British Journal of Pharmacology (1996) 117, 885–890 

# Modulation by nitric oxide and prostaglandin of the renal vascular response to angiotensin II (3-8)

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**1** The aim of this study was to investigate the renal vascular response to angiotensin II (3-8) (AIV). The effect of the nitric oxide synthase inhibitor, $N^G$-nitro-L-arginine methyl ester (L-NAME) or the cyclo-oxygenase inhibitor, indomethacin on the AIV-induced response was examined in anaesthetized spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY).

**2** Intrarenal infusion of AIV produced a biphasic vasoconstrictor response. The vasoconstriction developed rapidly to reach a maximum followed by a partial recovery to a sustained lesser level of vasoconstriction. The initial maximum response was enhanced by L-NAME but not affected by indomethacin treatment. The simultaneous administration of L-NAME and indomethacin prevented the partial recovery resulting in a greater sustained level of constriction.

**3** A stable vasoconstriction of relatively constant magnitude was observed with angiotensin II (AII) infusion. The AII vasoconstriction was enhanced by L-NAME but not changed by indomethacin. The combination of these inhibitors further enhanced the AII-induced vasoconstriction in WKY, but not in SHR.

**4** Pretreatment with the AII receptor antagonist, losartan, inhibited the vasoconstriction induced by AIV and AII.

**5** These results suggest that nitric oxide and prostaglandins may modulate the renal vascular response to AIV.

**Keywords:** Angiotensin II; angiotensin II (3-8); nitric oxide; prostaglandins; renal haemodynamics; spontaneously hypertensive rats

## Introduction

Angiotensin II (3-8) (AIV) is a carboxyl terminal hexapeptide fragment of angiotensin II (AII). Recent studies have shown the presence of specific AIV binding sites that are pharmacologically distinct from the AII receptors (Hall *et al.*, 1993; Hanesworth *et al.*, 1993; Miller-Wing *et al.*, 1993; Swanson *et al.*, 1992). The AIV receptor has been reported to be distributed in a variety of tissues including brain, heart, kidney, aorta, liver and lung. In addition the receptor numbers are 1.3 to 20 times greater than the [$^{125}$I]-Sar$^1$,Ile$^8$-AII binding receptor (Swanson *et al.*, 1992).

In spite of its high expression in several tissues, the physiological role of the AIV specific receptor has been poorly understood. AIV has been known to induce a pressor and dipsogenic effect like AII although its potency was less than AII (Fitzsimons, 1971; Wright *et al.*, 1989; Gardiner *et al.*, 1993). However recently, Swanson *et al.* (1992) have reported that intrarenal infusion of AIV increased renal cortical blood flow whereas the same dose of AII induced renal vasoconstriction. Topical application of AIV with L-arginine on rabbit pial arterioles has been reported to induce vasodilatation and the effect was prevented by methylene blue (Haberl *et al.*, 1991). These results suggest possible interaction between AIV-induced cardiovascular response and intrinsic vasodilator substances such as nitric oxide (NO) or prostaglandins. Furthermore, many studies suggest that there is an impaired ability of NO or prostaglandins to regulate cardiovascular function in hypertensive animals (Shepherd & Katušić, 1991; Jackson & Herzer, 1993; Ruilope *et al.*, 1994). It is also possible that AIV may contribute to the pathogenesis of hypertension.

To investigate these possibilities, we compared the effect of intrarenal infusion of AIV and AII on renal blood flow in rats pretreated with the NO synthase inhibitor, $N^G$-nitro-L-arginine methyl ester (L-NAME) and/or the cyclo-oxygenase inhibitor, indomethacin in spontaneously hypertensive rats (SHR).

## Methods

Male SHR and normotensive Wistar-Kyoto rats (WKY) of 9–14 weeks of age were obtained from SLC (Shizuoka, Japan). Rats were maintained in the animal care facility with ambient temperature of 23±1°C and humidity of 55%. Animals were fed a standard diet and had free access to tap water. Approval for these studies was obtained from the Animal Experimentation Committee of Tohoku University Pharmaceutical Institute.

