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Effects of Angiotensin II on the Renal Interstitial Concentrations of NO_2/NO_3 and Cyclic GMP in Anesthetized Rats

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ABSTRACT—The present study was conducted to determine whether exogenous angiotensin II (Ang II) may increase the renal interstitial fluid concentrations of NO_2/NO_3 (NO_x) and cyclic guanosine monophosphate (cGMP) concomitantly and which Ang II receptor subtypes may induce these changes in anesthetized rats, using a microdialysis method. Ang II (50 ng/kg per min, i.v.) significantly increased mean blood pressure (MBP), extraction rates of renal interstitial NO_x from 23.9 ± 1.0 to 31.2 ± 1.9 pmol/min, and cGMP from 4.1 ± 0.3 to 6.4 ± 0.5 fmol/min, and decreased renal blood flow (RBF). The AT_1-receptor antagonist CV11974 alone significantly increased RBF, but did not alter MBP, renal interstitial concentrations of NO_x and cGMP. A superimposition of Ang II on CV11974 did not affect MBP and RBF, but significantly increased renal interstitial concentrations of NO_x and cGMP. The AT_2-receptor antagonist PD123319 alone did not change any of the parameters. However, superimposition of Ang II on PD123319 increased MBP and decreased RBF without any effects on renal interstitial concentrations of NO_x and cGMP. These results suggest that Ang II stimulates NO production via the AT_2-receptor in the kidney.

Keywords: Nitric oxide, Microdialysis, AT_2-receptor, Cyclic GMP, Kidney

Angiotensin II (Ang II) plays an important role in the control of blood pressure, body fluid and electrolyte balance via Ang II receptors, in which subtypes have been defined as type 1 (AT_1) and type 2 (AT_2) receptors. It is well known that most renal actions of Ang II are mediated via the AT_1-receptor (1, 2). AT_2-receptors are also present in the kidney, but the action via the AT_2-receptor has not yet been made clear (3). Ichiki et al. (4) have reported that mice lacking the AT_2-receptor have a higher blood pressure, indicating that Ang II may dilate blood vessels via the AT_2-receptor. Siragy and Carey (5) have recently reported that Ang II infusion increases the renal interstitial concentration of cyclic guanosine monophosphate (cGMP) in rats and that an AT_2-receptor antagonist or an inhibitor of nitric oxide synthase, N^G-nitro-L-arginine methyl ester (L-NAME), blocks the increase of cGMP production in response to Ang II. Furthermore, they have also reported that in rats with 2-kidney, 1 wrap model (Grollman) hypertension, renal interstitial NO_2/NO_3 (NO_x) and cGMP were higher in the contralateral kidney than in the wrapped kidney (6). These findings suggest that Ang II may stimulate the nitric oxide (NO)-cGMP pathway in the kidney. Arima et al. (7) have demonstrated vasodilation via the AT_2-receptor in an isolated microperfused afferent arteriole. Moreover, we have recently found that tempol, a membrane-permeable radical scavenger, strongly dilates renal blood vessels in Ang II-induced hypertensive rats and that the renal vasodilation induced by tempol disappears after the treatment of L-NAME (8). Thus, Ang II may stimulate the production of NO via the AT_2-receptor and dilate renal blood vessels. Nevertheless, all the findings in the above literature are indirect and do not show any data about the effects of Ang II on renal interstitial concentration of NO_x, which are stable metabolites of NO.

The present study was therefore conducted to determine whether Ang II increases the renal interstitial concentrations of NO_x and cGMP concomitantly and which Ang II receptor subtypes (AT_1 or AT_2) are related to Ang II-induced changes in anesthetized rats.

MATERIALS AND METHODS

General procedures

Experiments were performed in 7 – 8-week-old male Sprague Dawley rats, which had been maintained on stan-

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dard laboratory chow. All surgical and experimental procedures were performed according to the guidelines for the care and use of animals as established by the Kagawa Medical University. The rats were anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p.). Polyethylene tubes (PE 50) were inserted into the right femoral artery and vein for measurement of blood pressure and for infusion of drugs or isotonic saline. The left kidney was exposed through a retroperitoneal flank incision, and renal blood flow (RBF) was continuously measured by placing an electromagnetic flow probe around the renal artery. A microdialysis probe was gently implanted into the renal cortex and perfused with lactated Ringer's solution at a rate of 2 μl/min by a microinfusion pump. The microdialysis probe was constructed in our laboratory as previously reported (9, 10). The dialysis membrane is made of cuprophan fiber with a molecular mass cut-off of 5,500 Da (Toyobo, Otsu). The dialysates were collected during 30-min sampling periods in plastic chilled tubes and were stored at −70°C until assay.

