Enhanced response to AVP in the interlobular artery from the spontaneously hypertensive rat

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Enhanced response to AVP in the interlobular artery from the spontaneously hypertensive rat. Am J Physiol Renal Physiol 288: F1023–F1031, 2005. First published December 14, 2004; doi:10.1152/ajprenal.00175.2004.—Arginine vasopressin (AVP) induces exaggerated intracellular free calcium (Ca\textsuperscript{2+}) responses in preglomerular smooth muscle cells from young spontaneously hypertensive rats (SHR) due to increased density of the AVP V\textsubscript{1a} receptor. The intention of the present paper was to examine the relative contribution of afferent arterioles (AA) and interlobular artery (ILA) in AVP- and norepinephrine-induced calcium signaling. The kidneys were perfused with agar solution in vivo, and thin cortical slices were enzyme digested to produce isolated agar-filled vascular fragments. Calcium responses were recorded in fura 2-loaded cells by Ca\textsuperscript{2+} imaging. Diameter changes were measured after AVP stimulation and mRNA for V\textsubscript{1a} was measured on isolated vessel fragments. SHR had significantly higher baseline calcium ratio and lower resting diameter compared with normotensive Wistar-Kyoto rats (WKY). Stimulation with AVP (10^{-7} M) induced responses homogeneously distributed between the segments and strata. Nifedipine treatment or removal of external calcium (Ca\textsuperscript{2+}) reduced the norepinephrine-induced peak response. Both norepinephrine- and AVP-induced sustained responses were abolished after Ca\textsuperscript{2+} removal in SHR and WKY (P < 0.01). Measurements of V\textsubscript{1a} receptor mRNA on isolated segments showed a threefold increase in ILA from SHR. The present findings indicate that the exaggerated Ca\textsuperscript{2+} and contractile response to AVP in SHR is mainly mediated through ILA vasoconstriction.

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mRNA to explore its correlation with AVP-induced calcium signaling.

MATERIALS AND METHODS

Animals. A total of 18 WKY rats and 19 SHR aged 6–8 wk were obtained from Harlan. Five animals were kept in each cage and fed ordinary rat chow containing 0.5% sodium, 0.6% potassium, 0.71% calcium, and 14.7% crude protein. The rats had free access to tap water. The experiments were performed with the approval of the Norwegian State Board for Biological Experiments with Living Animals.

Isolation of renal vessels. The animals were anesthetized with pentobarbital sodium (50–70 mg/kg). The left kidney was perfused with 5–10 ml warmed RPMI to remove blood from the vasculature, and thereafter 1 ml Seaprep agarose solution (2%) in RPMI (37°C) was infused into the kidney to establish a hydrostatic and elastic core to which the smooth muscle cells could contract, mimicking the effect of pressure in the vessel. The kidney was removed and chilled (4°C for 10 min) in RPMI for solidification of the agarose. About 100-μm-thick cortical slices were cut with a Thomas slicer and incubated for 30–60 min at 37°C in Ca²⁺-free RPMI with 246 U/ml collagenase (CS138, Sigma), 0.5 U/ml protease (P3417, Sigma), and 0.05 mg/ml trypsin inhibitor (T6522, Sigma) to dissociate the vessels. Trypsin inhibitor was used to counteract the cleavage of V1a receptor protein from clostripain contamination in the collagenase IV (26, 31). The vascular fragments were picked with a small pipette (diameter = 100 μm) and transferred to acid-washed (1 N HCl) coverslips in a perfusion chamber. The microvessels usually attached strongly to the cover glass. The arteriolar segments were loaded in 2.5 μM fura 2 acetoxymethyl ester in RPMI at room temperature for 45 min. Thereafter, fura 2 was removed and the cells were incubated for 20 min (30°C) to ensure complete hydrolyzation of the fura 2 ester. The cells were kept at 30°C for up to 2 h before recording.

