Nongenomic Vascular Action of Aldosterone in the Glomerular Microcirculation

SHUJI ARIMA,* KENTARO KOHAGURA,* HONG-LAN XU,* AKIRA SUGAWARA,* TAKAAKI ABE,* FUMITOSHI SATOH,* KAZUHISA TAKEUCHI,* and SADAYOSHI ITO*

Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University School of Medicine, Sendai, 980-8574, Japan.

Abstract. Aldosterone (Aldo) accelerates hypertension, proteinuria, and glomerulosclerosis in animal models of malignant hypertension or chronic renal failure. Aldo may exert these deleterious renal effects by elevating renal vascular resistance and glomerular capillary pressure. To test this possibility, directly examined were the action of Aldo on the afferent (Af) and efferent (Ef) arterioles (Arts). Examined were the effect of Aldo added to both the bath and lumen on the intraluminal diameter (measured at the most responsive point) of rabbits. Aldo caused dose-dependent constriction in both arterioles with a higher sensitivity in Ef-Arts. Vasoconstrictor action of Aldo was not affected by a mineralocorticoid receptor antagonist spironolactone and was reproduced by membrane-impermeable albumin-conjugated Aldo, suggesting that the vasoconstrictor actions are nongenomic. Pretreatment with neomycin (a specific inhibitor of phospholipase C) abolished the vasoconstrictor action of Aldo in both arterioles. In addition, the vasoconstrictor action of Aldo on Af-Arts was inhibited by both nifedipine and efonidipine, whereas that on Ef-Arts was inhibited by efonidipine but not nifedipine. The results demonstrate that Aldo causes nongenomic vasoconstriction by activating phospholipase C with a subsequent calcium mobilization thorough L- or T-type voltage-dependent calcium channels in Af- or Ef-Arts, respectively. These vasoconstrictor actions on the glomerular microcirculation may play an important role in the pathophysiology and progression of renal diseases by elevating renal vascular resistance and glomerular capillary pressure.

There is increasing evidence that renin-angiotensin-aldosterone system (RAAS) plays an important role in the pathogenesis and progression of renal diseases (1–5). Although angiotensin II (AngII) has been identified as the primary mediator of the underlying mechanisms for these actions are not well defined, Aldo may exert these deleterious renal effects by elevating renal vascular resistance and glomerular capillary pressure (P_GC). However, to our knowledge, there is so far no study demonstrating the direct vascular action of Aldo in the kidney, although Aldo causes renal arteriolar injury (11).

To test the possibility that Aldo may elevate P_GC, we microperfused rabbit afferent (Af) and efferent (Ef) arterioles (Arts), crucial vascular segments to the control of glomerular hemodynamics, and directly examined the vascular action of Aldo on these arterioles.

Materials and Methods

This study was performed in accordance with the Guide for Animal Experimentation, Tohoku University School of Medicine. We used methods similar to those described previously to isolate and microperfuse Af- and Ef-Arts (12–14). Briefly, young male New Zealand white rabbits (1.5 to 2.0 kg body weight), fed standard rabbit chow and tap water ad libitum, were killed with intravenous sodium pentobarbital (40 mg/kg) and given an intravenous injection of heparin (500 U). The kidneys were removed and sliced along the corticomedullary axis. Slices were placed in ice-cold MEM (Life Technologies BRL, Gaithersburg, MD) containing 5% BSA (Sigma Chemical, St. Louis, MO) and microdissected under a stereomicroscope (SZH-10; Olympus, Tokyo, Japan) as described previously. From each rabbit, a single superficial Af- and/or Ef-Art with its glomer-
ulus intact was microdissected. With a micropipette, the arteriole was transferred to a temperature-regulated chamber mounted on an inverted microscope (IMT-2; Olympus) with Hoffman modulation. The arteriole was cannulated with an array of glass pipettes as described previously (12–14) and perfused with oxygenated medium 199 (Life Technologies BRL) containing 5% BSA. Intraluminal pressure was measured by Landis’ technique, using a fine pipette introduced into the arteriole through the perfusion pipette, and was maintained at 60 mmHg in the case of Af-Arts. For Ef-Art perfusion, an Af-Art was microdissected together with the glomerulus and attached Ef-Art (approximately 250 to 300 μm in length). The Af-Art was cut short (approximately 50 μm) and cannulated as described above, except that the perfusion pipette was advanced to the end of the Af-Art. The tip of the pressure pipette was placed just beyond the distal end of the Af-Art, and intraluminal pressure at this point was maintained at 50 mmHg throughout the experiment to eliminate the hemodynamic influences of the Af-Art (13,14). In a previous study (13), we found that pressure in the Ef-Art at a point 50 μm distal to the glomerulus was approximately 35 mmHg, the physiologic level, under these experimental conditions.

