The renal vascular response to ANG II injection is reduced in the nonclipped kidney of two-kidney, one-clip hypertension

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Bivol, Liliana Monica, Øyvind Brune Vågnes, and Bjarne Magnus Iversen. The renal vascular response to ANG II injection is reduced in the nonclipped kidney of two-kidney, one-clip hypertension. Am J Physiol Renal Physiol 289: F393–F400, 2005. First published March 22, 2005; doi:10.1152/ajprenal.00319.2004.---The ANG II receptor 1 (AT1R) level in the nonclipped kidney of two-kidney, one-clip hypertension (2K1C) has shown to be unchanged despite a high circulating angiotensin (ANG) II level. To examine the vasoreactive response to ANG II in this kidney, injections of ANG II into renal artery were performed 6 wk after clipping of the kidney and compared with normotensive controls. The renal blood flow (RBF) response to 2.5 ng ANG II was measured by a Transonic transit-time flowmeter, before and after indomethacin and candesartan treatment, and analyzed by a computer program. The RBF response to 5 ng arginine-vasopressin (AVP) was examined for comparison with ANG II. The mRNA for AT1A and AT1B as well as Western blotting for AT1R in renal resistance vessels were determined, and plasma renin activity (PRA) was measured. Systolic blood pressure was 183 ± 4 mmHg in 2K1C rats compared with 113 ± 1 mmHg in controls (P < 0.001). PRA was significantly increased in 2K1C animals (P < 0.05). Injection of ANG II reduced RBF with 10 ± 2% in the nonclipped kidney and 24 ± 3% in controls (P < 0.001). After indomethacin, the RBF response increased from 10 ± 2 to 20 ± 3% (P < 0.02) in 2K1C rats and from 24 ± 3 to 34 ± 6% in controls (P < 0.01). The doses of candesartan needed to completely inhibit RBF response to ANG II were 30 μg/kg in the nonclipped kidney and 100 μg/kg in controls (P < 0.001). Western blotting and mRNA for AT1A and AT1B in the nonclipped kidney were similar to the controls. The results indicate that despite no difference in total AT1R levels, functional AT1R is downregulated in the nonclipped kidney of 2K1C rats.

Two-kidney, one-clip (2K1C) hypertension is an angiotensin II (ANG II)-dependent model of hypertension, and plasma as well as intrarenal ANG II concentrations are increased (14, 15). Removal of the clip is followed by rapid reversal of high blood pressure with normalization of renin values (14, 15). ANG II mediates its effect in the renal vasculature through two main G-coupled receptors, the AT1 (AT1R) and AT2 receptors, where AT1R mediates the vascular constrictor effects of ANG II (2). The rat has two types of AT1 receptors, the AT1A and AT1B receptors (AT1A and AT1B), which have 95% homology in their receptor amino acid sequences (16). The AT2 receptor has only 34% homology with the AT1R and is expressed at a low level, particularly in the renal vasculature (7).

The AT1A receptor plays an essential role in the development of 2K1C hypertension, as knockout mice lacking this receptor do not develop hypertension (3). Usually AT1A is downregulated when the ANG II levels are high, as during a low-salt diet, and upregulated when ANG II levels are low, as in high salt intake (25). In the nonclipped kidney, reports indicate that the modulation of the AT1R is different from controls and this seems also to be the case during long-term infusion of ANG II (13). The data concerning regulation of the AT1R in the nonclipped kidney are, however, controversial. In the early phase of the disease, with increasing concentrations of renin and ANG II as well as blood pressure levels, down-regulation of the AT1R has been described 2–7 days after clipping (17). Other investigators report AT1R to be unchanged up to 8 wk after clipping whereas downregulation of the AT1R is seen in the late stage of the disease (1). After 16 wk, decreased vascular and glomerular AT1R densities have been reported in both the clipped and nonclipped kidney of 2K1C hypertensive rats using ligand binding techniques (1).