Perfusion of vessels in chamber. The microscope chamber had a volume of 400 μl and was gravity fed (2 ml/min) through a perfusion inline heater (Warner TC344-B), which maintained the temperature in the chamber at 36–37°C. All agents administered to the vessels were dissolved in reservoirs feeding the microscope perfusion chamber. The switching between these solutions was done automatically in a programmed sequence with a Valvebank® (AutoMate Scientific). All vessels were perfused for 150 s before and after administration of agents with RPMI to obtain stable baselines in the start and end of the recordings. To study the AVP and norepinephrine responses, cells were perfused with these two hormones (10⁻⁷ M) for 150 s. To examine the importance of L-type voltage-operated calcium channels, the vessels were perfused with nifedipine (10⁻⁷ M) for 150 s, followed by AVP (10⁻⁷ M) or norepinephrine (10⁻⁷ M) and nifedipine for 150 s. Responses without external calcium were performed by perfusing the vessels in 2 mM EGTA (0 [Ca²⁺]o) for 150 s, followed by AVP (10⁻⁷ M) or norepinephrine (10⁻⁷ M) and EGTA for 150 s. The plateau value was defined as the maximum Ca²⁺ concentration ([Ca²⁺]i) after 5 s of stimulation. The plateau value was defined as the initial [Ca²⁺]i, recorded 30 s after the stimulation. The response values were calculated as the difference between baseline and peak or plateau Ca²⁺ ratio levels. Measurements were performed in six to eight animals with one or two recordings from each animal.

Measurement of intracellular fura-2 ratio. The fura 2 ratio was measured using an inverted Olympus IX-70 with a ×40 UAP objective. The cells were excited alternatively with lights of 340- and 380-nm wavelengths from a dual-excitation wavelength system (Delta-Ram) from Photon Technologies (PTI). After the signals passed through a barrier filter (510 nm), fluorescence images were recorded by an IC-200 intensified CCD camera and analyzed with ImageMaster 1.49 Software from PTI. To compare Ca²⁺ ratios with other findings, recordings were calibrated to free calcium concentration based on the ratio of 340/380 nm, as described earlier (16, 20). Vessels with a core of agarose as seen in Fig. 1 were used for the recordings. Regions of interest on the vessels were defined with the ImageMaster software to accurately collect the fura 2 fluorescence from the chosen arteriolar segments and at a minimum distance of 20 μm from the branching points between AA and ILA.

Measurement of arteriolar lumen diameter. Simultaneously with fura 2 measurements, diameter variation in response to AVP stimulation was recorded with a Vicon VC285–242 CCD camera connected to a VPH 7090 Thomson video recorder. Digital images (640 × 480 pixels) were later on grabbed from the videotapes with an Asus VB460 video card. Lumen area for a defined length of AA or ILA was measured in AnalySIS software at 5 s before and 5 s after stimulation, and mean baseline and peak diameters were calculated from the formula (mean lumen diameter) = (lumen area)/(lumen length).

Real-time PCR for V1a receptor on isolated AA and ILA. Quantization of V1a mRNA was done by real-time PCR and AA and ILA were collected from five WKY and five SHR. Vessels were isolated as described above and resuspended in Cells to Signal lysis buffer from Ambion. Each sample consisted of six to eight vascular segments and was resuspended in 50 μl buffer. First-strand cDNA was synthesized directly using chemicals from the Cells to Signal kit and primed by Pd (N10) primers. Each cDNA synthesis was performed in a total volume of 60 μl. The reaction mix was then added 0.6 μl glycogen (20 mg/ml), 6 μl potassium acetate (3 M), and 150 μl ice-cold absolute ethanol. The reaction was precipitated at −20°C overnight and centrifuged at 15,000 g for 10 min at 4°C. The precipitated cDNA was resuspended in 16 μl water and used as template for the amplification. Primers for amplification of V1a were selected for a 114-bp fragment containing the splicing site of the two V1a exons. The forward primer was 5’-cgatcgtgagttgctga-3’. The reverse primer was 5’-ctctattatc-cacggttgtc-3’. The Taqman probe was 5’-catcaacggctgtgctggt-3’, marked with FAM and 3’-TAMRA. The amplified V1a cDNA was normalized against amplified 18S ribosomal RNA to compensate for any changes due to RNA degradation, reverse transcription efficiency, or amplification success. The primers were made for a 68-bp fragment. The forward primer was 5’-agctcgtgtgctgagg-3’. The reverse primer was 5’-gatcgagggctactaaac-3’. The Taqman probe was 5’-gccccgtgctactaccggtggtggt-3’, marked with 5’-Yakima Yellow and 3’-TAMRA.
The amounts of V1α and 18S were quantified using a standard curve for known quantities of V1α or 18S RNA. The V1α standard curve was made by amplifying a 1,125-bp region of the V1α cDNA with the primers cctgggtgcctctaaac (forward) and etgcttcgctgctgctg (reverse). For the 18S standard curve, a 396-bp region of the rat 18S RNA cDNA was amplified using primers ctgcatcctgtctgta (forward) and ecagctgtagaggtc (reverse). The amplification products were then cloned into pBAD TOPO TA vectors and transfected into TOP 10 Escherichia coli cells (Invitrogen). Plasmids containing the cloned material were then purified from bacterial cultures using a Qiagen Plasmid Purification Midi kit. The purified plasmids were diluted to concentrations appropriate for the standard curve: 10^10, 10^8, 10^7, and 10^5 molecules/μl for 18S, and 10^4, 10^3, 10^2, and 10^1 molecules/μl for V1α. The primer and probe constructions were done using Primer Express software from Applied Biosystems. The quantification was done on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems) and with a qPCR Core Kit (Eurogentec). The primer concentrations were optimized before use in quantification. Forward primers for both V1α and 18S were used in a final concentration of 0.3 μM. Reverse primers for both V1α, and 18S were used in a final concentration of 0.9 μM. For each sample, 1 μg of total RNA in 15 μl was used for the cDNA synthesis. In each amplification reaction, 1 μl cDNA solution was used as a template. All amplifications of both V1α and 18S RNA were done using two parallel amplification reactions under standard ABI conditions using a 19-μl reaction volume.

