vasoconstrictions of afferent and efferent arterioles of AT1B$^{-/-}$ mice are mediated by the remaining AT1A receptor and that ANG II administration in the presence of AT1 receptor blockade does not produce an AT2 receptor-mediated renal vasodilation in AT1B$^{-/-}$ mice.

**DISCUSSION**

ANG II has powerful effects on renal hemodynamics that are mediated principally by the AT1 receptor. ANG II regulates renal blood flow and glomerular filtration rate by altering the vascular tone of afferent and efferent arterioles. Studies of the effects of ANG receptor blockers have been used widely to assess the role of ANG II in renal physiology and pathophysiology. However, the currently available pharmacological inhibitors of the AT1 receptor are unable to distinguish between the AT1A and AT1B receptors. Therefore, the recent development of mutant mouse strains defective in the expression of specific ANG receptor subtypes has provided a valuable tool to investigate the function of the individual receptor subtypes. AT1A$^{-/-}$ mice have a significantly reduced basal blood pressure and reduced systemic arterial pressure response to exogenous ANG II and exhibit a mild degree of renal abnormalities, including slight papillary hypoplasia and hyperplasia of renin-producing granular cells (11, 24, 28). In contrast, AT1B$^{-/-}$ mice exhibit a normal blood pressure, renal morphological phenotype, and pressor response to systemic bolus administration of ANG II (1, 23). Surprisingly, deletion of both AT1 receptor subtypes (23, 29) is necessary to mimic the severely reduced blood pressure and abnormal renal phenotype of mice deficient in angiotensinogen (14) and angiotensin-converting enzyme (2), indicating a potential compensatory role for the AT1B receptor in the regulation of blood pressure and kidney development. Our goal was to utilize mutant mouse strains of AT1 receptor subtypes to directly assess AT1A receptor function in the absence of AT1B receptors and AT1B receptor function in the absence of AT1A receptors, particularly as related to ANG II-regulated microvascular function in the kidney.

We previously reported (5) that the vasoconstrictor response of afferent arterioles of AT1A$^{-/-}$ and WT mice to low doses (0.1 and 1 nM) of ANG II is not different; however, the vasoconstrictor response of AT1A$^{-/-}$ mice to high-dose (10 nM) ANG II is only 60% of the magnitude of the response of WT mice. The aim of the present study was to determine the maximal afferent arteriolar AT1B receptor response to ANG II. Two possibilities were considered: AT1B receptors may have a maximal vasoconstrictor effect that was observed at 10 nM ANG II or may constrict further to 100 nM ANG II, demonstrating a powerful vasoconstrictor potential of the AT1B receptor on the renal preglomerular vessels. In the present study, we found that the magnitude of the response of afferent arterioles of AT1A$^{-/-}$ mice to 100 nM ANG II was only 25% of the response of WT mouse kidneys. Responses of afferent arterioles of AT1A$^{-/-}$ and WT mice to 0.1 and 1 nM ANG II were not significantly different (5). Therefore, there does not appear to be a shift in the dose-response curve to ANG II of the afferent arteriole of AT1A$^{-/-}$ mice but, rather, a suppressed maximal vasoconstrictor response, which is mediated by the single effect of the AT1B receptor.

Efferent arterioles of AT1A$^{-/-}$ mice did not respond to ANG II at any dose in our previous study (5). The aim of the present study was to determine whether higher doses of ANG II are required to stimulate potentially low-abundance AT1B receptors on the efferent arteriole. If higher doses of ANG II caused efferent arteriolar vasoconstriction in AT1A$^{-/-}$ mice, one possible explanation would be that the AT1B receptor is present on the efferent arteriole, but in much smaller numbers. However, efferent arterioles of AT1A$^{-/-}$ mice did not respond to 100 nM ANG II, supporting our hypothesis that the efferent arteriole expresses only the AT1A receptor.

Our finding of a lack of functional expression of the AT1B receptor in the mouse juxtamedullary efferent arteriole is difficult to reconcile with the recent work of Helou et al. (8). They reported that the thick muscular juxtamedullary efferent arterioles, which terminate as vasa recta of the rat kidney, express AT1A, AT1B, and AT2 receptor mRNA, whereas the thin juxtamedullary efferent arterioles, which terminate as peritubular capillaries, express only the AT1A and AT2 types, and at a much lower level (8). Helou et al. also reported a reduced ANG II-induced intracellular calcium concentration response in thin and thick juxtamedullary efferent arterioles that was blocked by an angiotensin receptor blocker, valsartan (8). Also, the ANG II-induced calcium responses were similar in afferent and efferent arterioles of a given nephron (7). It is interesting to note that juxtamedullary efferent arterioles of the rat and mouse kidney that lie on the innermost surface of the cortex give rise to peritubular capillaries and vasa recta. Therefore, it is possible that our functional studies of juxtamedullary efferent arterioles are not representative of the two subpopu-
lations of vessels studied by morphological identification and microdissection. Alternatively, there may be a species difference in the expression profile of AT1A and AT1B receptors on the muscular juxtamedullary efferent arterioles of the mouse and rat kidney.