### *Vascular reactivity*

*Surgical preparation* On the day of the experiment, rats were anaesthetized with sodium pentobarbitone (50 mg kg$^{-1}$, i.p.) with supplements given subcutaneously as required. The left femoral artery was cannulated for continuous measurements of blood pressure. This cannula was connected to a pressure transducer (model TP-200T; Nihon Kohden, Tokyo) and an AP-601G amplifier (Nihon Kohden). The left femoral vein was cannulated for drug injection. Through a flank incision the left renal artery was dissected from the renal vein and fitted with a electromagnetic flow probe (1.0 mm in diameter) connected to a flowmeter (model MFV-2100; Nihon Kohden). A 30-gauge needle connected with PE-10 polyethylene tubing was inserted into the left renal artery through the abdominal aorta for the continuous infusion of saline (1 ml h$^{-1}$) and for drug admin-

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istration. Mean arterial blood pressure (MAP) and renal blood flow (RBF) were continuously recorded on a WT-645G Rec-ticorder (Nihon Kohden). After surgery, rats were stabilized at least 60 min.

*Effect of L-NAME* Seven WKY and six SHR were prepared as described above. Rats were infused with AIV (1 $\mu g\ kg^{-1} min^{-1}$) and AII (10 $ng\ kg^{-1}\ min^{-1}$) into the renal artery in random order for 10 min each and changes in MAP and RBF were monitored as a control response. At least 15 min were allowed between the infusions for recovery of RBF from the preceding drug infusion. We selected these doses of angiotensins to induce about 20% decrease from basal RBF in preliminary experiments. After this period, L-NAME was infused at 30 $\mu g\ kg^{-1}\ min^{-1}$ into the renal artery. When MAP and RBF were stabilized, the renal vascular responses to AIV and AII were examined during L-NAME infusion.

*Effect of indomethacin and L-NAME* Six WKY and six SHR were prepared as described above. After the control period, indomethacin (5 $mg\ kg^{-1}$) was injected intravenously and the renal vascular responses to AIV and AII were examined. Then indomethacin (5 $mg\ kg^{-1}$, i.v.) and L-NAME (30 $\mu g\ kg^{-1}\ min^{-1}$ via the renal artery) were administered and the effect of AIV and AII were examined again.

*Effect of losartan* Five WKY and five SHR were prepared as described above. After the control period, losartan (1 $mg\ kg^{-1}$) was injected intravenously. When MAP and RBF had stabilized, the renal vascular response to AIV and AII were examined.

### Drugs

AII ($H_2N$-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) and AIV ($H_2N$-Val-Tyr-Ile-His-Pro-Phe-OH) were purchased from Peptide Institute (Osaka, Japan). L-NAME and indomethacin were from Sigma chemicals. Losartan was a generous gift from Banyu Pharmaceutical Co. (Tokyo, Japan). Indomethacin was dissolved in 0.1 M $Na_2CO_3$ and adjusted pH to 7 by 1 M HCl. Other drugs were dissolved in 0.9% NaCl.

### Statistics

Results are presented as means ± s.e. Student's paired $t$ test was used to evaluate the effect of inhibitors on basal values. The other data were evaluated by two-way analysis of variance followed by Scheffe's test for multiple comparisons of means. Results with $P<0.05$ were considered statistically significant.

## Results

Effects of L-NAME or indomethacin on MAP and RBF are shown in Table 1. Intrarenal infusion of L-NAME reduced RBF in both WKY and SHR. The administration of indomethacin alone did not change RBF. When L-NAME was