Chemicals. All chemicals used in this experiment were from Sigma, except fura 2 acetoxyethyl which came from Molecular Probes. The RPMI media contained (g/l) 7.65 NaCl, 0.40 KCl, 0.20 MgCl₂, 0.20 Na₂HPO₄, 1.34 HEPES, 1 glucose, 0 Na, 0.11 pyruvat, 0.35 CaHCO₃, 0.22 CaCl₂, RPMI vitamins (Sigma R7256), and amino acids (Sigma R7131); 20× SSC (in g/l): 175 NaCl, 88.2 Na₃citrate, adjusted to pH 7.0 with 10 N NaOH; malonic acid buffer: 11.6 malonic acid, 8.77 NaCl, adjusted to pH 7.5 with solid NaOH.

Statistics. Data were presented as means ± SE. Sets of data were tested by ANOVA. P values of ≤0.05 were considered statistically significant. Differences between means were calculated in SPSS 12.0.

RESULTS

Experiments were performed on pregglomerular vascular fragments containing both AA and ILA segments. The VSMC in the wall of the isolated vessels could easily be identified with endothelial cells covering the inside of the vascular lumen. There was no connective tissue on the vasculature, and an agar core protruded from the vessels as shown in Fig. 1.

Measurements of intracellular cytosolic calcium showed no significant difference in baselines between the 24 combinations of strain (WKY or SHR), segment (ILA or AA), agonist (AVP or norepinephrine), or treatment (untreated, nifedipine treated, or 0 [Ca²⁺]₀) (P > 0.3, n = 8–14 in each group). However, when the baseline values for each segment in WKY and SHR were pooled, a significant difference of baseline calcium ratio became evident. As shown in Fig. 2A, the baseline ratio in the AA segments was 0.82 ± 0.02 in WKY and 0.90 ± 0.02 in SHR (P < 0.005, n = 67 and 68, respectively). In the ILA segment, the baseline ratio was 0.81 ± 0.01 in WKY and 0.92 ± 0.02 in ILA (P < 0.001, n = 86 and 88, respectively). There were no significant differences between AA and ILA from the same strain (P > 0.6 for both WKY and SHR).

In both strains, the Ca²⁺ ratio increase to AVP showed a sharp initial peak followed by a stable plateau that normalized when the vessels were exposed to normal media. As indicated with representative tracings in Fig. 3, and averaged in Fig. 4, the ILA segment in SHR had a marked exaggerated response to AVP. The initial peak ratio increase was 0.21 ± 0.04 (n = 9) in AA from WKY and 0.20 ± 0.03 (n = 12) in AA from SHR (P > 0.7). In ILA, the ratio increase was 0.24 ± 0.03 (n = 14) in WKY and 0.49 ± 0.09 (n = 14) in SHR (P < 0.01). There was no significant difference between AA and ILA in WKY (P > 0.6), but the calcium response was more than two times higher in ILA compared with AA in SHR (P < 0.01). The increased response to AVP in ILA from SHR is demonstrated in Fig. 5, where two representative Ca²⁺ ratio recordings were selected to illustrate the difference between the AVP Ca²⁺ response in ILA and AA in WKY and SHR.