NO_x and cGMP in dialysate were measured by the Griess method after reduction of NO_3 to NO_2 in the cadmium column (11) and by an RIA kit (Amersham Pharmacia Biotech, Little Chalfont, UK), respectively. The minimum detection level of NO_x by the Griess method and cGMP by radioimmunoassay are 0.25 μmol/L and 2 fmol/L, respectively.

Experimental protocols

The following protocols were performed in four different groups of rats. In all series of experiments, dialysates were collected at 30-min intervals throughout the experiments. 1) Effects of Ang II on renal hemodynamics and renal interstitial concentrations of NO_x and cGMP were examined in 6 rats. After the collection of two consecutive control dialysates, Ang II was infused intravenously at a rate of 50 ng/kg per min for 60 min, and two additional dialysates were collected during the infusion of Ang II. 2) Effects of AT_1-receptor blockade and Ang II during blockade of AT_1-receptor on renal hemodynamics and renal interstitial concentrations of NO_x and cGMP were examined in 6 rats. After collection of two consecutive control dialysates, CV11974 (priming dose: 1 mg/kg, i.v.; sustaining dose: 0.1 mg/kg per min, i.v.) was administered and two more 30-min dialysates were collected. Ang II was then superimposed to CV11974 at a dose of 50 ng/kg per min for 60 min and two additional dialysates were collected during infusion of CV11974 and Ang II. 3) Effects of AT_2-receptor blockade and Ang II during blockade of AT_2-receptor on renal hemodynamics and renal interstitial concentrations of NO_x and cGMP were examined in 6 rats. In this protocol, after collecting two consecutive control dialysates, PD123319 (priming dose: 10 mg/kg, i.v.; sustaining dose: 50 μg/kg per min, i.v.) followed by Ang II (50 ng/kg per min for 60 min) were infused and dialysates were collected in the same manner as described above. 4) In 6 rats, we did a time control experiment in which saline solution was infused for 180 min and four dialysates were collected.

Statistical analyses

The values are presented as means ± S.E.M. Differences in the extraction rates of renal interstitial NO_x or cGMP were assessed by Student's paired *t*-test. The time course for changes in a variable was evaluated using two-way ANOVA followed by a Newman-Keuls *post hoc* test. A value of $P<0.05$ was considered statistically significant.

Chemicals

CV11974 was obtained from Takeda Chemical Industries Ltd. (Osaka). PD123319 ((*S*-[+]-1-[(4-[dimethylamino]-3-methylphenyl)methyl]-5-[diphenylacetyl]-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic acid), ditrifluoroacetate) and Ang II were purchased from Research Biochemicals International (Natick, MA, USA) and Protein Research Foundation (Minoo), respectively. CV11974 was initially dissolved in 100 mM Na_2CO_3 and then finally dissolved in 0.9% saline solution.

RESULTS

Effects of Ang II on renal hemodynamics and renal interstitial concentrations of NO_x and cGMP

The intravenous infusion of Ang II increased mean blood pressure (MBP) from 103 ± 4 to 134 ± 4 mm/Hg and decreased RBF from 8.2 ± 0.8 to 5.6 ± 1.0 ml/min (Fig. 1A). The extraction rates of NO_x and cGMP from the renal interstitial space, which reflect the interstitial concentration of these substances, increased from 20.0 ± 1.7 and 8.0 ± 1.0 to 32.2 ± 3.0 pmol/min and 16.3 ± 2.5 fmol/min, respectively (Fig. 2).

In the time control experiments, MBP, RBF and extraction rates of NO_x and cGMP did not change throughout the 180-min experimental period (Data were not shown).

Effects of AT_1-receptor blockade and Ang II during blockade of AT_1-receptor on renal hemodynamics and renal interstitial concentrations of NO_x and cGMP

The intravenous administration of CV11974 significantly increased RBF. MBP tended to decrease, but these changes were not statistically significant (Fig. 1B). The extraction rates of NO_x and cGMP were also not affected by the administration of CV11974 (Fig. 3). A superimposition of Ang II on CV11974 did not change MBP and RBF; however, the renal extraction rates of NO_x and cGMP significantly increased from 23.9 ± 1.0 and 4.1 ± 0.3 to 31.2 ± 1.9 pmol/min and 6.4 ± 0.5 fmol/min, respectively (Fig. 3).