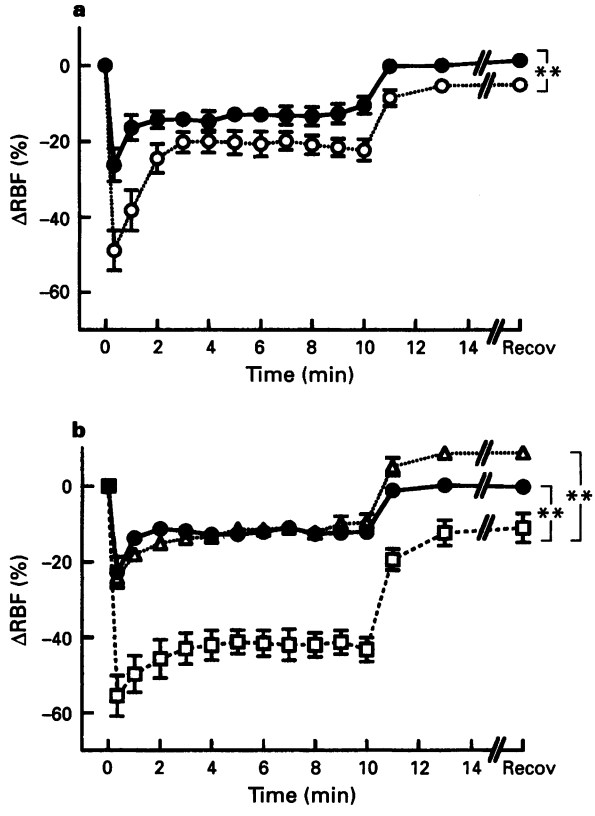
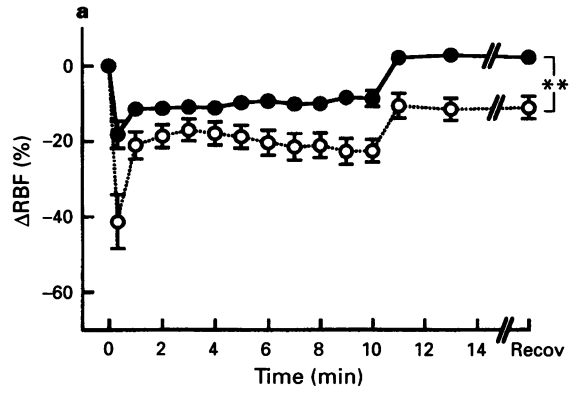



**Figure 1** (a) AIV-induced response of renal blood flow (RBF) in Wistar-Kyoto rats (WKY) before (●) or after treatment with $N^G$-nitro-L-arginine methyl ester (L-NAME, ○). (b) AIV-induced response of RBF in WKY before (●) or after treatment with indomethacin (Indo, △) or a combination of L-NAME and Indo (□). Values (shown as means ± s.e.) are expressed as percentage change relative to baseline immediately preceding intrarenal infusion of AIV in the presence of indicated inhibitors. $^{**}P<0.01$ between treatments.

**Table 1** Changes in MAP and RBF with L-NAME and/or Indo treatment

| | *L-NAME* | *Indo* | *Indo + L-NAME* |
|---|---|---|---|
| *WKY* | $n=7$ | $n=6$ | $n=6$ |
| MAP | | | |
| before drug | 100 ± 2 | 96 ± 5 | 94 ± 5 |
| after drug | 96 ± 4 | 94 ± 4 | 92 ± 4 |
| RBF | | | |
| before drug | 6.5 ± 0.6 | 6.5 ± 1.0 | 7.4 ± 0.9 |
| after drug | 4.7 ± 0.4* | 6.6 ± 1.1 | 4.6 ± 0.4** |
| *SHR* | $n=6$ | $n=6$ | $n=6$ |
| MAP | | | |
| before drug | 144 ± 7 | 139 ± 5 | 133 ± 3 |
| after drug | 142 ± 6 | 140 ± 4 | 141 ± 3 |
| RBF | | | |
| before drug | 6.8 ± 0.7 | 7.7 ± 0.4 | 7.6 ± 0.4 |
| after drug | 4.7 ± 0.3** | 8.0 ± 0.4 | 4.9 ± 0.4** |

Values are means ± s.e. L-NAME: $N^G$-nitro-L-arginine methyl ester; Indo: indomethacin; MAP: mean arterial blood pressure (mmHg); RBF: renal blood flow (ml $min^{-1}\ g^{-1}$ kidney weight). $^{*}P<0.05$, $^{**}P<0.01$ between before and after drug treatment.