In all recordings, the sustained Ca²⁺ ratio increase was lower than the initial ratio increase in both WKY and SHR (P < 0.0001). The number of recordings is given only after the initial peak value in each group and not after the plateau value because they were the same. The sustained ratio increase was 0.10 ± 0.06 in AA from WKY and 0.11 ± 0.04 in AA from SHR (P > 0.6). In ILA, the sustained ratio increase was 0.13 ± 0.03 in WKY and 0.18 ± 0.03 in SHR (P > 0.06). As shown with representative tracings in Fig. 3, and averaged in Fig. 4, vessels pretreated with nifedipine before AVP stimulation showed a peak response almost unchanged from the untreated vessels (P > 0.5). The ILA fragments from SHR showed an exaggerated calcium ratio increase to AVP also after nifedipine pretreatment. The initial peak ratio increase

![Graph A](image1.png)

![Graph B](image2.png)

Fig. 2. A: resting calcium ratio values in AA and ILA from Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). **P < 0.005 vs. corresponding vessels in WKY. B: resting diameters in AA and ILA from WKY and SHR. *P < 0.01 vs. corresponding vessel in WKY.
was 0.22 ± 0.05 (n = 10) in AA from WKY and 0.17 ± 0.03 (n = 8) in AA from SHR (P > 0.5). In ILA, the initial ratio increase was 0.22 ± 0.05 (n = 10) in WKY and 0.60 ± 0.14 (n = 13) in SHR (P < 0.05). No difference between AA and ILA was seen in WKY (P > 0.5), but in SHR a more than a threefold difference was found (P < 0.05).

The sustained ratio increase in AA was 0.03 ± 0.01 in WKY and 0.04 ± 0.02 in SHR (P > 0.8). In ILA, the sustained ratio increase was 0.08 ± 0.03 in WKY and 0.12 ± 0.02 in SHR (P < 0.01). The sustained response in nifedipine-treated vessels was significantly smaller than in untreated vessels (P < 0.05), except in AA in WKY (P > 0.2).

As shown in representative tracings in Fig. 3, and averaged in Fig. 4, vessels stimulated with AVP in calcium-free media (2 mM EGTA) had a reduced peak response in WKY and SHR compared with vessels stimulated in normal media. The differences in ratio increase observed in normal media between ILA from WKY and SHR were absent in calcium-free media. The peak ratio increase was 0.14 ± 0.05 (n = 8) in AA from WKY and 0.15 ± 0.03 (n = 14) in AA from SHR (P > 0.8). In ILA, the peak response was 0.20 ± 0.03 (n = 8) in WKY and 0.24 ± 0.05 (n = 14) in SHR (P > 0.5). The peak values in both segments and strains were similar (P > 0.1). The ILA segment in SHR was reduced compared with both untreated and nifedipine-treated ILA peak responses in SHR (P < 0.05). The sustained Ca\(^{2+}\) response in EGTA-treated vessels was not significantly different from zero in either WKY or SHR (P > 0.3).

To compare calcium signaling obtained with AVP in AA and ILA from both strains, vessels were stimulated with...
norepinephrine (10^{-7} M) under the same conditions as with AVP. As shown with representative tracings in Fig. 6, and averaged in Fig. 7, the basic norepinephrine ratio response was 0.66 ± 0.03 (n = 13) in AA from WKY and 0.58 ± 0.11 (n = 8) in AA from SHR (P > 0.1). The response was 0.80 ± 0.08 (n = 14) in ILA from WKY and 0.71 ± 0.07 (n = 8) in ILA from SHR (P > 0.1).

The sustained basic norepinephrine response was 0.50 ± 0.04 in AA from WKY and 0.42 ± 0.13 in AA from SHR (P > 0.2). The response was 0.60 ± 0.07 in ILA from WKY and 0.41 ± 0.12 in ILA from SHR (P > 0.2).

In vessels treated with nifedipine (10^{-7} M), the initial calcium response to norepinephrine was 0.29 ± 0.02 (n = 8) in AA from WKY and 0.43 ± 0.07 (n = 11) from SHR (P > 0.13). The response was 0.28 ± 0.06 (n = 8) in ILA from WKY and 0.38 ± 0.05 (n = 14) in ILA from SHR (P > 0.32). There were no differences between AA and ILA in each strain (P > 0.1). However, in all segments, the peak values were reduced compared with the untreated responses (P < 0.05).