The present study was designed to examine the integrated response to ANG II injection in the nonclipped kidney, and our working hypothesis was that the AT1R mRNA and protein density are downregulated in renal resistance vessels 6 wk after induction of renal vascular hypertension in the 2K1C hypertensive model, and this reduction in AT1R expression mediates reduced vascular reactivity to ANG II in the nonclipped kidney. The specificity of changes in the AT1R was assessed by evaluating renal reactivity to AVP. We also investigated the response before and after blockade of the cyclooxygenase system by indomethacin. To explore the effect of blocking available AT1R, candesartan, an AT1 receptor antagonist, was used to measure the amount of antagonist needed to block the response to ANG II completely in the nonclipped kidney and controls.

Materials and Methods

Animals. Experiments were performed in a total of 59 male Wistar rats (HanTac:WHM) from the Mollegaard Breeding Colony (Skensved, Denmark). The body weight was 180–200 g at the start of the experiments. The animals were housed in individual metal wire cages with constant temperature (25°C) and humidity and were exposed to a 12:12-h light-dark cycle. Rats had unrestricted water intake and were acclimatized for at least 1 wk under these conditions before the start of the study.

The experiments were performed in accordance with and under the approval of the Norwegian State Board for Biological Experiments, the Guide for the Care and Use of Laboratory Animals (National Academy Press, 1996), and the Guidelines of the Animal Welfare Act.

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The animals were randomized in 10 groups: 5 control groups and 5 2K1C groups. The first control (n = 9) and 2K1C groups (n = 6) were used for acute injections of ANG II before and after indomethacin treatment, the second control (n = 6) and 2K1C groups (n = 6) were used for Western blot and mRNA analysis of AT1A and AT1B, the third control (n = 6) and 2K1C groups (n = 6) were used for studies of RBF changes after injection of different doses of candesartan, the fourth control (n = 5) and 2K1C groups (n = 5) were used for studies of renal reactivity to acute injections of ANG II before and after candesartan treatment, and the fifth control (n = 5) and 2K1C groups (n = 5) were used for acute injections of AVP into the renal artery.

**Induction of 2K1C hypertension.** In the 2K1C animals, the right kidney was exposed through a lumbar incision and the right renal artery was clipped by placing of a rigid U-shaped silver partially occlusive clip with an internal opening of 0.25 mm, during isoflurane anesthesia (1 l/min, Forene-Abbott) mixed with 2 l/min O2 using an Ohmeda Isotec 3 anesthesia utility from BOC-Health Care. The left nonclipped kidney was used for RBF measurements 6 wk after clipping. All groups were fed by standard rat chow diet (Na: 0.25 g/100 g pellets, proteins: 14.7 g/100 g pellets).

**Measurement of blood pressure.** Systolic blood pressure was measured before clipping and during the development of high blood pressure by means of the tail-cuff method (Ugo Basile) in unanesthetized animals. The rats were prewarmed to 35°C for 10 min in a cupboard before blood pressure measurement.

**Hemodynamic study.** The acute experiments with ANG II injections in the left renal artery were performed 6 wk after clipping. Twelve hours before the acute experiments, the rats were deprived of food but were allowed free access to water. Anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (65 mg/kg), and the animals were placed on a servo-controlled heating table to keep the body temperature at 37°C. A tracheotomy was performed to facilitate free breathing, and the animals received oxygen through a facial mask in the acute experiments.

The right femoral artery was cannulated with a PE-10 catheter to monitor arterial pressure using a Statham P 23 XL pressure transducer, and a PE-10 catheter was introduced into the right femoral vein for the infusion of albumin, injection of indomethacin, candesartan, and supplementary doses of pentobarbital sodium. Bovine serum albumin at a concentration of 4.7 g/dl was infused initially at a rate of 50 μl/min to replace losses associated with surgery (1.25 ml/100 g) and then at 10 μl/min during the experiment.

The abdominal aorta and left kidney were exposed through a midline incision extended through the linea alba into the abdominal cavity. The intestines were pushed to the right side. The left iliac vessel was ligated below the renal arteries. The aorta was ligated above the renal arteries. The left renal vein was cut, and the kidneys were perfused with ice-cold PBS and thereafter with magnetic iron oxide suspended in PBS (1% w/vol). The kidneys were excised and placed in ice-cold PBS. All subsequent steps of vessel isolation were performed on ice. Kidneys were decapsulated, and the cortex was isolated by dissection. Cortical tissue was minced with a razor blade and homogenized in a glass homogenizer with a loose-fit pestle (Kontes). Iron oxide-containing vessels were removed from the homogenate with a magnet, and the magnetic tissue was passed through 21-, 23-, and 25-gauge needles and then filtered through a 125-μm-mesh sieve.