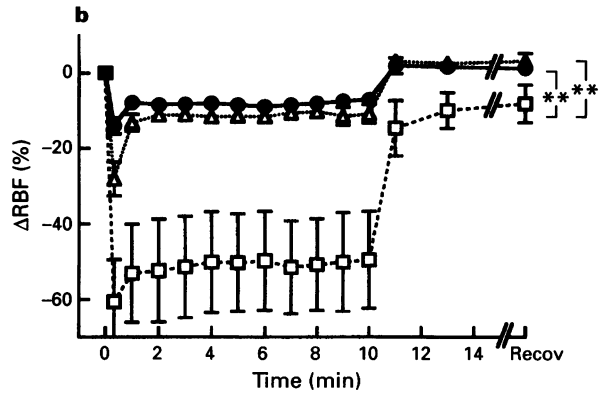
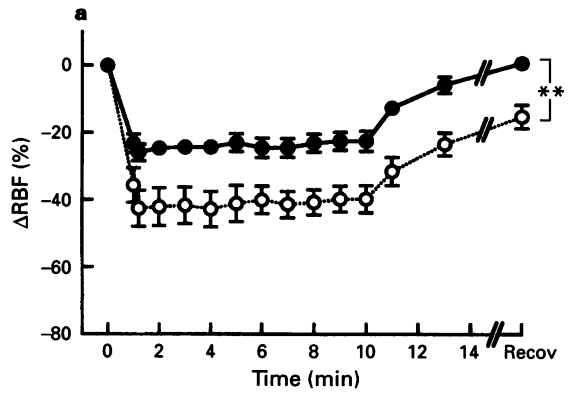




**Figure 2** (a) AIV-induced response of RBF in spontaneously hypertensive rats (SHR) before (●) or after treatment with L-NAME (○). (b) AIV-induced response of RBF in SHR before (●) or after treatment with Indo (△) or a combination of L-NAME and Indo (□). Values (shown as means ±s.e.) are expressed as percentage change relative to baseline immediately preceding intrarenal infusion of AIV in the presence of indicated inhibitors. $^{**}P<0.01$ between treatments.



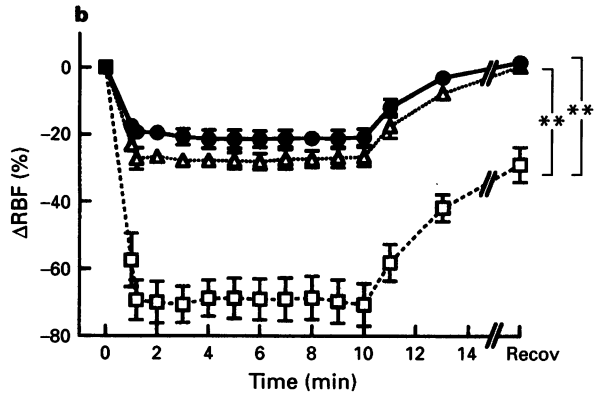




**Figure 3** (a) AII-induced response of RBF in WKY before (●) or after treatment with L-NAME (○). (b) AII-induced response of RBF in WKY before (●) or after treatment with Indo (△) or in combination with L-NAME and Indo (□). Values (shown as means±s.e.) are expressed as percentage change relative to baseline immediately preceding intrarenal infusion of AII in the presence of indicated inhibitors. $^{**}P<0.01$ between treatments.

administered in combination with indomethacin, a decrease in RBF was obtained in both strains. MAP was not affected by these treatments. Because the basal RBF was different depending on the drug treatment, the changes in RBF produced by AIV and AII are presented as percentage decreases from the basal level just prior to initiating the infusion.

The effects of L-NAME or indomethacin administration on AIV-induced RBF changes in WKY are shown in Figure 1. Intrarenal infusion of AIV induced a rapid decrease in RBF (peak: 0.28±0.03 min) followed by a partial recovery to a sustained vasoconstriction within 2 min. When the infusion of AIV was stopped, the RBF was quickly recovered. The AIV-induced changes in RBF were enhanced by intrarenal infusion of L-NAME ($P<0.01$). Pretreatment with indomethacin did not affect the AIV-induced changes in RBF. During the simultaneous administration of both inhibitors, the maximum decrease in RBF was greater than with L-NAME alone. However, in contrast to L-NAME alone, the combination of these inhibitors blocked the partial return to reduced vasoconstriction. Similar effects of L-NAME or indomethacin on the AIV-induced changes in RBF were observed in SHR (Figure 2). MAP did not show any significant effects of AIV infusion in either WKY or SHR even if treated with L-NAME or indomethacin.