In a very careful light- and electron-microscopic evaluation of renal arteries in WT and AT1A−/− mice, Inokuchi et al. (10) found that interlobular and afferent arterioles possessed an occasional abluminal overpopulation of vascular smooth muscle cells, increased wall thickness, and proximal expansion of renin-producing cells. They stated that the vascular smooth muscle cells of AT1A−/− mice clearly possess a contractile, but slightly synthetic phenotype compared with WT mice (10). An assessment of ultrastructure of the efferent arterioles of AT1A−/− mice was not reported in this study. Interestingly, overpopulation of vascular smooth muscle cells was specific to the intrarenal arteries (10). It is possible that these alterations in vascular structure may contribute to altered arteriolar function in AT1A−/− mice. However, we previously showed that afferent arteriolar responses to increases in renal perfusion pressure to 160 mmHg are preserved in AT1A−/− mice, with maximal responses yielding a 30% reduction in vessel diameter, which was not different from the responses in WT mice (5). To test the hypothesis that the reduced ANG II response observed in the afferent and efferent arterioles of AT1A−/− mice is not due to vascular smooth muscle cell developmental abnormalities, afferent and efferent arteriolar maximal vasoconstrictor responses were determined in response to norepinephrine. In the present study, we found that the norepinephrine-induced vasoconstrictor responses of afferent and efferent arterioles of AT1A−/− mice were not significantly different from those of WT mice. Therefore, the increased expression of renin and smooth muscle cell hyperplasia in the AT1A−/− afferent arteriole (24) does not appear to significantly affect the vasoconstrictor response to norepinephrine in this vessel. Unexpectedly, the afferent arteriolar responsiveness to norepinephrine in AT1A−/− mice was significantly reduced with aging. The mechanism of the suppressed vasoconstriction of afferent arterioles to norepinephrine in 1-yr-old AT1A−/− mice was not addressed in detail in the present study. It can be speculated that the degree of renin expression and smooth muscle cell hyperplasia may be altered with age and affect contractions to norepinephrine. In addition, the observance of a strong vasoconstriction of the efferent arteriole in the AT1A−/− mouse, in response to norepinephrine, indicates that the vascular smooth muscle cells are capable of vasoconstriction in response to another agonist. This is in agreement with the findings of Ryan et al. (26), who reported that the vasoconstrictor response to phenylephrine, U-46619, KCl, and 5-hydroxytryptamine in the isolated carotid artery was not altered in AT1A−/− compared with WT mice. Therefore, we conclude that the reduced vasoconstrictor response of the pre- and postglomerular vessels to ANG II in AT1A−/− mice is not solely limited by vascular smooth muscle cell contractile function, as assessed by norepinephrine, but is due to the loss of AT1A receptor function. Therefore, the suppressed afferent and absent efferent arteriolar response to ANG II in AT1A−/− mice appears to reflect the loss of AT1A receptor-mediated contraction specifically, rather than a global reduction in vasoconstrictor potential.

To directly assess the renal microvascular vasoconstrictor response of the AT1A receptor, we studied the diameter of afferent and efferent arterioles of AT1B−/− mice during application of ANG II. Because our earlier work supported the concept that AT1A and AT1B receptors are located on the afferent arteriole, we hypothesized that the afferent arteriolar vasoconstrictor response would be attenuated in the AT1B−/− mouse kidney. In addition, we expected that efferent arteriolar ANG II responses in AT1B−/− mice would be identical to those in WT mice if the AT1A receptor was the only subtype expressed on the efferent arteriole. To our surprise, the ANG II responses were almost identical in afferent and efferent arterioles of AT1B−/− and WT mice. Because the afferent and efferent arteriolar vascular smooth muscle cells of AT1B−/− mice responded to ANG II to the same degree as WT mice, the vasoconstrictor responses to norepinephrine were not conducted in the AT1B−/− mice. There are two possible explanations for the lack of an attenuation of the afferent arteriolar response to ANG II in the AT1B−/− mice: 1) The AT1B receptor compensates for the loss of the AT1A receptor in AT1A−/− null mice, and the observed response to ANG II reflects this enhancement. 2) The AT1A receptor expression may be enhanced in the absence of the AT1B receptor, as evidenced by the maximal ANG II responses of the AT1A receptors in AT1B−/− mice. There is evidence that the AT1B receptor compensates in the absence of the AT1A receptor by the small systemic pressure response to ANG II in AT1A−/− mice treated chronically with angiotensin-converting enzyme inhibition (22) and due to the difference in severity of AT1A null vs. AT1A/AT1B double-null renal phenotype. Taken together, there is strong evidence for mediation of ANG II-induced afferent arteriolar vasoconstriction by the AT1A and AT1B receptors, in addition to evidence for protein and mRNA expression of both subtypes on the afferent arteriole. The full recovery of the efferent arteriolar vasoconstrictor response to ANG II in the AT1B−/− mice supports the segmentally specific localization of the AT1A receptor as the only AT1 receptor subtype functionally expressed on the efferent arteriole of mouse juxtamedullary nephrons.

