Angiotensin II-dependent induction of AT$_2$ receptor expression after renal ablation


Angiotensin II-dependent induction of AT$_2$ receptor expression after renal ablation. Am J Physiol Renal Physiol 288: F207–F213, 2005. First published September 14, 2004; doi:10.1152/ajprenal.00216.2004.—Angiotensin (ANG) II can be associated with gene expression regulation. Thus we studied the possible role of ANG II in the regulation of AT$_2$ mRNA and protein expression. We utilized sham-operated renal ablation rats as well as renal ablation rats pretreated during the first 7 days of the development of renal damage with either the angiotensin-converting inhibitor ramipril, the AT$_1$ receptor antagonist losartan, or the AT$_2$ receptor antagonist PD-123319. Renal tissue was analyzed for histological changes and expression of AT$_2$ receptor mRNA (by RT-PCR) and protein (by immunohistochemistry). To explore the physiological role of AT$_2$ receptor overexpression in the development of renal damage, blood pressure, urinary protein excretion, and renal damage were evaluated. A time-dependent increase in the expression of AT$_2$ receptor mRNA and protein was observed at 7, 15, and 30 days after renal ablation. Because these effects were already evident at day 7, the effects of ramipril, losartan, or PD-123319 were tested at this time. The ramipril group and the PD-123319-pretreated group showed inhibition of AT$_2$ receptor expression, whereas the losartan-pretreated group showed a further increase in AT$_2$ receptor expression. Inhibition of the AT$_2$ receptor during renal ablation was associated with increased renal damage and a further increase in the blood pressure. This suggests that overexpression of AT$_2$ receptors after renal ablation is modulated by ANG II through its own AT$_2$ receptor and that functional expression of this effect may represent a counterregulatory mechanism to modulate the renal damage induced by renal ablation.

AT$_1$ receptor; renovascular hypertension; losartan; PD-123319

Angiotensin (ANG) II plays an important role in the pathophysiology of renovascular hypertension through vasoconstriction of the peripheral vasculature, hypertrophy, and hyperplasia of cardiovascular cells (7, 8). ANG type 1 (AT$_1$) and type 2 (AT$_2$) isoforms of the ANG II receptors have been described (9). However, expression of AT$_2$ receptors is high in fetal tissues but decreases during development (20). Expression of AT$_2$ receptors in the adult can be upregulated in cardiovascular diseases such as ischemia and myocardial infarct (15). Hence, expression of AT$_2$ receptors during the development of pathophysiological conditions may be relevant for the fate of the disease. Indeed, we (1) recently demonstrated an induction of the expression of AT$_2$ receptor mRNA, associated with an increased renal vasodilatation in hypertensive rats (1), suggesting that overexpression of AT$_2$ receptors might represent a counterregulatory mechanism of the prohypertensive effects induced by ANG II (1). This hypothesis is supported by other studies (4, 10, 15, 16) demonstrating 1) pressor hypersensitivity to ANG II in mice lacking the AT$_2$ receptor; and 2) prevention of the hypertensive response to AT$_1$ receptor blockade by selective antagonism of AT$_2$ receptors in a renal wrap hypertension model. Elevated concentrations of ANG II can be found in some of the pathologies where upregulation of this receptor has been shown. Thus ANG II represents a natural candidate to regulate this AT$_2$ receptor. However, no comprehensive study has investigated the role of ANG II in the induction of AT$_2$ receptor mRNA during the development phase of renal damage and the biological role of this AT$_2$ overexpression in the fate of the disease. Therefore, in the present study, we tested the hypothesis that during the development of renal damage after renal ablation, increased levels of ANG II induce the expression of AT$_2$ receptor mRNA probably through one of it receptors, and that this overexpression is associated with a modulation of renal damage. For this purpose, we studied 1) the time course expression of AT$_2$ receptor mRNA and protein at 7, 15, and 30 days after renal ablation; and 2) the effect on AT$_2$ receptor expression produced by 7 days of treatment with either ramipril (an angiotensin-converting enzyme inhibitor), losartan (a selective AT$_1$ receptor antagonist), or PD-123319 (a selective AT$_2$ receptor antagonist). The biological significance of AT$_2$ receptor overexpression was evaluated by chronic blockade of the AT$_2$ receptor with PD-123319 and evaluating the effect on renal damage by measuring blood pressure, urinary protein excretion, and renal sclerosis.