Microvessels recovered on the top of the sieve were resuspended in PBS, and iron oxide-containing vessels were extracted with a magnet (4).

**Western blotting.** Proteins from isolated renal preglomerular resistance vessels were electrophoretically separated (5 μg tissue/well) by SDS-PAGE on a 4–12% stacking gel with Tris glycine buffer (10% SDS, 24 mM Tris base, 192 mM glycine) for 45 min at 200 V and then transferred to a polyvinylidene difluoride transfer membrane (Amer sham) for 1 h at 100 V. For molecular weight detection, a Bio-Rad prestained low-range protein molecular standard was used, and the membrane was analyzed with the Quantity 4.1.1. program (Bio-Rad).

A molecular weight marker (Prestained SDS Standards Low Range, Bio-Rad) was used to determine molecular mass. The polyvinylidene difluoride membrane were then incubated for 1 h at room temperature with 3% dry skimmed milk in Tris-buffered salt solution (TBS; 19 mM Tris, 0.5 M NaCl). Thereafter the membrane was washed in TBS-Tween (TBS-t, 0.05% Tween 20) and incubated for 1 h at room temperature with rabbit anti-AT,R (SC-1173, Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:200 in TBS-t. The membrane was washed for 3 × 10 min with TBS-t at room temperature and incubated for 1 h at room temperature with alkaline phosphatase-conjugated monoclonal anti-rabbit IgG (1:10,000) in TBS-t. Detection was done using substrate tablets (B-5655, Sigma). β-Actin was used as a loading reference. Western blot detection for β-actin was done as described for AT,R but with monoclonal anti-β-actin diluted 1:1,000 as the primary antibody and alkaline-conjugated goat anti-mouse IgG.

A six-port external volume sample injection Cheminert valve (VICI Valco Instruments) was used to introduce 10 μl of a test agent into the intrarenal catheter. One minute before the agent was introduced, the infusion rate was increased to 120 μl/min, and the entire bolus of test agent was delivered to the kidney within 30–45 s. After recovery of the RBF to its baseline level (~2 min), the infusion rate was returned to 5 μl/min. The infusion rate chosen for administration of drugs was selected on the basis of previous studies (4).

The renal vascular effects were measured during injection of 1 and 2.5 ng ANG II and 5 ng AVP, two consecutive measurements for each dose. After these recordings were done, in both the control and 2K1C groups, 1 ml/kg of indomethacin (a nonselective cyclooxygenase inhibitor) was injected through the venous femoral catheter. Indomethacin was prepared by diluting 5 mg indomethacin in 1 ml distilled water, buffered by 5 mg sodium carbonate. Injections of ANG II were performed 20 min after indomethacin was given, following the same protocol as before indomethacin administration.

To test the role of candesartan, the drug was injected into the femoral vein and the RBF response was recorded in the nonclipped kidney and in controls using concentrations of 10, 20, and 30 μg/kg. Additional studies were also performed with 2.5 ng ANG II injections in controls and in the nonclipped kidney of 2K1C hypertensive rats before and after treatment with candesartan. In these experiments, candesartan was injected in different concentrations (10, 20, 30 μg/kg in the 2K1C group and 30, 80, and 100 μg/kg in the control group). Candesartan was prepared by diluting with 1 M solution of sodium carbonate in distilled water. Injections of ANG II were performed 10 min after candesartan was given, using the same protocol as before candesartan administration.

**Isolation of preglomerular vessels.** After anesthesia, a midline abdominal incision was made, and the abdominal aorta was cannulated below the renal arteries. The aorta was ligated above the renal arteries, the left renal vein was cut, and the kidneys were perfused with ice-cold PBS and thereafter with magnetic iron oxide suspended in PBS (1% w/vol). The kidneys were excised and placed in ice-cold PBS. All subsequent steps of vessel isolation were performed on ice. Kidneys were decapsulated, and the cortex was isolated by dissection. Cortical tissue was minced with a razor blade and homogenized in a glass homogenizer with a loose-fit pestle (Kontes). Iron oxide-containing vessels were removed from the homogenate with a magnet, and the magnetic tissue was passed through 21-, 23-, and 25-gauge needles and then filtered through a 125-μm-mesh sieve.