In contrast to AIV, the AII-induced decrease in RBF reached a maximum about 1 min after the start of intrarenal infusion and maintained the same level until the end of the infusion period (Figure 3 and 4). The recovery of RBF was also slower than with AIV. The AII-induced RBF change was enhanced by L-NAME infusion in WKY ($P<0.01$) and SHR ($P<0.05$). Pretreatment of indomethacin did not induce statistically significant changes in the AII-induced renal vasoconstriction in either strain. The simultaneous administration of indomethacin and L-NAME further enhanced the RBF decrease induced by AII in WKY, but such a synergistic action was not observed in SHR. AII infusion did not alter MAP throughout these experiments.

To clarify the effect of the simultaneous administration of indomethacin and L-NAME, we calculated the ratio of vasoconstriction during L-NAME treatment to that without L-NAME (Figure 5). At the peak response, the AIV-induced decrease in RBF with L-NAME infusion became 2.14±0.33 times that of the control response in WKY. Also, the administration of indomethacin and L-NAME enhanced the decrease in RBF to 2.34±0.21 times the response with indomethacin alone in WKY. Similar enhancement was observed in SHR. The AII-induced decrease in RBF was enhanced by L-NAME 1.67±0.19 times in WKY. With indomethacin treatment, the decrease in RBF was further enhanced by L-NAME in WKY (2.71±0.35 times, $P<0.01$). However, in SHR, L-NAME-induced enhancement of the AII response was not different in the presence and absence of indomethacin.

To test whether AIV and AII-induced renal response is mediated by the AII type 1 ($AT_1$) receptor, we examined the effect of $AT_1$ receptor antagonist, losartan (Dup 753) on the AIV or AII-induced renal vasoconstriction. Losartan treatment inhibited both the rapid transient phase and the following weaker phase of vasoconstriction induced by AIV. At peak of the response, the AIV-induced decrease in RBF was significantly reduced by losartan in both WKY (−25±6 to

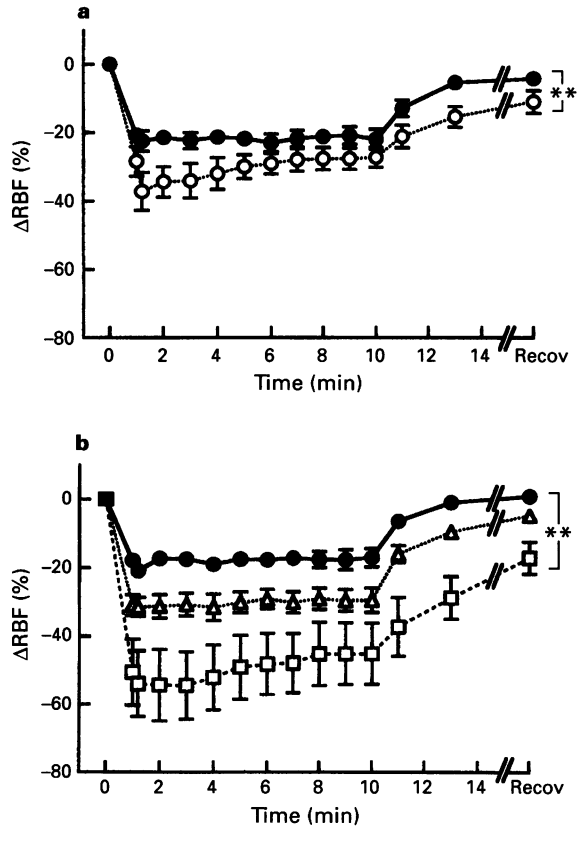



**Figure 4** (a) AII-induced response of RBF in SHR before (●) or after treatment with L-NAME (○). (b) AII-induced response of RBF in SHR before (●) or after treatment with Indo (△) or a combination of L-NAME and Indo (□). Values (shown as means±s.e.) are expressed as percentage change relative to baseline immediately preceding intrarenal infusion of AII in the presence of indicated inhibitors. $*P<0.05$, $**P<0.01$ between treatments.

