Essential Role of AT1A Receptor in the Development of 2K1C Hypertension

Luděk Červenka, Vladislav Horáček, Ivana Vaněčková, Jaroslav A. Hubáček, Michael I. Oliverio, Thomas M. Coffman, L. Gabriel Navar

Abstract—The aims of this study were to delineate the relative contribution of angiotensin II (ANG II) subtype 1A (AT1A) and 1B (AT1B) receptors to the development of two-kidney, one-clip (2K1C) Goldblatt hypertension in mice, to examine if increased nitric oxide synthase (NOS) activity counteracts the vasoconstrictor influences of ANG II in 2K1C hypertensive mice, and to determine the role of ANG II type 2 (AT2) receptors in 2K1C hypertension in mice. AT1A ANG II receptor knockout (AT1A−/−) and wild-type (AT1A+/+) mice underwent clipping of the right renal artery. Systolic blood pressure (SBP) was significantly lower in AT1A−/− compared with AT1A+/+ mice, and neither clip placement nor AT2 receptor blockade with PD 123319 (PD) altered SBP in AT1A−/− mice. A significant and sustained rise in SBP from 119±5 to 163±6 mm Hg was observed in the 2K1C AT1A+/+ mice from day 10 to day 26. Chronic PD infusion did not alter the course of hypertension in 2K1C/AT1A+/+. Acute PD infusion did not alter mean arterial pressure (MAP) in AT1A+/+, PD/AT1A+/+, 2K1C/AT1A+/+, PD/2K1C/AT1A+/+, AT1A−/−, PD/AT1A−/−, and PD/2K1C/AT1A−/− mice compared with basal levels. In contrast, acute PD infusion caused significant increases in MAP in 2K1C/AT1A−/− mice. The subsequent acute NOS inhibition caused greater increases in MAP in 2K1C/AT1A−/+ and PD/2K1C/AT1A−/+ mice than in AT1A+/+ and PD/AT1A+/+ mice. These results support the essential role of AT1A receptors in mediating 2K1C hypertension and support the hypothesis that augmented NO production serves as a counteracting system in this model of hypertension. (Hypertension. 2002;40:735-741.)

Key Words: mice ■ receptors, angiotensin II ■ hypertension, renovascular ■ nitric oxide synthase ■ nitric oxide

It is well recognized that the renin-angiotensin system plays the pivotal role in the development and maintenance of two-kidney, one-clip (2K1C) Goldblatt hypertension.1 Previous studies have shown that activation of angiotensin II (ANG II) type I (AT1) receptors predominantly mediates the effect of ANG II on the renal vasculature and is largely responsible for the development of hypertension in ANG II–dependent models of hypertension.2–4 However, two subtypes for AT1 receptors have been identified in mouse and rat (AT1A and AT1B).5 It has been demonstrated that the AT1A receptors are the predominant subtype in most tissues, with the exception of the adrenal cortex, pituitary gland, and glomerulus, where the AT1B receptors are also highly expressed.6,7 The critical role of AT1A receptors in blood pressure (BP) regulation has been confirmed by the development of AT1A receptor knockout mice (AT1A−/−). The AT1A−/− mice exhibit markedly lower BP and impaired ability for normal sodium handling by the kidney and urinary concentrating ability compared with their wild-type controls (AT1A+/+).8–11 In contrast, the AT1B receptor knockout mice (AT1B−/−) exhibit no abnormal phenotype.12 However, it has been shown that in the absence of AT1A receptors, AT1B receptors may partially replace the function of AT1A receptors in BP regulation.13 Moreover, it has been also demonstrated that when AT1A receptors are absent, AT1B receptors can play an important role in mediating ANG II effects in the renal vasculature.14,15 Taken together, these results indicate that under certain conditions, AT1B receptors partially compensate for the absence of AT1A receptors. In view of this information, we hypothesized that activation of AT1B might contribute to the development of 2K1C Goldblatt hypertension. Since the binding signatures of the AT1A and AT1B receptors are identical,16 making it impossible to distinguish their functions with the use of pharmacological antagonists, we used an AT1A receptor null model to determine its role in contributing to the development of hypertension.17 The major advantage of gene deletion models is that the absence of specific receptor protein is complete. On the other hand, the disadvantages of gene targeting studies are that some genetic alterations have