MATERIALS AND METHODS

Animals

Male Wistar normotensive rats (250–300 g) were used in the present experiments. The animals were maintained at a 12:12-h light-dark cycle (light beginning at 7 AM) and housed in a special room at constant temperature (22 ± 2°C) and humidity (50%), with food and water freely available in their home cages.

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General Methods

Renal ablation. After the animals underwent anesthesia with ether, the kidneys were exposed under aseptic conditions via two dorsal incisions. The right kidney was completely removed after the blood vessels and urethra were ligated. The left kidney underwent dissection of the renal artery branches. Two or three of these branches were ligated as described previously (11); thus two-thirds of the kidney became ischemic (5/6 nephrectomy). These incisions were then closed. At this point, the animals were subsequently divided into several groups: 1) sham-operated group (control; n = 35); 2) renal ablation group (n = 35); 3) renal ablation group pretreated with ramipril (2.5 mg·kg\(^{-1}\)·day\(^{-1}\) in the drinking water; n = 5); 4) renal ablation group pretreated with losartan (10 mg·kg\(^{-1}\)·day\(^{-1}\) in the drinking water; n = 17); and 5) renal ablation group pretreated with PD-123319 (3 mg·kg\(^{-1}\)·day\(^{-1}\) subcutaneously by an osmotic minipump; Alza; Palo Alto, CA; n = 13). Ramipril, losartan, or PD-123319 was administered for 7 days, starting on the day of surgery. 6) In the ANG II-treated group, normal rats were chronically infused with ANG II (200 ng·kg\(^{-1}\)·day\(^{-1}\) subcutaneously by an osmotic minipump n = 5) for 14 days. 7) In the ANG II plus nifedipine-treated group, normal rats were chronically infused with ANG II (200 ng·kg\(^{-1}\)·day\(^{-1}\)) and nifedipine (1 mg·kg\(^{-1}\)·day\(^{-1}\); n = 5) subcutaneously by an osmotic minipump for 14 days. The development of renal damage was evaluated by measuring mean arterial pressure (MAP), urinary protein excretion, and renal sclerosis.

Mean arterial blood pressure was measured by the telemetric method. Seven days before 5/6 nephrectomy, rats were subjected to implantation of an intraperitoneal device (TA11PA-C40 transducer) for telemetric measurements of blood pressure, according to the manufacturer’s instructions (Data Sciences International). Blood pressure was measured daily during a 10-min period. Blood pressure values were recorded in a personal computer for later analysis. Urinary excretion of protein was measured as previously reported (11).

Histological studies. Paraffin-embedded sections were stained with hematoxylin and eosin and periodic acid-Schiff reagent. Four-micrometer-thick sections were examined by two investigators in a blinded fashion. Glomerulosclerosis was defined as the presence of periodic acid-Schiff-stained tissue within the glomerular tuft, with loss of cellular elements, collapse of capillary lumen, and entrapment of hyaline material. The severity of glomerulosclerosis was classified as previously reported (18): grade 0 = no sclerosis, grade 1 = <25% of the glomerulus, grade 2 = 25 to 50% of the glomerulus, grade 3 = 50 to 75% of the glomerulus, and grade 4 = 75 to 100% of the glomerulus. The score percentage of the biopsy was calculated as number of glomeruli with damage/number of normal glomeruli.