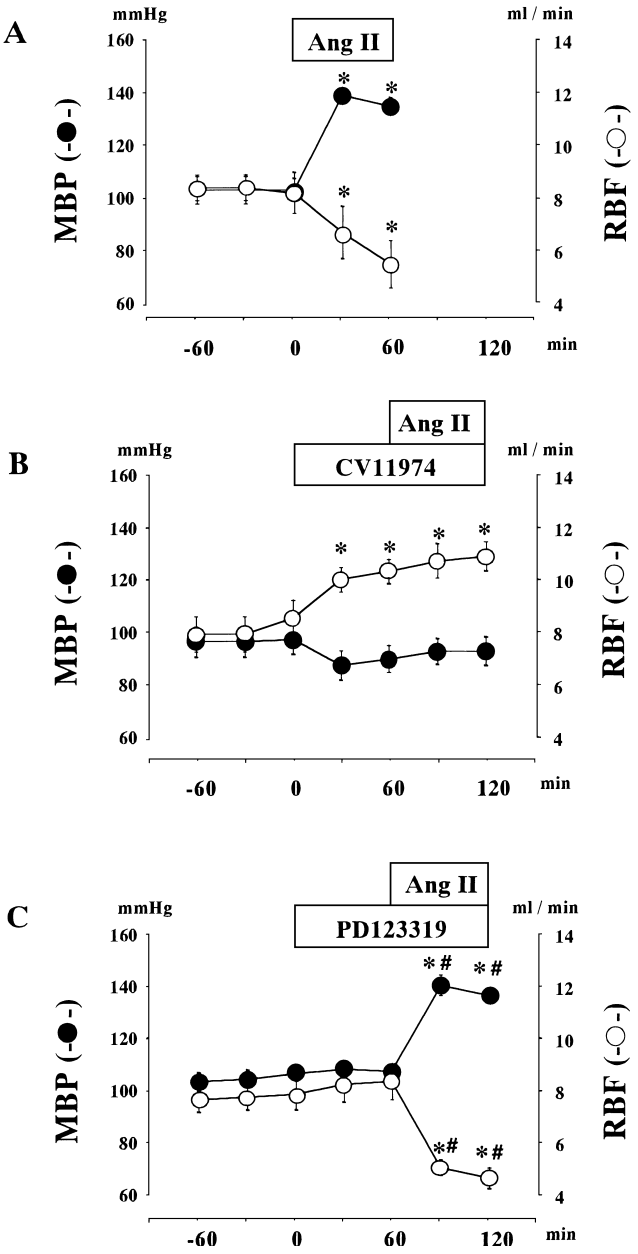

Fig. 1. Effects of angiotensin II (Ang II) on mean blood pressure (MBP, closed circles) and renal blood flow (RBF, open circles). A: Ang II alone. B: During blockade of AT_1-receptor by CV11974. C: During blockade of AT_2-receptor by PD123319. *P<0.05 vs values (at 0 min) obtained just before starting of Ang II, CV11974 or PD123319. $^{\#}P$<0.05 vs values (at 60 min) obtained just before starting of Ang II during infusion of CV11974 or PD123319.

Effects of AT_2-receptor blockade and Ang II during blockade of AT_2-receptor on renal hemodynamics and renal interstitial concentrations of NO_x and cGMP

PD123319 did not affect MBP, RBF (Fig. 1C), or the extraction rates of NO_x and cGMP (Fig. 4). A superimposition of Ang II on PD123319 increased MBP from 107 ± 1 to 136 ± 3 mm/Hg and decreased RBF from 8.3 ± 0.7 to 4.6 ± 0.4 ml/min (Fig. 1C), but did not affect the renal extraction rates of NO_x and cGMP (Fig. 4).

DISCUSSION

The aim of the present experiment was to determine whether exogenous Ang II stimulates the NO synthesis in the kidney and which Ang II receptor subtypes mediate these effects of Ang II. For measurements of NO_x and cGMP in the renal interstitial space, we have introduced a microdialysis method using a specially devised probe (9, 10). Due to the difficulty of direct evaluation of NO itself in the kidney, we have measured the accumulation of NO_2/NO_3, which are stable metabolites of NO. Furthermore, cGMP, which is a second messenger of NO, was also measured concomitantly with NO_x. The present study clearly demonstrates that intravenous administration of Ang II increased the extraction rates of NO_x and cGMP from the renal interstitial space in rats. However, several researchers have already suggested that Ang II stimulates NO synthesis in the kidney. Deng et al. (12) have reported that short-term Ang II infusion in rats increases the urinary excretion of NO_x, although Suto et al. (13) have suggested that urinary NO_x may not reflect systemic or renal synthesis of NO. Using the microdialysis method, Siragy and Carey (5) have also reported that Ang II increases the renal interstitial cGMP in conscious rats. However, they did not measure the renal interstitial concentrations of NO_x during Ang II infusion. Thus, this is the first report that clearly shows Ang II-induced increase in renal interstitial concentrations of NO_x.