The sustained ratio increases in nifedipine-treated vessels were 0.07 ± 0.02 in AA from WKY and 0.12 ± 0.05 in AA.
from SHR ($P > 0.37$). The response was $0.04 \pm 0.02$ in ILA from WKY and $0.14 \pm 0.03$ in ILA from SHR ($P = 0.07$). There were no differences between AA and ILA within each strain ($P = 0.08$). However, as with the initial responses, all sustained responses were reduced compared with untreated plateau values ($P < 0.05$).

In vessels treated with EGTA (2 mM), the calcium ratio response to norepinephrine was $0.28 \pm 0.04 (n = 8)$ in AA from WKY and $0.33 \pm 0.11 (n = 8)$ in AA from SHR ($P > 0.7$). The ratio increase was $0.28 \pm 0.04$ in ILA from WKY and $0.39 \pm 0.12$ in ILA from SHR ($P > 0.3$). There were no differences between AA and ILA in each strain ($P > 0.3$). The peak values were unchanged compared with nifedipine-treated values ($P > 0.7$) but reduced compared with untreated peak responses ($P < 0.05$).

The sustained ratio increase in zero calcium was not different from zero and reduced compared with untreated and nifedipine-treated vessels ($P < 0.05$ except in AA from SHR ($P = 0.08$).

Simultaneous with fura 2 recordings, the diameters of the vessels were measured 5 s before and 5 s after the stimulation with AVP to study the relationship between calcium response and diameter change. As seen in Fig. 2B, the mean afferent arteriolar resting diameter in WKY was $21.6 \pm 0.5 \mu m$ in AA and $37.8 \pm 2.1 \mu m$ in ILA. In SHR, the afferent arteriolar resting diameter was $14.4 \pm 1.2 \mu m$ in AA and $30.4 \pm 2.4 \mu m$ in ILA. The resting diameters in SHR and WKY were significantly different in the AA segment ($P < 0.01$) but not in ILA ($P > 0.2$).

In WKY, the diameter was reduced to $78 \pm 5$% of the resting diameter in AA and to $82 \pm 5$% of the resting diameter in ILA during AVP stimulation ($P > 0.5$; Fig. 8). In SHR, the diameter was reduced to $87 \pm 4$% of the resting diameter in AA and to $61 \pm 3$% of the resting diameter in ILA ($P < 0.001$). In AA, the change in diameter was not different between the strains ($P > 0.2$), but in ILA there was a significant difference in diameter change during AVP stimulation between the two strains ($P < 0.001$).

To further explore any segmental heterogeneity, V1α mRNA levels in AA and ILA from WKY and SHR were analyzed using real-time RT-PCR on isolated vessels. As shown in Fig. 9, the V1α mRNA level from SHR was significantly higher in the ILA segment ($342 \pm 9 \text{ V1}_\alpha \text{mRNA/10}^5 \text{ 18S RNA}$) compared with the AA segment ($84 \pm 5 \text{ V1}_\alpha \text{mRNA/10}^5 \text{ 18S RNA}$; $P < 0.001$). The corresponding values in ILA and AA from WKY were $76 \pm 11 \text{ V1}_\alpha \text{mRNA/10}^5 \text{ 18S RNA}$ and $68 \pm 9 \text{ V1}_\alpha \text{mRNA/10}^5 \text{ 18S RNA}$, respectively ($P > 0.5$). There was no difference in mRNA V1α level between AA from WKY and SHR.

**DISCUSSION**

The main new finding in the present study is the large difference in the Ca$_{\text{II}}^{2+}$ response between AA and ILA segments after stimulation with AVP in genetic hypertensive rats. The AVP-induced Ca$_{\text{II}}^{2+}$ response in the ILA segment from SHR was more than twice as large compared with AA in the same strain and AA and ILA from normotensive animals. This new observation was supported by a significantly greater contraction of ILA in SHR after exposure to AVP. The calcium response in ILA from SHR remained different from the other segments also after nifedipine treatment, but this difference disappeared after calcium removal, indicating that this ratio increase is mainly mobilization dependent. A further support of our findings is the three times higher level of V1α mRNA in ILA from SHR in vessels isolated with the agar perfusion/ enzyme digestion technique. To test the uniqueness of this exaggerated segment-specific response pattern in SHR, the AVP recordings were compared with similar measurements after norepinephrine stimulation. The norepinephrine-induced

![Fig. 7](http://ajprenal.physiology.org/)

**Fig. 7.** Averaged responses to AVP ($n = 8–14$). *$P < 0.05$ vs. corresponding untreated vessels. †$P < 0.05$ vs. corresponding nifedipine-treated and untreated vessels.