The bath was identical to the arteriolar perfusate except that it contained 0.1% BSA, and was exchanged continuously. Microdissection and cannulation of the arteriole were completed within 90 min at 8°C, after which the bath was gradually warmed to 37°C for the rest of the experiment. Once the temperature was stable, a 30-min equilibration period was allowed before taking any measurements. Images of the arteriole were displayed at magnifications up to ×1980 and recorded with a video system consisting of a camera (CS520MD; Olympus), monitor (PVM1445MD; Sony, Tokyo, Japan), and video recorder (HR-S101; Victor, Tokyo, Japan). When we analyzed the data, we first observed the whole record of each experiment and decided the site where the strongest constriction occurred in response to Aldo. We consistently measured the intraluminal diameter of this site throughout the experiments (including the control period or pretreatment period) with an image-analysis system (VM-30; Olympus).

Effect of Aldo on Luminal Diameter

After the equilibration period, increasing doses of Aldo (10^{-10} M, 10^{-9} M, 5 \times 10^{-9} M and 10^{-8} M; Sigma) were added to both the bath and lumen. Luminal diameter was measured immediately before adding Aldo and observed for at least 30 min at each dose.

Time Course and Reversibility of Aldo-Induced Constriction

We found that Aldo at the nanomolar level caused vasoconstriction in both arterioles (see Results). To exclude the possibility that these constrictions were induced by delayed effects of lower concentrations of Aldo, we examined the time course of Aldo action. After the equilibration period, vehicle or Aldo (at 5 \times 10^{-9} M or 10^{-8} M) was added to both the bath and lumen, and then luminal diameter was observed for 30 min. In addition, we also examined the reversibility of Aldo-induced constriction. After examining the 30-min time course, both the bath and arteriolar perfusate were changed to the vehicle without Aldo, and then diameter was observed for additional 30 min.

Effect of Spiromolactone on Aldo-Induced Constriction

We next studied the possible involvement of intracellular mineralocorticoid receptor (MR) in the Aldo-induced vasoconstriction. For this, we examined the effect of spiromolactone, a classic MR antagonist. On the day of the experiment, a fresh solution containing spiromolactone (Sigma) at 10^{-5} M was prepared in physiologic salt solution containing 0.1% DMSO (Sigma). After the equilibration period, Af- or Ef-Arts were treated with spiromolactone at 10^{-5} M for 60 min, which is considered to be a sufficient dose and time period to take effect (15). Then vasoconstrictor actions of Aldo were examined as in protocol 1. We have confirmed that spiromolactone vehicle has no effect on the luminal diameter or vascular reactivity of Af- or Ef-Arts.

Effect of Albumin-Conjugated Aldo on the Luminal Diameter

We found that pretreatment with spiromolactone had no effect on Aldo-induced vasoconstriction in either arteriole (see Results). To further confirm that intracellular MR does not mediate Aldo’s vasoconstrictor action, we next examined whether albumin-conjugated Aldo (Alb-Aldo), which will not enter the cytoplasm, may cause similar time- and dose-dependent vasoconstriction as induced by free-form Aldo. The Alb-Aldo (Peptide Institute, Osaka, Japan) was produced by binding one BSA (molecular weight, 66,000; Sigma) molecule per 12 molecules of Aldo (Sigma). Because both the bath and perfusate already contain high amounts of BSA, Aldo-bound BSA is thought not to affect the vascular reactivity in our preparations. Effects of Alb-Aldo on the luminal diameter were examined as in protocol 1.

Effect of Actinomycin D or Cycloheximide on Aldo-Induced Constriction

To examine whether Aldo causes nongenomic vasoconstriction in renal arterioles, we next examined the effect of actinomycin D and cycloheximide, inhibitors of transcription and protein synthesis, on Aldo-induced vasoconstriction. After the equilibration period, Af- or Ef-Arts were treated with actinomycin D (10^{-5} M, Sigma) or cycloheximide (10^{-5} M, Sigma) for 60 min. Then vasoconstrictor actions of Aldo were examined as in protocol 1. It has been demonstrated that this concentration of actinomycin D or cycloheximide prevents Aldo-induced genomic nuclear signaling (16) or de novo protein synthesis in rat aorta (17), respectively. We used same vehicle for actinomycin D or cycloheximide with that for spiromolactone.