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consequences for organ development and animal survival, as has been demonstrated in some mice in which genes of the renin-angiotensin system have been altered by gene targeting (eg, angiotensinogen and ACE knockout mice). However, the AT$_{1A}$−/− mice do not show major survival or kidney developmental problems and do not show significant changes in expression (either upregulation or downregulation) of other ANG II receptor subtypes. Therefore, the AT$_{1A}$−/− mice appear to be an optimal model to study the interplay of ANG II receptors in the regulation of BP and development of 2K1C hypertension.

The first aim of the present study was to delineate the relative contribution of AT$_{1A}$ and AT$_{1B}$ receptors to the development of 2K1C hypertension after unilateral renal arterial constriction (clipping).

Emerging evidence suggests that increased nitric oxide synthase (NOS) activity in ANG II–dependent models of hypertension serves as an important vasodilator countering system modulating the magnitude of the BP response. Accordingly, the second aim of the present study was to assess the effects of acute NOS inhibition on BP in sham-operated and 2K1C AT$_{1A}$−/− and AT$_{1A}$+/+ mice.

In view of the growing body of information suggesting that activation of ANG II type 2 (AT$_{2}$) receptors plays a counter-regulatory protective role in the regulation of BP that opposes the hypertensinogenic actions of ANG II mediated through AT$_{1}$ receptors, the third aim of the present study was to assess the effects of acute and chronic AT$_{2}$ receptor blockade in sham-operated and 2K1C mice in AT$_{1A}$−/− mice compared with AT$_{1A}$+/+ mice.

**Methods**

Protocols in the present study were designed according to the Guiding Principles in the Care and Use of Animals approved by the Council of the American Physiological Society and were approved by Czech Animal Care and Use Committee (protocol 26/2001).

**Animals**

Mice lacking AT$_{1A}$ receptors for ANG II (AT$_{1A}$−/−) were generated by homologous recombination in embryonic stem cells as described previously. The breeder pairs were obtained from the animal facility of the Durham Veterans Affairs Medical Center and were transferred to the Institute for Clinical and Experimental Medicine. The genotype of each animal used in the present study was determined by Southern blot analysis of DNA isolated from the tail. Animals were housed in a temperature- and light-controlled room and allowed free access to standard chow (0.75% NaCl and 22% protein) and water.

**Preparation of 2K1C Goldblatt Hypertensive Mice**

Male AT$_{1A}$+/+ and AT$_{1A}$−/− mice weighing 28 to 36 g were anesthetized with sodium pentobarbital (50 mg/kg IP). The right renal artery was isolated through a flank incision, and a silver clip (0.12-mm internal gap) was placed on the renal artery, as described previously. Sham-operated mice that underwent the same surgical procedure except for placement of the renal artery clip served as controls. Osmotic minipumps (model 1002, Alzet Co) containing PD 123319 (PD, an AT$_{2}$ receptor antagonist) at concentrations sufficient to allow the delivery of 30 mg/kg body wt per day were implanted into the abdominal cavity of the AT$_{1A}$+/+ and AT$_{1A}$−/− in 2K1C mice (PD/2K1C/AT$_{1A}$/+, n = 23 and PD/2K1C/AT$_{1A}$/−, n = 23). This dose of PD has been used in previous studies that examined the effect of chronic AT$_{2}$ receptor blockade on BP. Osmotic minipumps containing saline were implanted into the abdominal cavity of the 2K1C AT$_{1A}$/++ and AT$_{1A}$/−/− mice (2K1C/AT$_{1A}$/++).