![Fig. 8](http://ajprenal.physiology.org/)

**Fig. 8.** Contractile responses to AVP ($10^{-7}$ M) in WKY and SHR. *$P < 0.01$ compared with AA and corresponding segment in WKY.
responses were similar in both WKY and SHR and had the same magnitude in AA and ILA from both strains. Collectively, our data indicate that the AVP response in ILA of SHR is considerably different from norepinephrine.

Previous studies demonstrated that AVP injection into the renal artery produces exaggerated renal vasoconstriction in young SHR compared with normotensive WKY (13). The $Ca^{2+}$ response to AVP has also been shown to be enhanced in SHR compared with the normotensive control (21). This is most likely due to the increased density of V1a receptors in preglomerular vessels, which has been shown by Vagnes et al. (38) using ligand-binding technique and measurements of mRNA.

Hormonal heterogeneity in different vascular segments of the kidney has been found in the hydronephrotic kidney preparation (35) and in the juxtamedullary perfused model (17). In the hydronephrotic kidney preparation, the effect of AVP seems to be increased in ILA compared with AA. In the juxtamedullary perfused model, the effect of AVP was significantly lower in ILA compared with the distal part of AA using low concentrations ($10^{-8}-10^{-9}$ M) of AVP, but at higher concentrations ($10^{-7}-10^{-6}$ M) the contractile responses were similar. These contradictory findings may be due to use of different experimental models. Calcium signaling with AVP and measurements of V1a mRNA have until now only been performed on samples produced with the iron oxide/sieving technique of isolated preglomerular vessels, we have been able to explore differences between preglomerular segments to a greater extent than before.

In a recent paper, we observed greater variations in the $Ca^{2+}$ response in isolated preglomerular fragments from young SHR compared with age-matched WKY, and we decided to explore this observation further (39). In the present study, we found a nearly twofold increased calcium response in ILA compared with AA in SHR. In the normotensive controls, no differences between calcium signaling in AA and ILA were found, and the response in these two segments was not different from what was seen in AA from SHR. Similar to other findings (34), stimulation with norepinephrine did not show any difference in peak response between the two segments in WKY and SHR, indicating that the AVP-induced signaling in ILA from SHR is different from norepinephrine. We propose that the previously observed exaggerated calcium response to AVP in preglomerular segments from SHR results mostly from increased reactivity in the ILA segment.

Earlier studies showed that calcium entry via voltage-gated operated channels is the predominant mechanism for cytosolic calcium increase in preglomerular vessels (5, 8), but several later studies also demonstrated that calcium mobilization from intracellular stores is taking place in preglomerular vessels (10, 12, 24, 32, 33). Flow studies after injection of AVP showed that the contribution of IP3-mediated mobilization of internal $Ca^{2+}$ stores constituted two-thirds of the $Ca^{2+}$ response in both SHR and WKY (12). Further support for the role of mobilization of internal $Ca^{2+}$ stores in preglomerular vessels is given in the present study, as nifedipine treatment did not affect the AVP-induced peak response. The sustained response was reduced in both WKY and SHR, as predicted due to the blocking effect nifedipine has on L-type $Ca^{2+}$ channels. However, pretreatment with nifedipine on norepinephrine-stimulated vessels gave a significant reduction in both peak and plateau values, similar to what was found by Salomonsson and Arendshorst (34) in microdissected AA from WKY and SHR. This observation is supported by findings of Bauer and Parekh (1), who found that nifedipine influenced the vasoconstrictive effect of AVP and norepinephrine differently. These authors found that to reduce renal blood flow by 25%, coinfusion with nifedipine required the norepinephrine dosage to be increased fourfold, whereas the AVP dosage only needed to be increased twofold. In our study, the AVP-induced peak responses were only reduced in ILA from SHR during removal of external calcium, whereas the peak response after norepinephrine stimulation was also reduced after nifedipine treatment. These findings indicate that the norepinephrine-induced $Ca^{2+}$ response is more dependent on entry mechanisms in the initial phase than AVP.