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diluted 1:2,000 in TBS-t. Quantitative analyses of the blot were done by use of the Quantity one 4.1.1 program (Bio-Rad).

Real-time PCR. Total RNA was purified by the use of TRIZol (Invitrogen). Quantitation of AT1A mRNA was done by real-time PCR. All amplifications were done at the same time. First-strand cDNA was synthesized from isolated total RNA using a First-Strand cDNA Synthesis Kit (Amersham Biosciences) and primed by Pd (N)6 primers. Primers for amplification of AT1A were selected for a 70-bp sequence. The forward primer was 5′-tctgctaccgctcagtga-3′. The TaqMan probe was 5′-acctcaagccggttccagcag-3′, marked with FAM and 3′-TAMRA. Primers for amplification of AT1B were selected for a 100-bp sequence. The forward primer was 5′-gcgcctgataaatcgattg-3′. The reverse primer was 5′-gcgaggaccaacctgcggag-3′. The TaqMan probe was 5′-aatggcggctcgtggtcagcag-3′, marked with FAM and 3′-TAMRA. The amplified AT1A and AT1B cDNA were normalized against amplified 18S ribosomal RNA to compensate for any changes due to RNA degradation, reverse transcriptase efficiency, or amplification success. The primers for 18S were made for a 68-bp fragment. The forward primer was 5′-gggctcctcagggtga-3′, marked with 5′-TAMRA. The amplified AT1A and AT1B cDNA were quantified using a standard curve for known quantities of AT1A, AT1B, or 18S DNA. The standard curve was made by amplifying the 100-bp AT1B sequence mentioned above. For the AT1A cDNA with the primers accgctatggaataccgatg (forward) and aaagtctgcatcttttttttgaagtggagtgaga-3′. The reverse primer was 5′-gatccgagggcctcactaaac-3′. The primer and probe constructions were done using Primer Express software from Applied Biosystems. The quantification was done on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems) and with a qPCR Biosystems. The quantification was done on an ABI PRISM 7900 HT

RESULTS

Effect of clipping on systolic blood pressure. Systolic blood pressure was measured by the tail-cuff method 1 wk before clipping and thereafter weekly for the next 6 wk after clipping in conscious rats until the acute hemodynamic study was performed (Fig. 1). The systolic blood pressure declined slightly in the control group (n = 9) from 130 ± 4 mmHg before to 113 ± 1 mmHg after 6 wk (P < 0.02), whereas systolic blood pressure of 2K1C hypertensive rats (n = 6) increased from 133 ± 1 mmHg before clipping to 183 ± 4 mmHg after 6 wk (P < 0.001). The difference in systolic blood pressure between the two groups at 6 wk was significantly different (P < 0.001).

Baseline RBF in animal studies. RBF was 8.6 ± 0.7 ml/min in the control kidneys (n = 9) and 10.0 ± 1.1 ml/min in the nonclipped kidney (n = 6) before ANG II injection (P = 0.35). The corresponding values were 5.4 ± 0.4 and 5.6 ± 0.6 ml·min⁻¹·g kidney wt⁻¹, respectively (P > 0.7).

RBF response to ANG II injections at 6 wk after clipping. In the control kidneys and in the nonclipped kidney of 2K1C hypertensive rats, ANG II injections showed a dose-dependent

Fig. 1. Systolic blood pressure in 2-kidney, 1-clip (2K1C) hypertensive animals (n = 6) and in control group (n = 9) at 6 wk after clipping. •, Time of clipping. Systolic blood pressure increased significantly in 2K1C hypertensive rats (P < 0.001). Systolic blood pressure decreased during the observation period in the control rats (P < 0.02). Values are means ± SE.* P < 0.01.
RBF response. Injection of 1 ng ANG II in the nonclipped kidney of the 2K1C group induced a smaller renal vascular response compared with controls. Calculated as percentage of baseline or absolute difference from baseline RBF, the responses were 5 ± 2% or 0.7 ± 0.2 ml/min in 2K1C and 13 ± 2% or 1.2 ± 0.1 ml/min in control rats (P < 0.01). The corresponding data after 2.5 ng ANG II injections were 0.9 ± 0.1 ml/min or 10 ± 2% in 2K1C rats and 2.2 ± 0.2 ml/min or 24 ± 3% in controls (P < 0.01) (Fig. 2). The renal vasoconstriction to 2.5-ng ANG II injections was greater than that produced by 1 ng ANG II (P < 0.01).