Effect of Neomycin on Aldo-Induced Constriction

We next examined a possible involvement of phospholipase C (PLC), which mediates nongenomic actions of Aldo in cultured vascular smooth muscle cells (VSMCs) (18,19), in the Aldo-induced vasoconstriction. After the equilibration period, Af- or Ef-Arts were treated with neomycin (300 μM; Sigma), a specific inhibitor of PLC, for 60 min. Then vasoconstrictor actions of Aldo were examined as in protocol 1. It has been demonstrated that neomycin at this concentration inhibits Aldo-induced rapid (within 30 s) increase in intracellular inositol 1,4,5-triphosphate (IP_3) in rat VSMCs (20). We used the same vehicle for neomycin with that for spiromolactone.

Effect of Calcium Channel Blockade on Aldo-Induced Constriction

It has been demonstrated that calcium (Ca^{2+}) influx to VSMC through L-type and/or T-type voltage-dependent calcium channels (VDCC) is required to elicit sustained vasoconstriction in Af- and Ef-Arts (14,21). Thus, we next examined the possible contribution of Ca^{2+} influx through these calcium channels to Aldo-induced vasoconstriction. After the equilibration period, Af- and Ef-Arts were treated with nifedipine (L-type calcium channel blocker, 1 μM; Sigma) or efonidipine (both L- and T-type calcium channel blocker, 1 μM; Nissan Chemical Industries, Tokyo, Japan) for 30 min. Then vasoconstrictor actions of Aldo were examined as in protocol 1. We (14,22) and Hayashi et al. (21) have previously reported that nifedi-
Aldosterone or efonidipine at this concentration completely abolishes AngII- and norepinephrine-induced vasoconstriction in Af- or Ef-Arts, respectively. We used the same vehicle for nifedipine or efonidipine with that for spironolactone.

Statistical Analyses
Values were expressed as mean ± SEM, and all statistical analyses were carried out with absolute values. A paired t test was used to examine whether the diameter at a given concentration differed from the control or pretreated value within each group. ANCOVA was used to examine whether dose-response curves differed between groups, and a two-sample t test was used to examine whether the change in diameter at a given concentration differed between groups. A value of P < 0.0125 (0.05/4) was considered significant using Bonferroni’s adjustment for multiple comparisons.

Results
Effect of Aldo on Luminal Diameter
Basal luminal diameter of Af- and Ef-Arts was 17.1 ± 0.3 (n = 8) and 14.9 ± 0.4 μm (n = 7), respectively. As shown in Figure 1, Aldo caused dose-dependent constriction in both arterioles (the constriction occurred within 10 min and persisted throughout the observation period). In Af-Arts, significant constriction was observed from 5 × 10⁻⁹ M, which decreased the diameter by 3.4 ± 0.7 μm (or 20% ± 4%, P < 0.001). Aldo at 10⁻⁸ M similarly decreased the diameter by 4.9 ± 0.5 μm (or 29% ± 3%). On the other hand, Aldo began to cause significant constriction from 10⁻⁷ M (by 2.2 ± 0.3 μm or 15% ± 2%, P < 0.01) in Ef-Arts. Thus, sensitivity to Aldo was higher in Ef-Arts compared to Af-Arts. Aldo at 5 × 10⁻⁹ M or 10⁻⁸ M similarly decreased the diameter of Ef-Arts by 3.5 ± 0.4 μm (or 23% ± 3%) or 3.9 ± 0.2 μm (or 26% ± 2%), respectively. In Ef-Arts, the decrease in diameter induced by 10⁻⁸ M Aldo (but not 5 × 10⁻⁹ M Aldo) was significantly (P < 0.01) greater than that induced by 10⁻⁹ M Aldo. In addition, when compared Af- and Ef-Arts, vasoconstrictor actions of Aldo were significantly stronger (P < 0.01) in Ef-Arts only at 10⁻⁸ M (which decreased the diameter of Af-Arts by 0.8 ± 0.4 μm or 5% ± 2%).