In our experiments, the sustained responses after norepinephrine and AVP stimulation were reduced after nifedipine treatment and abolished after removal of external calcium in both strains. The incomplete ability of nifedipine to abolish the plateau response supports the presence of a second calcium entry pathway in addition to L-type voltage-regulated $Ca^{2+}$ channels, as earlier suggested by Salomonsson and Arendshorst (33, 34). Also, the fact that removal of external calcium completely abolishes the sustained response suggests a major dependence on entry mechanisms both after norepinephrine and AVP stimulation.
In the present study, we measured the basic diameters and the contractile effect of AVP in AA and ILA. The resting diameters obtained by the methods used in the present study were similar to what we and others reported earlier in young SHR and WKY using the microsphere method (19, 22). The diameter of the ILA cannot be measured by the use of microspheres, but in casts the diameter has been measured to be within the limits of 25 to 60 μm (15, 30). The contractile response to AVP was significantly greater in ILA from SHR compared with the AA and the corresponding segment in WKY. Similar to our results, stimulation with AVP in the hydronephrotic kidney model (7), and the perfused juxtaglomerular nephron preparation (17), has indicated considerable contractile responses in the ILA segment. In accordance with our data from WKY, Harrison-Bernard and Carmines (17) found a diameter change in ILA from Sprague-Dawley rats that was similar to the distal and central part of AA, when using the same concentration of AVP that was used in the present study (10^{-7} M). Similar to our data, Touyz et al. (36) found an increased contractile response to AVP in third-order branches of arteries from the mesenteric bed in 17-wk-old SHR compared with WKY and Wistar rats.

In contrast to baseline calcium values obtained from the renal vasculature using the iron oxide/sieving technique (11, 21), and also when other isolation techniques have been used (28, 34), the baseline $Ca^{2+}$ level in this study was higher in the hypertensive strain. However, similar to our results, Brown et al. (4) found that cardiac myocytes had increased $Ca^{2+}$ resting levels in SHR compared with WKY. Touyz and Schiffrin (37) found that the basal $[Ca^{2+}]_i$ in mesenteric arteries from 17-wk-old SHR was $134 \pm 8$ nM, significantly higher than the baselines found in WKY ($98 \pm 12$ nM) and in Wistar rats ($99 \pm 10$ nM). These changes were already seen in 5-wk-old rats and are similar to our data when our results are converted to $Ca^{2+}$ concentration from ratio values. Touyz and Schiffrin used pressurized arterioles, which are mimicked by the agarose core in our vessels. Pressurizing and the use of intact vasculature are most likely important factors in physiological conditions, and this might explain why the results in the present paper differ from recordings done of single cells (11, 21) or unpressurized arterioles (28, 34). Preglomerular arterioles have been shown to be constricted at a young age in SHR (19, 22), and based on the data presented in the present study, it is not unlikely that increased vascular tone in the SHR renal vasculature is linked to a higher steady-state level of $[Ca^{2+}]_i$.

The ligand-binding method used earlier in our laboratory (38) is not suitable to test isolated segments of the vascular bed because of the large amounts of protein needed. Using the agar perfusion/enzyme digestion method, pregglomerular segments were isolated and the $V_{1a}$ mRNA levels were measured with real-time RT-PCR. The results showed a threelfold higher level of $V_{1a}$ in ILA from SHR, and this finding is consistent with the increased calcium signaling and vasoconstriction induced by AVP in this segment.

Some methodological reservations should be made in the interpretation of data from the present study. The preparation consists of two different cell types, VSMC and endothelial cells. Consequently, there are two possible sources of calcium transients, and the measured $[Ca^{2+}]_i$ are averaged values derived from the two cell layers. As a consequence, we presented our $Ca^{2+}$ recordings as ratio values and used converted $[Ca^{2+}]_i$ values only for comparison with other studies. It is well established that the endothelium is an important modulator for vascular responses. Therefore, we argue that arterioles with the endothelial cell layer intact most likely exhibit physiological responses representative of vessels in vivo, especially because differences in endothelium-derived relaxing factors are reported to differ between the two strains used in this study (25, 40).

In conclusion, $Ca^{2+}$ signaling, contractile responses, and $V_{1a}$ mRNA levels demonstrate that the exaggerated responsiveness to AVP is localized to the ILA segment in SHR. This condition could reduce blood flow to the glomeruli and thereby lower the glomerular filtration rate and induce salt and water retention, which is regarded as one of the possible mechanisms of hypertension in SHR.

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GRANTS

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