Tubulointerstitial fibrosis evaluation in every case was performed by a pathologist unaware of the origin of each kidney section. For the scoring of the tissue affected by fibrosis, sections were stained with Masson’s trichrome. Ten consecutive noncrossed fields of cortex (640 × 477 mm at ×10) per biopsy were analyzed by light microscopy (model BX51; Olympus America, Melville, NY) and captured by a digital video camera (Cool Snap Pro, Media Cybernetics, Silver Spring, MD). Pictures were processed on a computer and analyzed with Image-Pro Plus 5.0 (Media Cybernetics) and Photoshop 7.0 (Adobe Systems, San Jose, CA). By taking advantage of the capabilities of color recognition of this software, blue positive areas were selected and quantified in pixel units, previous exclusion of glomeruli, and vessels from the field. For each examined field, the number of positive areas was expressed as a fraction of the tubulointerstitium (blue positive areas divided by the overall field area). Finally, for each biopsy, the mean fractional number of blue positive areas was obtained by averaging the values obtained from 10 examined fields.
3, and 10 ± 1 mL/24 h. However, these values were markedly increased in the renal ablation group; MAP increased to 115 ± 6, 118 ± 9, and 116 ± 4 mm Hg (P < 0.05). Urinary protein excretion increased to 39.1 ± 12.8, 87.6 ± 25.2, and 131.4 ± 45.6 mg protein/24 h (P < 0.05) and urine volume increased to 42 ± 4, 37 ± 2, and 44 ± 5 mL/24 h (P < 0.05) at 7, 15, and 30 days after renal ablation. Moreover these changes in renal parameters were associated with progressive renal tissue injury. Renal ablation induced progressive and time-dependent renal damage. Seven days after nephrectomy, glomeruli showed segmental sclerosis and collapse; when the percentage of damage was calculated, we found that 3.3% of the glomeruli were affected. Moreover, we found the presence of collagen in the connective tissue in <5% of the cortical area. Thus grade I interstitial fibrosis was reported. Fifteen days after renal ablation, the number of glomeruli with segmental sclerosis and collapse increased to 37% and the amount of collagen in the connective tissue was within 25–40% of the cortical area; thus the presence of grade II interstitial fibrosis was reported. Finally, 30 days after renal ablation, the number of glomeruli with segmental sclerosis and collapse increased to 48% and the amount of collagen in the connective tissue was within >40% of the cortical area, in addition to tubular dilation and nephritis. Thus the presence of grade III interstitial fibrosis was reported.

Time-Dependent AT$_2$ Receptor Expression

AT$_2$ receptor mRNA expression in the renal tissue from sham-operated rats was very low, whereas that in renal ablation rats was markedly increased in a time-dependent manner (Fig. 1, top). To evaluate whether the increased levels of AT$_2$ receptor mRNA were associated with increased levels of AT$_2$ receptor protein, we detected the AT$_2$ receptor renal content by immunohistochemistry. AT$_2$ receptor protein expression was present in the sham-operated rats, but was significantly increased in a time-dependent manner in the renal hypertensive rats (Fig. 1, bottom).

Effect of ANG II system blockade on AT$_2$ receptor expression. Figure 2, top, is a representative example of the PCR products obtained after 7 days of surgery from sham-operated and renal ablation rats as well as renal ablation rats pretreated with either ramipril, losartan, or PD-123319. The amount of AT$_2$ receptor mRNA was analyzed by scanning and expressed as the ratio to GAPDH as arbitrary units in the different groups. AT$_2$ receptor mRNA was increased in the renal tissue from renal ablation rats compared with the sham-operated rats. Furthermore, AT$_2$ mRNA was reduced in the renal tissue from ramipril-treated renal ablation rats (Fig. 2). Moreover, ANG II receptor blockade showed contrasting results; pretreatment with losartan further increased the expression of AT$_2$ receptor mRNA, whereas PD-123319 inhibited the increase in this expression (Fig. 2). Thus the densitometric values for renal ablation rats were, respectively, 0.277 ± 0.012, 0.195 ± 0.005, 0.483 ± 0.011, and 0.184 ± 0.04 arbitrary units for untreated, ramipril-, losartan-, and PD-123319-pretreated animals. Similarly, immunohistochemistry demonstrated that induction of the AT$_2$ protein expression in the renal ablation rats was...
potentiated by losartan and inhibited by PD-123319 (Fig. 2, bottom). The corresponding colorimetric values were, respectively, 170 ± 53, 313 ± 75, 120 ± 15, 1,831 ± 164, and 111 ± 11 arbitrary units for the sham-operated, renal ablation, losartan-pretreated renal ablation and PD-123319-pretreated renal ablation rats. The combination of both ANG II blockers (losartan and PD-123319) showed prevention of the losartan-induced potentiation of AT2 mRNA (0.483 ± 0.011 and 0.254 ± 0.04 arbitrary units for losartan- and losartan + PD-123319-pretreated animals).