Time Course and Reversibility of Aldo-Induced Constriction
As shown in Figure 2, Aldo vehicle had no effect on the luminal diameter of either arteriole. Before, 30 min, and 60 min after the treatment, the diameter was 17.0 ± 0.6 μm, 17.2 ± 0.6 μm, and 17.0 ± 0.6 μm in Af-Arts (n = 7) and 15.0 ± 0.2 μm, 15.0 ± 0.2 μm, and 15.0 ± 0.3 μm in Ef-Arts (n = 7). Aldo at 5 × 10⁻⁹ M or 10⁻⁸ M began to cause significant (P < 0.01) constriction within 5 min in both arterioles. At 10 min after the application of 5 × 10⁻⁹ M or 10⁻⁸ M Aldo, luminal diameter decreased from 17.5 ± 0.5 μm to 14.1 ± 0.8 μm (by 3.4 ± 0.5 μm or 19% ± 3%, n = 8) or from 16.8 ± 0.4 μm to 12.5 ± 0.4 μm (by 4.3 ± 0.3 μm or 26% ± 2%, n = 7) in Af-Arts, whereas from 15.0 ± 0.2 μm to 12.1 ± 0.2 μm (by 3.0 ± 0.3 μm or 20% ± 2%, n = 7) or from 15.0 ± 0.3 μm to 11.2 ± 0.3 μm (by 3.7 ± 0.3 μm or 25% ± 2%, n = 6) in Ef-Arts. However, thereafter, Aldo at each concentration did not cause further constriction until 30 min in both arterioles.

Removing Aldo from the bath and lumen gradually dilated both arterioles, and the diameter tended to return to the basal level. Significant dilation was already observed at 10 min after removing Aldo in each case, and at 30 min, arteriolar diameters were not different from their basal value (at time 0) except for Af-Arts pretreated with 10⁻⁸ M Aldo. Thus, it became clear that vasoconstrictor actions of Aldo on renal arterioles were reversible.

Effect of Spironolactone on Aldo-Induced Constriction
Pretreatment with spironolactone at 10⁻⁵ M did not affect the luminal diameter of either arteriole; luminal diameter before and after the treatment was 16.9 ± 0.3 μm and 16.6 ± 0.3 μm (n = 6) or 15.0 ± 0.3 μm and 15.2 ± 0.4 μm (n = 7) in Af- or Ef-Arts, respectively. As shown in Figure 3, spironolactone had no effect on Aldo-induced vasoconstriction in either arteriole. In spironolactone-treated arterioles, Aldo began to cause significant constriction from 5 × 10⁻⁹ M (by 3.2 ± 0.6 μm or 19% ± 3%) in Af-Arts or from 10⁻⁹ M (by 2.0 ± 0.2 μm or 14% ± 2%) in Ef-Arts, and at 10⁻⁸ M the diameter decreased by 4.1 ± 0.6 μm (or 24% ± 3%) or 3.4 ± 0.4 μm (or 22% ± 2%), respectively.

Effect of Alb-Aldo on Luminal Diameter
Basal luminal diameter of Af- or Ef-Arts was 17.0 ± 0.6 μm or 14.5 ± 0.3 μm (n = 7, each), respectively. As shown in Figure 3, Alb-Aldo caused similar dose-dependent vasoconstriction (occurring within 10 min) with free-form Aldo in both arterioles; Alb-Aldo began to cause significant (P < 0.001) constriction from 5 × 10⁻⁹ M (by 3.4 ± 0.5 μm or 20% ± 2%) in Af-Arts or from 10⁻⁹ M (by 1.9 ± 0.4 μm or 13% ± 2%) in Ef-Arts, and at 10⁻⁸ M, the diameter decreased by 4.4 ± 0.6 μm (or 25% ± 3%) or 3.6 ± 0.4 μm (or 24% ± 3%), respectively.
Effect of Actinomycin D or Cycloheximide on Aldo-
Induced Constriction

Pretreatment with actinomycin D or cycloheximide did not affect the luminal diameter of either arteriole. The diameter of Af-Arts before and after the treatment was 17.0 ± 0.6 μm and 16.7 ± 0.7 μm for actinomycin D (n = 6) or 17.5 ± 0.3 μm and 17.3 ± 0.3 μm for cycloheximide (n = 6), whereas that of Ef-Arts was 14.8 ± 0.4 μm and 14.8 ± 0.4 μm for actinomycin D (n = 7) or 14.8 ± 0.3 μm and 14.8 ± 0.3 μm for cycloheximide (n = 7). As shown in Figure 4, neither actinomycin D nor cycloheximide had effect on Aldo-induced vasoconstriction in either arteriole. In actinomycin D–treated arte-