**Figure 1.** Experimental design for blood pressure responses to acute AT$_{2}$ receptor blockade and consecutive NOS inhibition (A) and for time-control mice (B). SAL indicates infusion of an isosorbide dinitrate solution.

n = 22 and 2K1C/AT$_{1A}$/−/−, n = 23). Osmotic minipumps containing either PD or saline vehicle were also implanted into sham-operated AT$_{1A}$/+/+ and AT$_{1A}$/−/− mice (PD/AT$_{1A}$/+/+, n = 19; AT$_{1A}$/++, n = 18 and PD/AT$_{1A}$/−, n = 24; AT$_{1A}$/−/−, n = 22). Because the osmotic minipumps have an operating time of 14 days, new pumps containing PD or saline vehicle were implanted intraoperatively on day 13, as described above, after removal of old pumps.

**Systolic Blood Pressure Measurements**

SBP was measured by a tail-cuff apparatus (RTPB; Kent Scientific Co) in conscious mice 3 days before and then on days 3, 7, 10, 15, 18, 21, 24, and 26 after clip placement (or sham operation). SBP values were derived from an average of 5 measurements per animal at each time point. Two preliminary training sessions were performed during 1 week before starting the experiment.

**Acute Studies**

On day 27, mice were anesthetized with pentobarbital sodium (50 mg/kg IP) and placed on a servo-controlled surgical table that maintained body temperature at 37°C and a tracheostomy was performed with PE-90 tubing. The animals were allowed to breathe air enriched with O$_2$. The right carotid artery was cannulated with a PE-10 catheter connected to PE-50 for continuous BP measurements. Mean arterial pressure (MAP) was monitored with a pressure transducer (model MLT 1050) and recorded with a computerized data acquisition system (PowerLab/4SP, AD Instruments). The right jugular vein was catheterized with PE-10 tubing for fluid infusion.

An isotonic saline solution containing 1% albumin (bovine; Sigma Chemical Co) was infused at a rate of 5 μl/min throughout the experiment. After surgery, mice were allowed a 15-minute recovery period. After the recovery period, the first 15-minute control period for basal MAP determination was started. Thereafter, consecutive blockade of AT$_{1}$ receptors and NOS was performed by chronic infusion of PD (50 μg/kg body wt per minute) and nitro-L-arginine-methyl-ester (L-NAME) (250 μg/kg body wt per minute). After 5-minute delays in each experimental period, the MAP was recorded for 25 minutes. In previous studies, it was shown that this dose of PD yielded plasma concentrations near 3×10$^{-5}$ mol/L, a concentration that is reported to be highly selective for AT$_{1}$ receptors. The dose of L-NAME used in the present study was the same as used in our previous study in ANG II–infused mice; it is the lowest dose that elicited near-maximal inhibition of the hypotensive effect of acetylcholine in B2R+/+ mice. Time-control mice received a constant saline infusion throughout the experiment. To maintain a standard protocol, PD was also administered acutely to the rats treated chronically with PD. The experimental design is outlined in Figure 1. The animals groups are shown in Table 1.
At the end of the experiment, the animals were euthanized with excess intravenous pentobarbital. The kidneys and hearts were excised, drained, and weighed. Tissue weight (mg) was normalized per gram of body weight.

### Statistical Analysis

All values are expressed as mean±SEM. Two-way repeated-measures ANOVA was used to detect differences within each experimental group. For comparison between AT1A+/+ and AT1A−/− mice, repeated-measures ANOVA was used with a test of interaction to determine whether the average change after experimental manipulation (clip placement and pharmacological treatment) was different between AT1A+/+ and AT1A−/− mice. One-way ANOVA was used for heart and kidney weight data. Statistical significance was defined as *P*<0.05.

### Results

The basal values of kidney weight and heart weight are summarized in Table 2.