**RBF response to ANG II injections after indomethacin treatment.** Indomethacin treatment did not change RBF baseline. RBF was 8.6 ± 0.7 ml/min before and 8.6 ± 0.9 ml/min after indomethacin in controls (P > 0.9) and 10 ± 1.1 ml/min before and 9.4 ± 1.1 ml/min after indomethacin in the nonclipped kidney of 2K1C rats (P > 0.7). In both groups, the RBF responses to ANG II were increased after indomethacin treatment. Using 1 ng ANG II, the RBF response increased in the control group from 1.2 ± 0.1 ml/min or 13 ± 2% to 1.8 ± 0.3 ml/min or 20 ± 2% after indomethacin treatment (P < 0.01), and after 2.5-ng ANG II injections, the response increased from 2.2 ± 0.2 ml/min or 24 ± 3% to 3.5 ± 0.7 ml/min or 34 ± 6% (P < 0.01). In the 2K1C group, 1-ng ANG II injections increased the RBF response from 0.7 ± 0.2 ml/min or 5 ± 2% to 1.0 ± 0.1 ml/min or 11 ± 2% (P < 0.01), and from 0.9 ± 0.1 ml/min or 10 ± 2% to 1.8 ± 0.1 ml/min or 20 ± 3% (P < 0.02) after 2.5-ng ANG II injections after indomethacin treatment (Fig. 2). Mean arterial pressure (MAP) did not change during injection of neither 1 ng nor 2.5 ng ANG II.

**RBF response to candesartan and effect of ANG II injections after candesartan treatment.** The studies with candesartan were done with another batch of rats, and the mean baseline RBF was lower than in the first group in our study. In the nonclipped kidney of 2K1C rats (n = 6), the RBF response to injections of candesartan was significantly smaller than in the control rats (n = 5) (Fig. 3). Baseline RBF values and response to candesartan are given in Table 1. In the same way, increasing concentrations of candesartan reduced the renal vasoconstriction produced by ANG II in a dose-related manner in both groups. In the nonclipped kidney of 2K1C rats (n = 5), the concentration of candesartan needed to completely inhibit the RBF response to 2.5-ng ANG II injections was much smaller (30 μg/kg) than that needed in the control animals (n = 5; 100 μg/kg) (P < 0.001) (Fig. 4). There was no change in MAP during the initial 10 min after candesartan administration, either in the control or in 2K1C rats. MAP in control rats was 94 ± 2 mmHg before candesartan treatment and 88 ± 4 mmHg after, whereas in the 2K1C group MAP was 179 ± 5 mmHg before and 177 ± 6 mmHg after.

**RBF response to AVP injections.** AVP (5 ng) elicited the same RBF response in the control kidneys as in the nonclipped kidneys from 2K1C hypertensive animals. In the control kidneys (n = 5), the response was 0.8 ± 0.1 ml/min or 7.7 ± 0.6% and in the nonclipped kidney of the 2K1C (n = 5) group the RBF decline was 0.7 ± 0.1 ml/min or 6.8 ± 0.6% (P = 0.3).

PRA. Plasma renin activity was 8.1 ± 0.8 in controls and 28.0 ± 14.3 ng·ml⁻¹·h⁻¹ in 2K1C hypertensive animals (P < 0.05).

mRNA of AT₁A receptor. There was no difference in the amount of AT₁A mRNA in smooth muscle cells from pre-
glomerular renal resistance vessels isolated from the nonclipped kidney in 2K1C animals and normal controls. The values were 26,662 ± 6,116 vs. 26,183 ± 5,253 molecules AT1A·10⁸·18S⁻¹ RNA, respectively (P = 0.9).

mRNA of AT1B receptor. The mRNA level for AT1B was 1,363 ± 255 molecules AT1A·10⁸·18S⁻¹ RNA in isolated vessels from the nonclipped kidney, not significantly different from controls (1,913 ± 388 molecules AT1B·10⁸·18S⁻¹ RNA) (P = 0.4). The amount of mRNA for AT1B was ~5–7% of the mRNA level for AT1A (P < 0.001) in both the controls and the nonclipped kidney.