Effect of ANG II infusion on AT2 mRNA expression. To evaluate whether high ANG II levels were responsible for induction of AT2 mRNA, we chronically infused rats with a dose of 200 ng·kg⁻¹·min⁻¹ of ANG II. Comparison of the hemodynamics effects produced by chronic ANG II infusion with the effect in the control rats showed that ANG II increased blood pressure from 105 ± 2 to 132 ± 6 mmHg. Furthermore, chronic ANG II infusion increased renal AT2 mRNA and protein expression to levels similar to those in rats with renal ablation (Fig. 3, top). To evaluate whether the effect of ANG II on AT2 mRNA expression was dependent on the hypertensive or nonhypertensive effects of ANG II, we prevented the hypertension by treating the ANG II-infused rats with the antihypertensive drug nifedipine. Treatment with nifedipine decreased blood pressure values of the ANG II-infused rats from 132 ± 6 to 115 ± 2 mmHg. However, reduction of the
blood pressure was not associated with the ANG II-dependent increase in AT$_2$ mRNA expression (Fig. 3, bottom).

AT$_2$ receptor biological role in renal damage after renal ablation. Daily evaluation of blood pressure as well as urinary protein excretion and urine volume showed that changes in these parameters are present from the first day after induction of the renal damage and they reach a stable value 7 days after the 5/6 nephrectomy. Each curve represents the means ± SE of 5 different experiments. *$P < 0.05$ vs. the corresponding response in the sham-operated group. **$P < 0.05$ vs. the corresponding response in the hypertensive group.

DISCUSSION

Apart from the implications discussed below, our results demonstrate the following: 1) expression of the AT$_2$ receptor (mRNA and protein) is increased in the renal tissue of renal ablation (compared with sham-operated) rats; 2) ANG II synthesis inhibition was associated with reduction in the induced AT$_2$ mRNA expression; 3) ANG II type 1 receptor blockade was associated with overexpression of the AT$_2$ receptor, whereas ANG II type 2 receptor blockade was associated with inhibition of the AT$_2$ overexpression; 4) ANG II infusion increased AT$_2$ expression and this effect was not affected by preventing the hypertensive effect of ANG II; and 5) inhibition
of the AT$_2$ receptor was associated with potentiation of the development of renal and vascular damage after renal ablation.

Our observation that AT$_2$ receptor expression is increased during the development of renal damage and hypertension agrees with previous results where AT$_2$ receptor mRNA expression was greater in young spontaneously hypertensive rats (SHR) compared with age-matched Wistar-Kyoto (WKY) and adult SHR (18). Moreover, kidneys from newborn and 7-day-old SHR showed significantly greater densities of AT$_2$ receptors than 4-mo-old SHR and WKY rats (22). Furthermore, increased AT$_2$ receptor gene expression and protein had been shown in the kidneys from 5/6 nephrectomized rats. However, these observations and our data seem to disagree with a recent publication in which a significant reduction in AT$_2$ receptor protein has been shown in the wrapped kidney model (21). This apparent discrepancy may be explained in terms that AT$_1$ receptors are downregulated and no changes are observed in the contralateral kidney; thus a relatively greater expression of AT$_2$ receptors in the wrapped kidney may be present in this model of hypertension. The above findings beg the question as to the mechanism responsible for the induction of AT$_2$ receptor expression in the 5/6 nephrectomy rat, which is an ANG II-dependent model of hypertension. We (5) and other authors (7) have reported a direct effect of ANG II on mRNA expression in renal tissue. Thus we explored the role of ANG II in the regulation of AT$_2$ receptor protein and mRNA after renal ablation by testing the hypothesis that either inhibition of the ANG II system by inhibition of the angiotensin-converting enzyme or blockade of ANG II receptors with either AT$_1$ or AT$_2$ receptor blockers could prevent the increased expression of the AT$_2$ receptor in renal tissue after renal ablation. Indeed, treatment with ramipril prevented the induction of AT$_2$ expression, suggesting that ANG II could be responsible for the regulation of AT$_2$ receptor expression. However, ANG II receptor blockade showed contrasting results. Interestingly, AT$_1$ receptor blockade did not inhibit the AT$_2$ receptor overexpression in the renal ablation rats but it resulted in an increased AT$_2$ receptor expression, suggesting that AT$_1$ receptors were not involved in the regulation of the AT$_2$ receptor expression or that, in the presence of AT$_1$ receptor blockade, ANG II through stimulation of AT$_2$ receptors, could mediate the increased mRNA expression; this view gains weight when one considers that AT$_2$ receptor blockade inhibited such overexpression. In keeping with this hypothesis, other lines of evidence have shown that ANG II did the following: 1) upregulated AT$_2$ receptor mRNA via the stimulation of AT$_2$ receptors in rat cortical cells (7) and 2) increased AT$_2$ receptor function either directly or indirectly (via the activation of the renin-angiotensin system by sodium depletion) (12, 13). However, inhibition of ANG II synthesis or ANG II receptor blockade is associated with important changes in blood pressure. Thus the question that arises is whether ANG II or hypertension is responsible for the induction of AT$_2$ expression. Further experiments were performed to explore this question. Chronically infused ANG II further supported the role for ANG II in the regulation of AT$_2$ receptors, and the combination with nifedipine ruled out the participation of hypertension in this ANG II-dependent effect.