### SBP in 2K1C AT1A+/+ and AT1A−/− Mice and Effect of Chronic AT2 Receptor Blockade

Basal SBP was significantly higher in AT1A+/+ mice compared with AT1A−/− mice (119±5 versus 79±7 mm Hg). As shown in Figures 2A and 2B, SBP remained unchanged in AT1A+/+, PD/AT1A+/+, AT1A−/−, PD/AT1A−/−, PD/2K1C/AT1A−/−, and PD/2K1C/AT1A−/− mice for the duration of the study. SBP in 2K1C/AT1A+/+ exhibited progressive increases during the duration of study, reaching a value of 163±6 mm Hg on day 26. Chronic PD infusion did not influence the course of SBP elevation in 2K1C AT1A+/+ mice (160±5 mm Hg).

### MAP in Anesthetized Mice and Effects of Acute AT2 Receptor Blockade and NOS Inhibition

As shown in Figures 3A and 3B, 2K1C/AT1A+/+ and PD/2K1C/AT1A+/+ mice had a significantly higher MAP

### Table 1

<table>
<thead>
<tr>
<th>Group Name</th>
<th>n</th>
<th>Experimental Manipulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2K1C/AT1A+/+</td>
<td>14</td>
<td>Acute AT2 receptor blockade followed by acute NOS inhibition</td>
</tr>
<tr>
<td>2K1C/AT1A+/+</td>
<td>8</td>
<td>Time control (saline infusion throughout the experiment)</td>
</tr>
<tr>
<td>PD/2K1C/AT1A+/+</td>
<td>15</td>
<td>Acute AT2 receptor blockade followed by acute NOS inhibition</td>
</tr>
<tr>
<td>PD/2K1C/AT1A+/+</td>
<td>8</td>
<td>Time control</td>
</tr>
<tr>
<td>2K1C/AT1A−/−</td>
<td>14</td>
<td>Acute AT2 receptor blockade followed by acute NOS inhibition</td>
</tr>
<tr>
<td>2K1C/AT1A−/−</td>
<td>9</td>
<td>Time control</td>
</tr>
<tr>
<td>PD/2K1C/AT1A−/−</td>
<td>14</td>
<td>Acute AT2 receptor blockade followed by acute NOS inhibition</td>
</tr>
<tr>
<td>PD/2K1C/AT1A−/−</td>
<td>9</td>
<td>Time control</td>
</tr>
<tr>
<td>AT1A+/+</td>
<td>10</td>
<td>Acute AT2 receptor blockade followed by acute NOS inhibition</td>
</tr>
<tr>
<td>AT1A−/−</td>
<td>10</td>
<td>Time control</td>
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<tr>
<td>PD/AT1A+/+</td>
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<td>Acute AT2 receptor blockade followed by acute NOS inhibition</td>
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<td>Acute AT2 receptor blockade followed by acute NOS inhibition</td>
</tr>
<tr>
<td>AT1A−/−</td>
<td>10</td>
<td>Time control</td>
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<tr>
<td>PD/AT1A−/−</td>
<td>14</td>
<td>Acute AT2 receptor blockade followed by acute NOS inhibition</td>
</tr>
<tr>
<td>PD/AT1A−/−</td>
<td>10</td>
<td>Time control</td>
</tr>
</tbody>
</table>