Figure 2. Time course and reversibility of aldosterone-induced vasoconstriction in afferent arterioles (left) and efferent arterioles (right). Aldosterone at 5 × 10⁻⁹ M (solid triangles) or 10⁻⁸ M (solid circles) caused time-dependent constriction until 10 min; however, thereafter, aldosterone at each concentration did not cause further constriction until 30 min in either arteriole. Removing aldosterone from the bath and arteriolar perfusate gradually dilated both arterioles, and the diameter tended to return to the basal level. Thus, it became clear that vasoconstrictor actions of aldosterone on renal arterioles were reversible. On the other hand, aldosterone vehicle (open circles) observed for 60 min had no effect on the luminal diameter of either arteriole. *P < 0.01 compared with 0 min, #P < 0.01 compared with 30 min.

Figure 3. Effect of spironolactone or albumin conjugation on the vasoconstrictor action of aldosterone in afferent arterioles (left) and efferent arterioles (right). In either arteriole, vasoconstrictor action of aldosterone was not affected by pretreatment with 10⁻⁵ M spironolactone (solid circles) and was reproduced by membrane-impermeable albumin-conjugated aldosterone (solid squares), suggesting that the vasoconstrictor actions are nongenomic. *P < 0.01 compared with prealdosterone value.

Effect of Actinomycin D or Cycloheximide on Aldo-
Induced Constriction

Pretreatment with actinomycin D or cycloheximide did not affect the luminal diameter of either arteriole. The diameter of Af-Arts before and after the treatment was 17.0 ± 0.6 μm and 16.7 ± 0.7 μm for actinomycin D (n = 6) or 17.5 ± 0.3 μm and 17.3 ± 0.3 μm for cycloheximide (n = 6), whereas that of Ef-Arts was 14.8 ± 0.4 μm and 14.8 ± 0.4 μm for actinomycin D (n = 7) or 14.8 ± 0.3 μm and 14.8 ± 0.3 μm for cycloheximide (n = 7). As shown in Figure 4, neither actinomycin D nor cycloheximide had effect on Aldo-induced vasoconstriction in either arteriole. In actinomycin D–treated arte-
rioles, Aldo began to cause significant constriction from $5 \times 10^{-9}$ M (by 2.7 ± 0.6 µm or 16% ± 3%) in Af-Arts or from $10^{-9}$ M (by 2.4 ± 0.4 µm or 16% ± 3%) in Ef-Arts, and at $10^{-8}$ M, the diameter decreased by 3.9 ± 0.5 µm (or 23% ± 2%) or 3.9 ± 0.4 µm (or 26% ± 2%), respectively. In cycloheximide-treated arterioles, Aldo began to cause significant constriction from $5 \times 10^{-9}$ M (by 3.2 ± 0.8 µm or 19% ± 5%) in Af-Arts or from $10^{-9}$ M (by 1.9 ± 0.4 µm or 12% ± 2%) in Ef-Arts, and at $10^{-8}$ M, the diameter decreased by 4.2 ± 0.7 µm (or 24% ± 4%) or 3.8 ± 0.6 µm (or 25% ± 4%), respectively.

Effect of Neomycin on Aldo-Induced Constriction
Pretreatment with neomycin at 300 µM did not affect the luminal diameter of either arteriole; luminal diameter before and after the treatment was 17.7 ± 0.5 µm and 17.3 ± 0.5 µm.

Figure 4. Effect of cycloheximide and actinomycin D on the vasoconstrictor action of aldosterone in afferent arterioles (left) and efferent arterioles (right). In either arteriole, vasoconstrictor action of aldosterone was not affected by pretreatment with $10^{-5}$ M cycloheximide (solid circles) or $10^{-3}$ M actinomycin D (solid squares), further suggesting that the vasoconstrictor actions are nongenomic. *$P < 0.01$ compared with prealdosterone value.