Western blotting. Protein expression of AT1R is presented as the ratio of AT1R to β-actin. There was a tendency for AT1R protein expression to be lower in renal preglomerular resistance vessels isolated from nonclipped kidney (1.125 ± 0.006 pixel intensity ratio) vs. controls (1.563 ± 0.0146 pixel intensity ratio), but the difference was not statistically significant (P = 0.07). A representative tracing is shown in Fig. 5A. The specificity of the Western blot analysis was documented by preadsorbing the anti-AT1R polyclonal antibody with synthetic AT1R peptide antigen (Santa Cruz Biotechnology). No protein was detected in analysis performed with preadsorbed antibody (Fig. 5B). Nonadsorbed polyclonal antibodies were shown to bind to an ~49-kDa protein, in accordance with the 50-kDa molecular mass described by Santa Cruz Biotechnology.

Kidney weight. The right clipped kidney in 2K1C hypertensive animals (n = 6) was 0.73 ± 0.016 g, smaller than nonclipped kidney (1.81 ± 0.06 g, P < 0.01). There was no significant difference between the kidney weights in the control group (n = 9) (1.50 ± 0.04 vs. 1.55 ± 0.05 g).

**DISCUSSION**

The present study provides new information concerning the role of ANG II in the nonclipped kidney of 2K1C hypertensive animals. The renal vascular response to ANG II injection is substantially decreased compared with control animals, but the responses were found to be dose dependent, indicating that all AT1R are not completely saturated 6 wk after clipping. The present study also showed that the response to acute injection of candesartan, an AT1R receptor antagonist, induced a minor RBF increase in the nonclipped kidney compared with controls. Similarly, when 2.5 ng ANG II were injected into the kidney in animals treated with different concentrations of candesartan, the amount of drug needed to inhibit the ANG II response completely was lower in the nonclipped kidney compared with the control rats, indicating that the available AT1R was substantially reduced in the nonclipped kidney. Furthermore, when studies were performed to examine the specificity of the ANG II response using another vasoactive substance such as AVP, the response was similar in control and nonclipped kidneys. Our investigations also demonstrate that the responses depend on an operative cyclooxygenase system, as indomethacin treatment enhances the response to ANG II in the nonclipped kidney, nearly to the same degree as in the controls. Surprisingly, Western blot analyses of AT1R and the mRNA receptor expression for AT1A and AT1B were similar in the nonclipped kidney of 2K1C rats 6 wk after clipping, i.e., in a phase where hypertension is stabilized with still increased PRA.

In the 2K1C animal model of hypertension, clipping of one renal artery is followed by development of hypertension in 2–3 wk, and after 4–5 wk the hypertension is established with increased plasma renin levels (14, 19). 2K1C hypertension is a

Table 1. Renal blood flow in the nonclipped kidney and controls after different doses of candesartan injections and total change in RBF from baseline