AT1A−/− indicates angiotensin II AT1A receptor knockout mice; AT1A+/+, wild-type controls; PD, PD123319 infused chronically; 2K1C, Goldblatt hypertensive mice.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW (g)</th>
<th>HW (mg/g BW)</th>
<th>NCKW (mg/ g KW)</th>
<th>CKW (mg/g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2K1C/AT1A+/+</td>
<td>22</td>
<td>37.9±2.2</td>
<td>5.41±0.18†</td>
<td>9.37±0.32*</td>
<td>5.16±0.18*</td>
</tr>
<tr>
<td>PD/2K1C/AT1A+/+</td>
<td>23</td>
<td>36.6±1.8</td>
<td>5.07±0.23†</td>
<td>9.28±0.37*</td>
<td>5.08±0.22*</td>
</tr>
<tr>
<td>AT1A−/−</td>
<td>18</td>
<td>36.7±1.9</td>
<td>4.92±0.19*</td>
<td>7.12±0.19</td>
<td>7.08±0.22</td>
</tr>
<tr>
<td>PD/AT1A−/−</td>
<td>19</td>
<td>37.4±1.6</td>
<td>4.86±0.23*</td>
<td>7.14±0.34</td>
<td>7.12±0.36</td>
</tr>
<tr>
<td>2K1C/AT1A−/−</td>
<td>23</td>
<td>36.7±1.7</td>
<td>3.98±0.19</td>
<td>9.22±0.38*</td>
<td>5.24±0.27*</td>
</tr>
<tr>
<td>PD/2K1C/AT1A−/−</td>
<td>23</td>
<td>37.4±1.8</td>
<td>4.01±0.22</td>
<td>9.32±0.42*</td>
<td>5.11±0.31*</td>
</tr>
<tr>
<td>AT1A+/+</td>
<td>22</td>
<td>37.2±2.1</td>
<td>3.92±0.18</td>
<td>7.08±0.22</td>
<td>7.01±0.11</td>
</tr>
<tr>
<td>PD/AT1A+/+</td>
<td>24</td>
<td>37.6±1.9</td>
<td>4.02±0.13</td>
<td>7.18±0.20</td>
<td>7.14±0.13</td>
</tr>
</tbody>
</table>

Weights were measured 27 days after placement of clips and implantation of minipumps. BW indicates body weight; HW, heart weight; NCKW, nonclipped kidney weight; CKW, clipped kidney weight.

*P*<0.05 vs unmarked values; †*P*<0.05 vs all other values.
compared with AT1A/+ and PD/AT1A/+ mice measured on day 27 in anesthetized animals (136 ± 5 and 133 ± 5 versus 101 ± 5 and 102 ± 6 mm Hg, P < 0.05). There were no significant differences in MAP among AT1A−/−, PD/AT1A−/−, 2K1C/AT1A−/−, and PD/2K1C/AT1A−/− mice (67 ± 5, 66 ± 5, 66 ± 6, and 67 ± 5 mm Hg).

Acute PD infusion did not alter MAP in AT1A+/+, PD/AT1A+/+, 2K1C/AT1A+/+, PD2K1C/AT1A+/+, AT1A−/−, PD/AT1A−/−, and PD/2K1C/AT1A−/− mice compared with basal levels (+2 ± 2, +2 ± 2, +2 ± 2, −1 ± 1, +2 ± 2, ±1, and −1 ± 1 mm Hg). In contrast, acute PD infusion caused a significant increase in MAP in 2K1C/AT1A−/− mice (+10 ± 3 mm Hg, P < 0.05 versus basal values).

As shown in Figure 4A, 2K1C/AT1A+/+ and PD/2K1C/AT1A+/+ mice responded to acute NOS inhibition with greater increases in MAP than AT1A+/+ and PD/AT1A+/+ mice (+51 ± 4 and +49 ± 5 versus +20 ± 5 and +21 ± 4 mm Hg, P < 0.05). In contrast, acute NOS inhibition elicited similar increases in MAP of AT1A−/−, PD/AT1A−/−, 2K1C/

Figure 2. Changes in SBP after clip placement or sham operation in wild-type (AT1A+/+) (A) and knockout mice (AT1A−/−) (B). PD indicates PD 123319 infusion by osmotic minipumps. *P < 0.05 vs unmarked groups.