The identification and characterization of the AT$_2$ receptor-induced expression in our model provided the rationale to investigate the biological role of this ANG II receptor subtype in the development of renal damage. Several studies have suggested that AT$_2$ receptor activation is associated with vasodilator effects (1, 3, 13), suggesting a role in the regulation of blood pressure (6, 17). Renal hypertension after renal ablation develops in a time-dependent manner and blood pressure is significantly higher as early as 3 days after the renal ablation, reaching a plateau value 1 wk after the surgery. Thus, to evaluate the role of AT$_2$ receptors in the development of hypertension, we tested the effect of AT$_2$ receptor blockade on blood pressure during those first days of development of hypertension. Our results showing that PD-123319 potentiated the development of hypertension (Fig. 4) are particularly relevant, as blood pressure was increased from the first day after the 5/6 nephrectomy. These findings imply that 1) by inhibiting the early functional expression of AT$_2$ receptors, we are eliminating a mechanism that protects against the increase in blood pressure; and 2) an overexpression of AT$_2$ receptors during the development of hypertension represents a counterregulatory mechanism to modulate the increase in blood pressure. The mechanism by which AT$_2$ receptor blockade could increase blood pressure can be suggested as the elimination of a vasodilatory effect of ANG II through the AT$_2$ receptor. Moreover, renal tissue from PD-123319-treated rats showed ischemic damage observed in the remnant tissue, associated with vascular hypertrophy and severe reduction of the vascular lumen, suggesting a renal vasoconstrictor effect in the presence of AT$_2$ receptor blockade. Nevertheless, either mechanism (loss of the vasodilatory actions of ANG II or basal vasoconstriction) would potentiate the vasoconstrictor effects of ANG II, thereby increasing blood pressure and renal damage. The findings that AT$_2$ receptor antagonism enhanced renal damage contrast with a recent publication (2) where beneficial effects have been reported after AT$_2$ receptor antagonism in 5/6 nephrectomized rats. However, in that study, the authors did not show changes in blood pressure or vascular hypertrophy after AT$_2$ receptor blockade. Thus we suggest that increased renal damage in these rats is the result of the ischemic process rather than a specific effect on matrix accumulation or cell proliferation. Admittedly, against this view, it could be argued that such a potentiation may be attributable to a possible partial agonistic activity of PD-123319 at AT$_1$ receptors. However, this possibility can be excluded as PD-123319 failed to increase MAP in sham-operated rats.

The findings of this study suggest that during the development of renal damage after renal ablation, increased ANG II concentrations induce AT$_2$ receptor overexpression and that functional expression of this mechanism represents a counterregulatory system to prevent the extension of the renal damage induced by the renal ablation.

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REFERENCES