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<th>Control kidney from baseline</th>
<th>Nonclipped kidney from baseline</th>
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<tr>
<td>RBF, baseline</td>
<td>4.1 ± 0.6</td>
<td>5.2 ± 0.1</td>
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<td>Candesartan</td>
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<td>10 µg</td>
<td>5.4 ± 0.7</td>
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<td>20 µg</td>
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<td>30 µg</td>
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Values are means ± SE expressed as ml/min. RBF, renal blood flow; 2K1C, 2-kidney, 1-clip hypertension. *P < 0.01, †P < 0.04, significant difference between 2K1C and control rats.
The AT1R mediates the vasoconstrictive effect of ANG II in all vascular beds by a G protein-coupled mechanism (2). The role of the AT1A receptor in development of 2K1C hypertension is clearly demonstrated as the AT1A receptor knockout animals are not able to develop hypertension (3). Data regarding the regulation of the AT1R in the nonclipped kidney during development and maintenance of 2K1C hypertension are more controversial. After 5 wk, blood pressure is stabilized at high levels, plasma renin and ANG II levels are still high, but AT1R level findings published are contradictory. Previous studies have reported the receptor to be upregulated (20), unchanged (6, 26), or downregulated (12) in different phases of 2K1C hypertension. All of these studies were performed in whole kidney tissue in contrast to the present study, where we examined isolated preglomerular vessels. We were not able to find any change in mRNA expression of either AT1A and AT1B or the receptor protein. Our results are similar to those of Amiri et al. (1), who found normal receptor densities performed in isolated preglomerular and glomerular structures at this stage of hypertension. However, at a late stage of the disease, they found downregulation of receptor densities, a stage of hypertension where PRA and probably also ANG II are reduced (1).

It is of interest to note an apparent dysregulation of mRNA AT1A and AT1B and receptor protein in the nonclipped kidney of 2K1C rats. AT1Rs in the systemic and renal vasculature are usually downregulated by chronic increases in plasma ANG II. On the other hand, AT1R in the proximal tubule appears to exhibit regulation in the opposite direction, such that high levels of ANG II upregulate proximal tubular AT1R (13). In our study, AT1R density in the preglomerular vessels tends to be decreased by roughly 30% in the nonclipped kidney of 2K1C rats, but the difference did not achieve statistical significance (P = 0.07). At the same time, mRNA expression for AT1A and AT1B was almost identical in the two groups.

One possible explanation is that the high level of renin and ANG II results in more AT1R sites that bind endogenous ANG II whereas fewer sites are available to exogenous ANG II. The reactivity to injected (exogenous) ANG II is lower in the kidney exposed to higher concentrations of endogenous ANG II than the controls. Evidence supporting this idea comes from studies of the renal vasculature in animals maintained on low- and high-salt diets for several weeks (25). A high-salt diet increases the mRNA and density of AT1R, whereas a low-salt diet decreases mRNA and number of receptors. The mechanism of AT1R density changes in this experimental setting is probably due to variations in circulating ANG II, with internalization of AT1R when ANG II levels are high and recovery of AT1R when ANG II levels are low (18). The functional consequences of high and low receptor levels were studied by the injection method, where the renal vascular responses to ANG II injection were closely related to the numbers of AT1R available (25). In our study, we have used the same injection technique to investigate the vascular response to ANG II in the nonclipped kidney of the 2K1C hypertensive model. By acute injection of a bolus dose of ANG II, the acute vasoactive response can repeatedly be examined as has been done in spontaneously hypertensive rats and normotensive controls (4, 25). The acute vascular reaction represents the integrated response to ANG II in the nonclipped kidney, a response that is significantly decreased in this kidney. Conclusively, in contrast to the nonclipped kidney, in the normal rat there is
good correlation between the receptor level and the functional vascular response to ANG II.

Studies in vitro have shown that in normal mesangial cells the AT1R level is downregulated by both an ANG II-dependent pathway and a cAMP-dependent pathway (18). On the contrary, the same investigators found an upregulation of AT1R in adrenal or pituitary glands (8, 13). This may be due to different expression of the two types of AT1R. AT1A is expressed (>90%) in most cardiovascular tissues, whereas AT1B is predominantly expressed in endocrine tissues such as the adrenal and pituitary gland (27). In the present study, we examined the mRNA expression for AT1A and AT1B, which was similar in 2K1C rats and controls and makes it very unlikely that different levels of AT1A and AT1B should play a role. The antibody used in Western blotting cannot separate the two receptors. Although the receptor protein was numerically lower in 2K1C rats, mRNA levels for AT1A and AT1B were similar.