**Discussion**

The major finding of this study is that targeted disruption of the AT1A receptor gene prevents the development of 2K1C Goldblatt hypertension in mice. These data are in agreement with previous studies indicating that AT1A receptors play an essential role in BP control and moreover suggest that for the development of 2K1C hypertension, the presence of AT1A receptors is critical. In view of previous observations that in the absence of AT1A receptors, AT1B receptors may partially replace the function of AT1A receptors in BP regulation and ANG II-mediated vasoconstriction, one would expect that the clip placement in AT1A−/− mice should lead to some increase in BP mediated by AT1B. The explanation for the lack of AT1B receptor-mediated contribution to the development of 2K1C hypertension might be that in the presence of increased ANG II levels, as was reported in AT1A−/− mice, all AT1B receptors are already fully occupied and

**Figure 3.** MAP measured 27 days after clip placement or sham operation in wild-type (AT1A+/+) (A) and knockout mice (AT1A−/−) (B). PD indicates PD 123319 infusion by osmotic minipumps. *P < 0.05 vs unmarked groups.

AT1A−/−, and PD/2K1C/AT1A−/− (+11 ± 4, +10 ± 5, +15 ± 5, and +12 ± 4 mm Hg) (Figure 4B). MAP did not change significantly in the time-control group of mice, and arterial BP remained within their control range.
II

NO synthesis resulted in exaggerated BP increases in ANG II. Previous studies that demonstrated that acute inhibition of AT1B receptors in BP regulation in AT1A knockout mice lowered ANG II levels in those animals. However, the role of AT1B receptors in BP regulation in AT1A knockout mice indicates that ANG II elicited BP increases in AT1A−− mice only after pretreatment with ACE inhibitor (which presumably lowered ANG II levels in those animals). However, the role of AT1B receptors in BP regulation in AT1A−− mice requires further investigation.

The second major observation of the present study was that acute inhibition of NOS led to greater increases in BP in 2K1C/AT1A−− mice compared with sham-operated AT1A+/+ mice. This finding is consistent with results from previous studies that demonstrated that acute inhibition of NO synthesis resulted in exaggerated BP increases in ANG II–dependent models of hypertension and further supports the hypothesis that a compensatory increase in NOS activity partially counteracts the enhanced vasoconstrictor influence of ANG II in this model of hypertension. Whether the enhancement of NOS activity in 2K1C hypertensive mice could be ascribed to higher BP levels causing a greater endothelial shear stress, which is a potent stimulus for NO release, or could be due to direct actions of ANG II to stimulate NO production by activation of AT1 receptors, as indicated in a previous study, or by both pathways remains to be elucidated in future studies. However, since either acute or chronic AT2 receptor blockade did not alter BP responses in 2K1C hypertensive mice, it would seem that the augmentation of NO production under these conditions is not dependent on activation of AT2 receptors, as previously suggested.

Of interest is our observation that chronic AT2 receptor blockade did not alter BP in sham-operated AT1A+/+ and AT1A−− mice and did not worsen the course of hypertension in 2K1C hypertensive AT1A+/+ mice. Moreover, acute AT2 receptor blockade did not significantly influence MAP in anesthetized sham-operated AT1A+/+ and AT1A−− mice and in 2K1C AT1A+/+ mice. These data indicate that AT2 receptors do not play a key regulatory role in acute and chronic regulation of BP in sham-operated AT1A+/+ and AT1A−− mice. In view of the findings that chronic AT2 receptor blockade did not worsen the developmental and maintenance phases of 2K1C Goldblatt hypertension in AT1A+/+ mice, we suggest that activation of AT2 receptors does not play a major counterbalancing role against the vasoconstrictor actions of ANG II mediated by the activation of AT1 receptors in this mouse model of hypertension. In previous studies, it has been demonstrated that the doses of PD (applied either chronically or acutely) used in the present study are sufficient to result in micromolar blood plasma concentrations that have been reported to be highly selective for AT2 receptors. Higher doses have been reported to lead to concentrations that also interfere with AT1 receptors. Thus, it seems unlikely that incomplete blockade of AT2 receptors would be responsible for the lack of BP responses to PD administration. Nevertheless, the greater increase in BP in response to acute PD in 2K1C AT1A−− mice suggests that the clipping procedure does augment circulating ANG II levels and that in the absence of functional AT1A receptors, the effects of ANG II on AT1 receptors exert an antihypertensive action. Moreover, the findings that chronic PD treatment prevented BP changes elicited by acute PD administration in 2K1C/AT1A−− mice indicate that the chronic dose of PD was sufficient to block AT2 receptors. The finding that AT2 receptor blockade did not significantly influence the course of BP in 2K1C/AT1A−− mice suggests that the clipping procedure does augment circulating ANG II levels and that in the absence of functional AT1A receptors, the effects of ANG II on AT1 receptors do not play a major role in chronic BP regulation.