To identify Ang II receptor subtypes, which mediate Ang II-induced increase in renal interstitial NO_x and cGMP, we used the selective AT_1-receptor antagonist CV11974 or AT_2-receptor antagonist PD123319. During the blockade of AT_1-receptor by CV11974, Ang II did not change the MBP, indicating a complete blockade of the AT_1-receptor. In the present study, our results show that CV11974 itself did not affect the renal interstitial concentrations of NO_x and cGMP. Combined administration of CV11974 and Ang II significantly increased renal interstitial concentrations of NO_x and cGMP. Similar to our results, Siragy and Carey (5) have reported that during blockade of AT_1-receptor by Losartan, Ang II increases renal interstitial cGMP. The same authors also reported that Losartan does not affect the renal interstitial concentrations of NO_x or cGMP in the normotensive rat, but increased renal NO_x and cGMP concentrations in rats with 2-kidney, 1 figure-8 wrap hypertension (6). These findings indicate that Ang II-induced increases in NO_x and cGMP are not mediated via the AT_1-receptor. On the other hand, Pueyo et al. (14) have reported that although rat aortic endothelial cells contain only the AT_1-receptor, Ang II

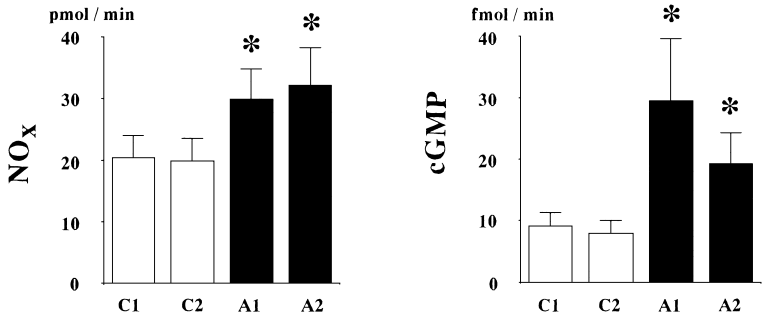

Fig. 2. Effects of angiotensin II (Ang II) on the extraction rates of NO_2/NO_3 (NO_x) and cyclic guanosine monophosphate (cGMP) from the renal interstitial space. Open and closed columns indicate control (C1 and C2) and Ang II (A1 and A2), respectively. **P*<0.05 vs C2.

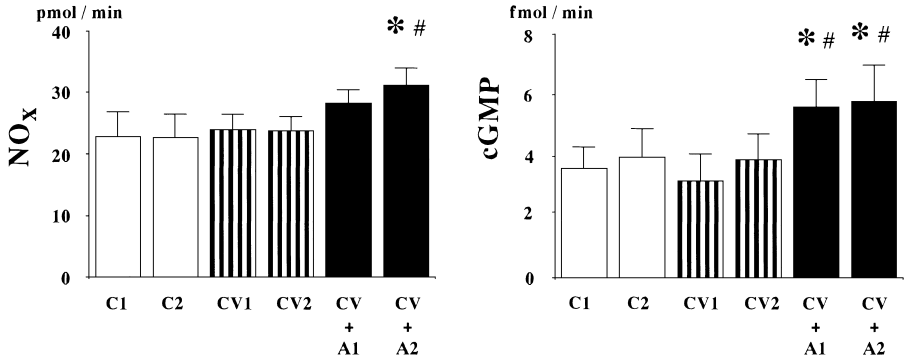

Fig. 3. Effects of angiotensin II (Ang II) during blockade of AT_1-receptor by CV11974 on the extraction rates of NO_2/NO_3 (NO_x) and cyclic guanosine monophosphate (cGMP) from the renal interstitial space. Open, hatched and closed columns indicate control (C1 and C2), CV11974 alone (CV1 and CV2) and CV11974 plus Ang II (CV + A1 and CV + A2), respectively. **P*<0.05 vs C2, #*P*<0.05 vs CV2.