Another possibility is that the postreceptor signal transduction is attenuated in this model where systemic and intrarenal amounts of ANG II are increased. It has been shown that attenuation of the cellular receptor-dependent response on prolonged or repeated agonist exposure occurs through several mechanisms, such as rapid uncoupling from G protein, sequestration of receptors into endosomal vesicles, or downregulation of the total number of receptors (23, 25). In the present study we did not show any difference in AT1R levels between control rats and the nonclipped kidney of rats with 2K1C hypertension using Western blot techniques and real-time measurements of mRNA for AT1R. A further strength of the present study is that receptor mRNA and protein are studied in isolated renal vessels and not in the whole kidney. The regulation of tubular AT1R may be different from that in vascular receptors but should contribute neither directly to the acute vascular response nor to the levels of AT1R and mRNA of AT1A and AT1B receptors as seen in the present study. The relationship between internalized and noninternalized AT1R is unknown, but a substantial reduction of the numbers of ligand-bound noninternalized AT1R may explain our findings both after ANG II injection and on the effect of candesartan on RBF. A further exploration of the mechanisms involved in AT1R transduction would be to study AT1R’s ability to activate Ca\(^{2+}\) signaling pathways in the vessels of the nonclipped kidney in 2K1C hypertension.

The substantial reduced vascular response to ANG II injection in the nonclipped kidney may also be due to upregulation of systems causing renal vasoconstriction. In the present study, we have shown that inhibition of the cyclooxygenase system is attenuated in this model where systemic and intrarenal amounts of ANG II are increased. It has been shown that attenuation of the cellular receptor-dependent response on prolonged or repeated agonist exposure occurs through several mechanisms, such as rapid uncoupling from G protein, sequestration of receptors into endosomal vesicles, or downregulation of the total number of receptors (23, 25). In the present study we did not show any difference in AT1R levels between control rats and the nonclipped kidney of rats with 2K1C hypertension using Western blot techniques and real-time measurements of mRNA for AT1R. A further strength of the present study is that receptor mRNA and protein are studied in isolated renal vessels and not in the whole kidney. The regulation of tubular AT1R may be different from that in vascular receptors but should contribute neither directly to the acute vascular response nor to the levels of AT1R and mRNA of AT1A and AT1B receptors as seen in the present study. The relationship between internalized and noninternalized AT1R is unknown, but a substantial reduction of the numbers of ligand-bound noninternalized AT1R may explain our findings both after ANG II injection and on the effect of candesartan on RBF. A further exploration of the mechanisms involved in AT1R transduction would be to study AT1R’s ability to activate Ca\(^{2+}\) signaling pathways in the vessels of the nonclipped kidney in 2K1C hypertension.

The role of another vasoactive substance was investigated in the present study. AVP, which has shown to have an increased response in genetic hypertension (9), had only a minor response in the nonclipped kidney, and the response was not different from control rats. The data may suggest that there is no difference between the role of AVP in autoregulation of RBF in control kidneys and in the nonclipped kidney of 2K1C rats.

Another possible explanation for the reduced vascular response may be the role of the nitric oxide system (3). This system has been shown to be upregulated in 2K1C hypertension, and this could play a significant role in the reduced response to ANG II (3). Whether the upregulation of the nitric oxide system is due to increased shear stress in hypertension or due to stimulation of ANG II is unclear (24, 29). Other hormonal systems may also play a role. Atrial natriuretic factor (ANF) is also found to be increased in the 2K1C hypertensive model 3–7 wk after clipping, and the receptor for ANF is reduced in both the clipped and nonclipped kidney as might be expected during an increased level of circulating hormone (10). The role of ANF to regulate blood flow in the nonclipped kidney is unclear, and ANF may play a role in the modulation of high blood pressure in this stage of hypertension (19).

In conclusion, the present study demonstrates for the first time that in the established stage of 2K1C hypertension, the vascular response to ANG II injection in the nonclipped kidney is substantially reduced despite normal vascular levels of AT1R determined by Western blotting and measurements of AT1A and AT1B mRNA. This may be explained by excessive internalization and desensitization of AT1R by prolonged exposure to high plasma and renal levels of ANG II. A defect in the AT1R, in G protein coupling to the receptor, or changes in Ca\(^{2+}\) signaling pathways, cannot be excluded. The present study also provides data that the cyclooxygenase system is not enhanced, as the response before and after indomethacin treatment was similar in controls and 2K1C hypertensive animals.

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F400  ANG II RESPONSE IN TWO-KIDNEY, ONE-CLIP HYPERTENSION