Figure 5. Effect of neomycin, a phospholipase C (PLC) inhibitor, on the vasoconstrictor action of aldosterone in afferent arterioles (left) and efferent arterioles (right). Pretreatment with 300 µM neomycin (solid circles) abolished the vasoconstrictor action of aldosterone in either arteriole, indicating the critical role of PLC activation in it. *$P < 0.01$ compared with prealdosterone value.
Effect of Calcium Channel Blockade on Aldo-Induced Constriction

Pretreatment with nifedipine at 1 \( \mu \)M did not affect the luminal diameter of either arteriole; luminal diameter before and after the treatment was 17.3 ± 0.5 \( \mu \)m and 17.7 ± 0.4 \( \mu \)m \((n = 8)\) or 15.0 ± 0.3 \( \mu \)m and 14.9 ± 0.3 \( \mu \)m \((n = 7)\) in Af- or Ef-Arts, respectively. As shown in Figure 5, Aldo did not cause any constriction in such arterioles; the decrease in diameter induced by Aldo at 10\(^{-8}\) M was 0.5 ± 0.3 \( \mu \)m and 0.8 ± 0.5 \( \mu \)m in Af- and Ef-Arts, respectively. Thus, pretreatment with neomycin abolished the vasoconstrictor action of Aldo in either arteriole.

Pretreatment with efonidipine at 1 \( \mu \)M did not affect the luminal diameter of either arteriole; luminal diameter before and after the treatment was 17.3 ± 0.5 \( \mu \)m and 17.7 ± 0.4 \( \mu \)m \((n = 8)\) or 15.0 ± 0.3 \( \mu \)m and 14.9 ± 0.3 \( \mu \)m \((n = 7)\) in Af- or Ef-Arts, respectively. As shown in Figure 6, Aldo did not cause any constriction in nifedipine-treated Af-Arts; the decrease in diameter induced by Aldo at 10\(^{-8}\) M was 0.6 ± 0.3 \( \mu \)m \((3\% ± 1\%)\). On the other hand, nifedipine had no effect on Aldo-induced vasoconstriction in Ef-Arts. In nifedipine-treated Ef-Arts, Aldo began to cause significant constriction from 10\(^{-9}\) M (by 2.1 ± 0.4 \( \mu \)m or 14\% ± 2\%) and at 10\(^{-8}\) M, the diameter decreased by 4.0 ± 0.4 \( \mu \)m \((27\% ± 3\%)\).

Pretreatment with efonidipine at 1 \( \mu \)M did not affect the luminal diameter of either arteriole; luminal diameter before and after the treatment was 17.0 ± 0.6 \( \mu \)m and 17.7 ± 0.4 \( \mu \)m \((n = 6)\) or 15.0 ± 0.3 \( \mu \)m and 14.9 ± 0.3 \( \mu \)m \((n = 7)\) in Af- or Ef-Arts, respectively. In contrast to nifedipine, efonidipine inhibited Aldo-induced vasoconstriction in both arterioles. As shown in Figure 6, Aldo did not cause any constriction in efonidipine-treated Af-Arts; the decrease in diameter induced by Aldo at 10\(^{-8}\) M was 0.7 ± 0.4 \( \mu \)m \((4\% ± 2\%)\). In efonidipine-treated Ef-Arts, Aldo did not cause significant constriction until the concentration reached 10\(^{-8}\) M, which slightly decreased the diameter by 1.7 ± 0.3 \( \mu \)m \((11\% ± 2\%\), \(P < 0.01\)).

Discussion

Development of proteinuria and elevated P\(_{GC}\) in the mineralocorticoid-salt hypertension model (23) suggests that mineralocorticoids cause hemodynamic injury to the glomerulus. However, their vascular action on the glomerular hemodynamics has not been defined. In the study presented here, we examined the possible vascular action of Aldo, a representative mineralocorticoid, on the renal microvessels and found that Aldo at the nanomolar level causes dose-dependent vasoconstriction in both Af- and Ef-Arts, with a higher sensitivity in the Ef-Art. Although the decrease in diameter (approximately 15\% to 30\%) induced by Aldo appears to be weaker than that induced by other vasoconstrictors such as AngII or norepinephrine (13), it represents more than twice increase in vascular resistance (because the vascular resistance is proportional to the reciprocal of fourth power of radius). Thus, from a physiological point of view, Aldo may play an important role in the regulation of vascular resistance in the renal microcirculation.