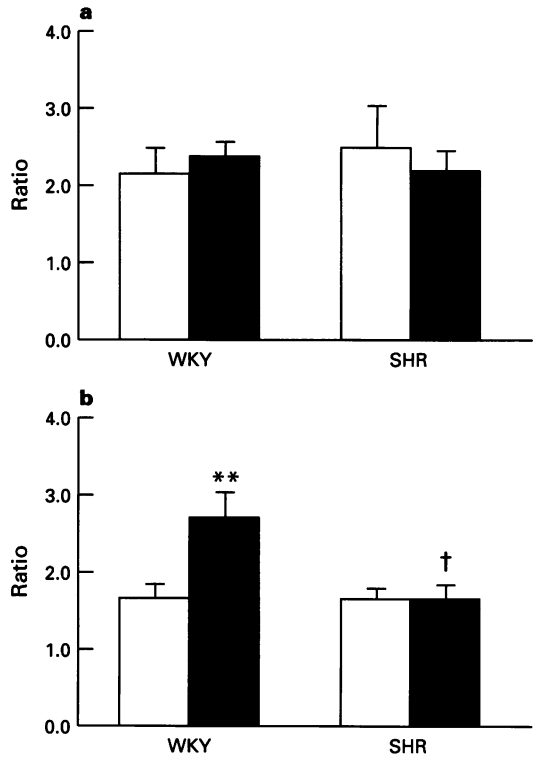



**Figure 5** Effect of L-NAME on renal vascular response to AIV and AII in the presence (solid column) or absence (open column) of indomethacin (Indo). Values (shown as means±s.e.) are ratio of vasoconstriction with L-NAME treatment to that without L-NAME. $**P<0.01$ compared to the group without Indo treatment. $\dagger P<0.05$ compared to WKY.

$-11\pm3\%$, $P<0.05$) and SHR ($-20\pm7$ to $-5\pm1\%$, $P<0.05$). The AII-induced decrease in RBF was also inhibited by losartan in both WKY ($-32\pm5$ to $-18\pm2\%$, $P<0.01$) and SHR ($-21\pm2$ to $-8\pm1\%$, $P<0.01$).

## Discussion

The present study supports the possibility of interactions of NO and prostaglandins with the AIV- or AII-induced decrease in RBF. These responses of angiotensins are mainly due to their effects on the renal vasculature because systemic BP was completely unaffected during their intrarenal infusion. The renal vasoconstriction induced by AIV may be mediated by the $AT_1$ receptor because maximum responses induced by AIV or AII were equally inhibited by losartan pretreatment. However, we found some differences between the AIV- and AII-induced renal vascular responses.

The AIV-induced vascular response in RBF was different in its time course characteristics from the AII-induced vasoconstriction. Intrarenal infusion of AIV induced a rapid decrease in RBF within 0.4 min, whereas AII takes more than 1 min to reach the maximum response. When the infusion of AIV was stopped, the RBF recovered more quickly than with the AII infusion. Gardiner *et al.* (1993) have also reported a rapid response to AIV when given by intravenous bolus injection. Because pretreatment with L-NAME or indomethacin did not change the time required to reach the maximum response and to recover in the present study, neither NO nor prostaglandins are thought to induce these differences in speed of response between the two angiotensins. Also, it is impossible to explain the slower response of AII by the idea that the vasoconstriction is mediated by AIV degraded from AII rather than AII itself because the potency of AIV was about 100 fold less than AII in this study. The faster recovery of the AIV-induced response may be explained by its rapid metabolism reported earlier in the isolated perfused kidney (Misumi *et al.*, 1983).