Previous studies have shown that administration of an AT2 receptor agonist potentiated the antihypertensive action of AT1 receptor blockade in the spontaneously hypertensive rats and decreased BP in normotensive rats. Furthermore, AT2 receptor blockade worsened the course of renal wrap hypertension. Moreover, AT1 receptor knockout mice have slightly elevated resting BP and enhanced pressor sensitivity to ANG II. In addition, the selective intrarenal inhibition of AT2 receptors with antisense oligodeoxynucleotides caused an increase in BP and elicited BP hypersensitivity to ANG II. Taken together, these studies suggest that the activation of AT2 receptors mediates a vasodilator cascade including bradykinin and NO, resulting in increased production of guanosine 3’5’ cyclic monophosphate. This vasodilatory pathway might serve as a vasodilator pathway that counteracts the vasoconstrictor actions of ANG II mediated through AT1.
receptors. On the other hand, there are also studies that failed to demonstrate a role for AT2 receptors as a mediator of vasodilator actions. It was reported that chronic AT2 receptor blockade did not worsen the course of hypertension in ANG II–infused rats. In addition, the immunization against AT II in AT1A

infused rats. In addition, the immunization against AT1 II present in AT1A

activation in response to ANG II during AT2 receptors blockade nor ANG II–induced vasorelaxation during AT1 receptors blockade were observed. Nevertheless, our observation that acute AT2 receptor blockade increased BP in 2K1C/AT1A−/− mice indicate that at least to some extent and under specific conditions, AT2 receptors participate in acute BP regulation. Although it has been reported that the AT1A−/− mice do not exhibit significant changes in expression for other ANG II receptors, we do not currently know whether the AT2 receptors are upregulated in 2K1C/AT1A−/− mice, and if they are, to what extent. Our results suggest that they are upregulated; however, further studies are needed to address this issue.

As expected, heart weights from AT1A+/+ mice were greater compared with AT1A−/− mice, and induction of hypertension caused further increases in heart weight compared with sham-operated AT1A+/+ mice. The nonclipped kidneys from 2K1C groups were larger than kidneys from sham-operated groups, thus indicating that the nonclipped kidneys undergo hypertrophy. Of interest is the observation that clip placement induced hypertrophy of nonclipped kidneys to the same extent in AT1A+/+ mice as well as in AT1A−/− mice, suggesting that neither intact AT1A receptors nor elevated arterial pressure are essential for the process of kidney hypertrophy.

Perspectives

Based on results of the present study that clearly show that targeted disruption of the AT1A receptor gene prevents the development of 2K1C Goldblatt hypertension in mice, we suggest the essential role of AT1A receptors in the development of hypertension in this model. Although AT1B are present in AT1A−/− mice, the present data indicate that they do not participate in the hypertensive response after unilateral renal arterial stenosis. The exaggerated BP responses to acute NOS inhibition in 2K1C hypertensive mice further support the notion that a compensatory increase in NOS activity counteracts the vasoconstrictor influences of ANG II in this model. Since the AT1 receptor blockade did not either modify the development of hypertension or the BP responses to NOS inhibition in 2K1C hypertensive mice, it appears that the activation of AT2 receptors does not play a major role in this mouse model of hypertension. We suggest that future perspectives provided by our data include research into the role of interaction of AT1A receptors and NOS activity during the development of 2K1C hypertension. To reconcile the contradictory findings regarding the role of AT1 receptors in BP regulation will require further studies.

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