The concentrations of Aldo required for significant constriction were higher than physiologic plasma levels (approximately 0.1 nM in human (24) or approximately 0.5 nM in rabbit (25)); however, under some pathologic conditions, plasma Aldo concentration (PAC) rises above 1 nM, which causes significant constriction in Ef-Arts in the study presented here. Berl et al. (26) have reported that mean PAC of eight patients with chronic renal failure were 523 pg/ml (approximately 1.4 nM), which increased to 2393 pg/ml (6.6 nM) when patients were placed on a low-sodium diet. Greene et al. (7) reported

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\text{Figure 6. Effect of calcium channel blockade on the vasoconstrictor action of aldosterone in afferent arterioles (left) and efferent arterioles (right). Vasoconstrictor action of aldosterone on afferent arterioles was inhibited by both nifedipine (L-type calcium channel blocker; solid circles) and efonidipine (both L- and T-type calcium channel blocker; solid squares), whereas that on efferent arterioles was inhibited only by efonidipine. Thus, calcium mobilization through L- or T-type calcium channels seems to be required for aldosterone-induced constriction in afferent or efferent arterioles, respectively. }^{*}P < 0.01 \text{ compared with prealdosterone value.}
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that PAC increases 10-fold in five of six nephrectomized rats from 50 to 526 pg/ml (approximately 1.5 nM). Thus, Aldo may cause vasoconstriction in Ef-Arts under some pathologic conditions such as chronic renal failure, in which hyperaldosteronism (several- to tenfold increase in PAC) is a common finding in both experimental animals (7) and humans (26,27). Through its vasoconstrictor action on the postglomerular Ef-Art, Aldo would elevate PGCC. Glomerular hypertension is now believed to be responsible, at least in part, for the development of glomerular dysfunction and glomerular structural damages in renal diseases (28–30). Thus, this vasoconstrictor action may account for the pathogenic role for hyperaldosteronism in the progression of renal diseases (5–10).

In Af-Arts, further higher concentrations of Aldo were required for significant constrictor. However, because PAC is known to elevate in SHRSP (PAC of M-strain SHRSP at age of 12 wk reaches approximately 4.5 nM (31)), Aldo may also cause vasoconstriction in Af-Arts under some other pathologic conditions, such as severe hypertension. Because the Af-Art accounts for most of the preglomerular vascular resistance and increase in its vascular resistance contributes to the pathogenesis of essential hypertension (32), the vasoconstrictor action on Af-Arts may play a role in the BP elevating effect of Aldo besides its “volume-retaining” actions in severe to malignant hypertension.

It is generally accepted that Aldo exerts its action by binding to high-affinity intracellular MR, which act as transcription factors and mediate delayed genomic effects (18,33). This delay (generally hours) would be a consequence of the time necessary for transcribing new mRNA and subsequent translation of that message into cell-specific proteins. On the other hand, several recent studies have demonstrated the rapid nongenomic effects of Aldo in cardiovascular tissues. In cultured VSMC, physiologic concentrations (subnanomolar) of Aldo induces a prompt (<1 min) increase in intracellular Ca2+ concentration, which was not inhibited by a MR antagonist spironolactone (19). Moreover, in humans, intravenous application of Aldo significantly increases systemic vascular resistance within a few minutes (34,35), suggesting a nongenomic vasoconstrictor action because of the short time frame. In the study presented here, vasoconstrictor action of Aldo was also compatible with nongenomic actions; the major characteristics was its relatively early onset (apparent within 5 min), which was not affected by spironolactone at 1000-fold excess concentrations and was reproduced by membrane-impermeable Alb-Aldo. Furthermore, neither actinomycin D nor cycloheximide had effect on Aldo-induced vasoconstriction in either arteriole. Taken together, these results strongly suggest that in the glomerular microcirculation Aldo causes direct nongenomic vasoconstriction, which may be mediated by specific membrane receptors distinct from the intracellular MRs. This idea is supported by recent studies indicating the presence of membrane receptors that mediate rapid nongenomic actions of Aldo (18,36,37).