It is of interest that the intrarenal infusion of AIV induced a sustained vasoconstriction of lesser magnitude than the initial rapid transient constriction. In contrast, AII produced a stable vasoconstriction. The difference between AIV and AII may be attributed to a differential activation of vasodilator mechanisms. In addition to the $AT_1$ receptor-mediated rapid vasoconstriction, AIV may induce a slower counteractive vasodilatation which was not observed during AII infusion. Swanson *et al.* (1992) have reported that the intrarenal infusion of AIV increased superficial blood flow in the rat kidney cortex as measured by laser Doppler flowmetry. The biphasic vasoconstriction induced by AIV observed in the total RBF measurements in our study may be attributed to the combination of the dilatation of the superficial vasculature of the renal cortex and the constriction of other renal vasculature. Although further studies will be required to determine whether the present results are mediated by the AIV-specific receptor proposed by Swanson *et al.* (1992), intrinsic vasodilators such as NO and prostaglandins are thought to participate in this AIV-induced vasodilatation, because the simultaneous administration of L-NAME and indomethacin inhibited the partial return to the reduced vasoconstriction in this study. AIV may stimulate the release or accumulation of these vasodilators as kallikrein does in the kidney of Dahl salt-sensitive rats (Uehara *et al.*, 1994). The stimulating factor cannot be simply attributed to an increase in shear stress of the vasculature because AII infusion did not produce such a vasodilator effect while the comparable vasoconstriction was observed.

Another difference between AIV and AII-induced renal vascular response is the strain differences. The AII-induced renal vasoconstriction in SHR was affected less by NO and prostaglandins than in WKY. The maximum response in RBF with AIV infusion was not different between SHR and WKY even if pretreated with L-NAME and/or indomethacin. However, the AII-induced vasoconstriction was enhanced more in WKY than SHR by the combination of L-NAME and indomethacin. This result suggests that the vasoconstrictor effect of AII is diminished to a greater extent by NO or prostaglandins in the normotensive rat than in the SHR. It is possible that the lesser counteractive mechanisms to the pressor action of AII may relate to the pathogenesis of hypertension.

In the present study, the renal vasoconstriction produced by AIV or AII infusion was enhanced by L-NAME treatment. The AII-induced vasoconstriction was potentiated by other NO synthase inhibitors, $N^G$-nitro-L-arginine (Ito *et al.*, 1991; Chu & Beilin, 1993; Matsumura *et al.*, 1995) or $N^G$-monomethyl-L-arginine (Conrad & Whittemore, 1992). These results including our study suggest an inhibitory modulation of NO on $AT_1$ receptor-mediated renal vasoconstriction. In contrast to L-NAME, indomethacin treatment did not alter the vascular response to AIV or AII in this study. However, prostaglandins may also modulate the vascular response to AII as reported earlier by Aiken & Vane (1973) because the combined treatment with L-NAME and indomethacin induced a synergistic enhancement of the AII-induced renal vasoconstriction in WKY in the present study.

A possible explanation for the synergistic action is a compensatory role of NO and prostaglandins. When the synthesis of one of these factors was inhibited, the vasodilator response is not fully blocked because of the compensatory activity of the other vasodilator. Similar compensation by these factors had been reported in the renal response to acetylcholine (Salom *et al.*, 1991). Acetylcholine-induced increases in RBF and urine volume were inhibited by the combination of $N^G$-monomethyl-L-arginine and meclofenamate but not by the separate treatment with these inhibitors. However, the mechanisms responsible for the compensation by NO and prostaglandins are unknown. It is still controversial whether NO affects prostaglandin synthesis. However, Doni *et al.* (1988) have shown that exogenous administration of NO inhibits the bradykinin-stimulated release of prostaglandin $I_2$ from cultured endothelial cells. The inhibitory effect of NO on cyclo-oxygenase activity has also been reported by other investigators (Kanner *et al.*, 1992; Stadler *et al.*, 1993). Moreover, there are some studies suggesting inhibitory properties of prostaglandins on NO synthase activity (Marotta *et al.*, 1992). If one or more of these inhibitory mechanisms are working between NO and prostaglandin systems in normal conditions, the lesser activity of one vasodilator can be compensated for by the other.

In conclusion, intrarenal infusion of AIV induced a biphasic vasoconstrictor response consisting of a rapid onset to a maximum followed by a partial recovery to a sustained vasoconstriction. The initial rapid vasoconstriction induced by AIV was exaggerated by L-NAME and inhibited by losartan. The recovery from the initial constriction was inhibited by simultaneous pretreatment with L-NAME and indomethacin. These results suggest that NO and prostaglandins may interact with the renal response to AIV in a different manner from AII-induced vasoconstriction.

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*(Received July 6, 1995*
*Revised October 9, 1995*
*Accepted November 9, 1995)*