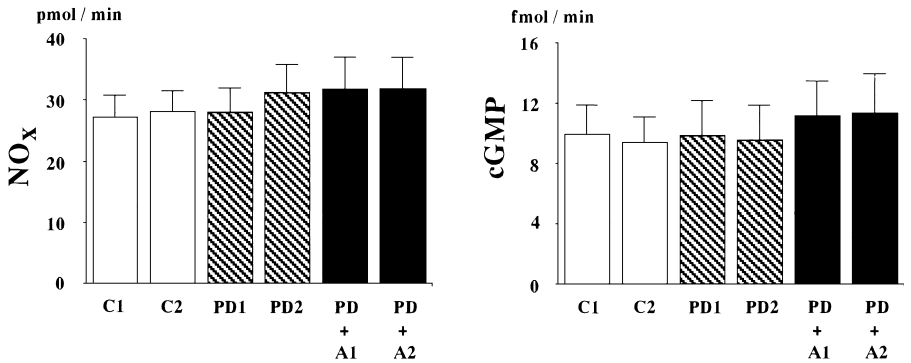

Fig. 4. Effects of angiotensin II (Ang II) during blockade of AT_2-receptor by PD123319 on the extraction rates of NO_2/NO_3 (NO_x) and cyclic guanosine monophosphate (cGMP) from the renal interstitial space. Open, hatched and closed columns indicate control (C1 and C2), PD123319 alone (PD1 and PD2) and PD123319 plus Ang II (PD + A1 and PD + A2), respectively.

stimulates NO_x and cGMP production in cultured aortic endothelial cells, indicating that Ang II may stimulate the production of NO via the AT_1-receptor. However, Tsutsumi et al. (15) reported that Ang II alone increases aortic cGMP level, and that the combination of Ang II and AT_1-receptor antagonist (CV11974) further increases it in AT_2-receptor transgenic mice. Since these Ang II-induced changes were completely blocked by inhibitors of NO synthase, bradykinin type 2 receptor and AT_2-receptor, the authors concluded that Ang II increases the aortic cGMP through the bradykinin/NO pathway and these effects of Ang II might be mediated via the AT_2-receptor. Thus, it is likely that Ang II stimulates the production of NO and cGMP via the AT_2-receptor in the kidney. However, based

on the present findings, we could not refer to the contribution of bradykinin.

In another group of rats, we demonstrated that Ang II-induced increases of NO_x and cGMP were inhibited by PD123319, indicating that Ang II stimulated the production of NO via AT_2-receptor. Siragy and Carey (5, 6) and Gohlke et al. (16) have also reported that PD123319 inhibits the Ang II-stimulated cGMP production in the kidney and aorta, respectively. Siragy et al. (17) further reported that in AT_2-null mice, cGMP levels are unchanged after Ang II infusion. In contrast to AT_2-null mice, Ang II significantly increased cGMP in wild type mice.

On the other hand, it has been reported that shear stress, which is exerted by the blood stream on the endothelial cell surface, regulates endothelial autocoids production such as NO (18, 19) and modifies the vascular action of Ang II (20). Based on the present results, we have analyzed the relationship between RBF and renal interstitial concentration of NO_x or cGMP. Ang II alone decreased RBF, but stimulated the renal production of NO_x or cGMP. During blockade of AT_1-receptors by CV11974, Ang II did not affect RBF, but stimulated the renal production of NO_x or cGMP. In addition, Ang II after the treatment of AT_2-receptor antagonist decreased RBF, but did not affect the renal production of NO_x or cGMP. There was significant dissociation between changes in RBF and the renal production of NO_x or cGMP. Thus, it is likely that Ang II-induced NO production might not be indirectly mediated by hemodynamic changes, but was directly mediated via the AT_2-receptor in the kidney.

Although our results demonstrate that Ang II increases the renal interstitial concentrations of NO_x and cGMP, it is beyond the scope of the present experiment to identify the source of NO_x and cGMP. However, Miyata et al. (21) have reported that AT_2-receptor mRNA is distributed in the various tubular and vascular segments of the renal cortex and medulla. In addition, nitric oxide synthase and soluble guanylate cyclase were also found in renal vascular tissue and tubules (22, 23). Thus, it is likely that increased concentrations of renal interstitial NO_x and cGMP reflect both vascular and tubular origin.

In summary, we demonstrated that Ang II markedly increased MBP, renal interstitial concentrations of NO_x and cGMP and decreased RBF. The AT_1-receptor antagonist CV11974 alone increased RBF, but did not alter MBP, NO_x and cGMP. A superimposition of Ang II on CV11974 did not affect MBP and RBF, but significantly increased NO_x and cGMP. The AT_2-receptor antagonist PD123319 alone did not affect any of the parameters. The superimposition of Ang II on PD123319 increased MBP and decreased RBF, but did not affect the renal interstitial concentrations of NO_x and cGMP. These results suggest that Ang II stimulates NO production via the AT_2-receptor in the kidney.

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