To study the mechanism or mechanisms for Aldo-induced vasoconstriction, we first examined the possible contribution of PLC because several studies have demonstrated the involvement of PLC (and the phosphoinositide pathway) in the intracellular signaling for nongenomic Aldo action on the VSMCs (18,19). As expected, we found that pretreatment with neomycin (a specific inhibitor of PLC) completely abolishes Aldo-induced contraction in both Af- and Ef-Arts, demonstrating the involvement of PLC and phosphoinositide pathway in the intracellular signaling for Aldo-induced constriction of renal arterioles. In addition, we have also found that both nifedipine (L-type calcium channel blocker) and efonidipine (L/T-type calcium channel blocker) inhibited the vasoconstrictor action of Aldo on Af-Arts, whereas efonidipine but not nifedipine inhibited that on Ef-Arts. Thus, our results indicate that Aldo causes nongenomic vasoconstriction by activating PLC with a subsequent calcium mobilization through L- or T-type VDCC in Af- or Ef-Arts, respectively. However, the precise mechanism of post-PLC activation for Aldo-induced constriction is still unclear. Activation of PLC induces hydrolytic breakdown of phosphoinositides into IP3 and diacylglycerol (DAG); IP3 induces calcium release from the sarcoplasmic reticulum and DAG activates protein kinase C (PKC) (38). Indeed, Christ et al. (20,39) have demonstrated that Aldo, presumably through its nongenomic action, stimulates the production of both IP3 and DAG-PKC in VSMC. Thus, IP3 and/or DAG-PKC pathways may act to elicit Aldo-induced vasoconstriction in renal arterioles.

With regard to this possibility, using AngII as a vasoconstrictor agent, Nagahama et al. (40) have studied the relative role of IP3 and DAG-PKC pathways in PLC-mediated renal arteriolar vasoconstriction. They found that IP3-induced calcium release from the sarcoplasmic reticulum with a subsequent extracellular calcium entry through L-type VDCC plays an important role in the Af-Art constriction-mediated by PLC activation, whereas DAG-PKC pathway which requires extracellular calcium entry independent of L-type VDCC plays a predominant role in the Ef-Art. However, post-PLC mechanism for Aldo may be different from that of AngII. Thus, further studies that more precisely characterize the intracellular mechanism or mechanisms by which Aldo causes vasoconstriction are required. In addition, the biochemical nature of the receptors mediating Aldo-induced vasoconstriction is also to be established.

In addition to such nongenomic vascular actions, genomic renal deleterious actions of Aldo have also been reported. Rocha et al. (11,41) have demonstrated that spironolactone or eplerenone, a selective Aldo receptor blocker, reduces proteinuria and malignant nephrosclerotic lesions without altering BP in saline-drinking SHRSP or in AngII-infused and saline-drinking Wistar rats with chronic nitric oxide synthesis inhibition. Thus, it is also possible that Aldo may exert genomic vascular actions on the glomerular microvessels when applied much longer (several hours or more) than this study (30 min for each dose). Alternatively, both genomic and nongenomic pathways may synergistically contribute to the vascular actions of Aldo on the glomerular microcirculation because it is proposed that rapid modulation of intracellular signaling and second messengers by nongenomic actions in turn influences the genomic actions of Aldo (15,18,42). Further studies examining
possible genomic vascular actions of Aldo on the renal microvessels, together with their possible interactions with nongenomic actions, are clearly required.

In summary, we found that Aldo causes dose-dependent vasoconstriction in both Af- and Ef-Arts, with a higher sensitivity in Ef-Arts. Thus, in addition to its tubular and fibrotic actions, Aldo may contribute to the pathophysiology and progression of renal diseases by elevating renal vascular resistance and FvGc through its hemodynamic actions on the glomerular microcirculation. We also found that these vasoconstrictor actions were presumably nongenomic and mediated via membrane-bound receptors because they were not affected by spironolactone, actinomycin D or cycloheximide, and were reproduced by the membrane-impermeable conjugate of BSA-Aldo.

In addition, our results indicate that the vasoconstrictor actions are mediated through activation of PLC with a subsequent calcium mobilization via L- or T-type calcium channels in Af- or Ef-Arts, respectively. However, our results obtained in isolated arterioles may represent an in vitro phenomenon and may have no direct relevance to the in vivo Aldo action. Although Romagni et al. (43) recently demonstrated that Aldo at physiologic concentrations causes direct and rapid (presumably nongenomic) vasoconstriction in human resistance arteries in vivo, to our knowledge, no study has provided direct evidence that Aldo causes renal vasoconstriction in vivo. Thus, to clarify the physiologic and pathophysiologic significance of the possible (nongenomic) vascular actions of Aldo in the kidney, studies investigating the renal hemodynamic effects of Aldo in vivo (for example, effects of Aldo on BP, renal blood flow, renal vascular resistance, and GFR) are required.

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