Functional and histological localization of the AT1A receptor to the renal arterioles has been reported by Kimura et al. (15) using targeted replacement of the AT1A receptor loci by the lacZ gene. The AT1A receptor was localized in the afferent and efferent arterioles of heterozygous mutant mouse kidney (15). ANG II similarly constricted the afferent and efferent arterioles of WT and heterozygous mice, but the responses were absent in homozygous-null hydronephrotic mice, arguing against AT1B receptor-mediated ANG II constriction (15). Our finding of AT1A and AT1B receptor function on the afferent arteriole is not in agreement with this argument (15) and may reflect an alteration in AT1B receptor function during the induction of hydronephrosis in AT1A receptor-null mice.

As has been shown previously in the rat kidney (4, 7, 15), the degree of afferent and efferent arteriolar ANG II vasoconstriction in WT and AT1B−/− mice was quite similar in magnitude and does not indicate a greater sensitivity of efferent than afferent arterioles to ANG II. Also, ANG II receptor blockade with candesartan completely blocked the ANG II constrictions in the AT1B−/− mice. We previously reported that candesartan blocks the ANG II-induced vasoconstriction in WT and AT1A−/− mice (5). In all genotypes studied, there was no evidence for afferent or efferent arteriolar vasodilation in response to ANG II in the presence of AT1 receptor blockade.
Therefore, our results do not support AT₂ receptor-mediated vasodilation in afferent or efferent arterioles in the isolated perfused mouse kidney.

In agreement with our previous study (5), we have demonstrated a significant difference in baseline efferent arteriolar diameter between AT₁A−/− and WT mice. Baseline efferent arteriolar diameter was 48% larger in AT₁A−/− than WT mice. Therefore, AT₁A receptor deletion affects baseline efferent arteriolar diameter and may reflect an effect of ANG II on arteriolar structure and/or allowance of a greater influence of vasodilator agents. Baseline diameters of afferent arterioles of AT₁A−/− mice, as well as afferent and efferent arterioles of AT₁B−/− mice, were not different from those of WT mice. Therefore, expression of the AT₁A receptor in the presence of a deletion of the AT₁B receptor restored the normal efferent arteriolar diameter.

Overall, our functional data on the vasoconstrictor response to ANG II in AT₁A and AT₁B Receptor-null mice indicate that the AT₁A receptor is the predominant renal microvascular AT₁ receptor subtype. There does appear to be compensation for the vasoconstrictor response to ANG II by the AT₁A and AT₁B receptors when the other subtype is not present. In contrast, Zhou et al. (33) showed that the vasoconstrictor response to ANG II is unaltered in the adrenal aorta and femoral artery of AT₁A−/− compared with WT mice, suggesting that the response is mediated predominantly by the AT₁B receptor. These findings have not been confirmed in the AT₁B−/− mice. Therefore, there appears to be a heterogeneity in the expression profile of the AT₁A and AT₁B receptors within the renal microcirculation, as well as in the systemic circulation. We conclude that the AT₁A and AT₁B receptors mediate the afferent arteriolar vasoconstriction to ANG II, whereas the AT₁A receptors alone mediate the efferent arteriolar responses to ANG II.

ACKNOWLEDGMENTS

The authors acknowledge the excellent technical assistance of Sara K. Smelcer and Justin D. Westervelt. The authors thank Dr. Samir S. El-Dahr for critically reviewing the manuscript and Dr. Anders Ljunggren (Astra Hassle, Gothenburg, Sweden) for generously providing the AT₁ receptor antagonist candesartan (Atacand). Portions of this work have been published in abstract form (FASEB J 18: A288, 2004; FASEB J 19: A1144, 2005).

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-62003 to L. M. Harrison-Bernard.

REFERENCES


