Enhanced Ca\(^{2+}\) response to AVP in preglomerular vessels from rats with genetic hypertension during different hydration states

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Submitted 27 September 2004; accepted in final form 12 January 2005

ARGinine VASOPressin (AVP) exerts important biological effects on target cells in the central nervous system and in peripheral organs, ranging from regulation of water permeability and balance, blood pressure and vascular tone, baroreceptor modulation, and adrenocorticotropic release (1, 23, 38, 46). Intracellular signaling of AVP is initiated by one of the following three G-protein-coupled receptors: V\(_{1a}\), V\(_{1b}\) (or V\(_{3}\)), and V\(_2\). V\(_{1a}\) receptors are localized to vascular smooth muscle cells (VSMC) of conduit and resistance arteries and arterioles, glomerular mesangial cells, liver cells, and brain (31–33). Vascular V\(_{1a}\) receptors are responsible for vasoconstriction

Vagnes, Oyvind B., Frank H. Hansen, Jian J. Feng, Bjarne M. Iversen, and William J. Arendshorst. Enhanced Ca\(^{2+}\) response to AVP in preglomerular vessels from rats with genetic hypertension during different hydration states. Am J Physiol Renal Physiol 288: F1249–F1256, 2005. First published January 18, 2005; doi:10.1152/ajprenal.00363.2004.—Exaggerated arginine vasopressin (AVP)-induced calcium signaling and renal vasoconstriction, characteristic in young spontaneously hypertensive rats (SHR) during euvolemia, are related to greater amounts of V\(_{1a}\) receptor mRNA and V\(_{1a}\) protein in preglomerular resistance arterioles. The present study determined whether V\(_{1a}\) receptor density and calcium signal transduction in the renal vasculature of young SHR is regulated appropriately during physiological changes in hydration state. \(^{[3]}\)H\(_{\text{AVP}}\) ligand binding documented two- to threefold greater density of V\(_{1a}\) receptors in euvolemic SHR vs. Wistar-Kyoto (WKY) rats. Parallel changes in V\(_{1a}\) receptor density were observed in both strains during chronic water loading (plus \(\sim 50\) fmol/mg) and during dehydration (minus \(\sim 50\) fmol/mg). Affinity was unchanged. Real-time RT-PCR demonstrated that V\(_{1a}\) mRNA in preglomerular arterioles was three times greater in euvolemic SHR. Dehydration decreased expression \(\sim 50\%\) in renal vessels independent of rat strain; water loading increased V\(_{1a}\) mRNA. Thus V\(_{1a}\) receptor regulation correlated with changes in mRNA in a normal manner in response to chronic changes in AVP concentration, albeit set at a higher level in SHR. In dehydrated animals, AVP increased the cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_{\text{cyt}}\)]\(_b\)) by 60 \(\pm\) 5 and 112 \(\pm\) 3 mM cytosolic Ca\(^{2+}\) in WKY and SHR, respectively \((P < 0.01)\), whereas in hydrated animals the [Ca\(^{2+}\)\(_{\text{cyt}}\)]\(_b\) increase was 168 \(\pm\) 10 and 220 \(\pm\) 18 mM, respectively \((P < 0.05)\). In all hydration states, calcium signaling was greater in SHR compared with WKY \((P < 0.05)\). Calcium signaling paralleled changes in the receptor density and mRNA. Mechanisms other than hydration state per se are likely to be responsible for the two- to threefold difference in the V\(_{1a}\) receptor density between WKY and SHR in the renal vasculature at the critical age of 6 wk.

renal circulation; afferent arteriole; receptor regulation; spontaneously hypertensive rat; calcium ion; arginine vasopressin; spontaneously hypertensive rat

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to those observed in normotensive animals. We also tested the hypothesis that exaggerated preglomerular cytosolic Ca\textsuperscript{2+} (Ca\textsuperscript{2+}) responses noted for the renal vasculature of euolemic SHR persist during different hydration states. We propose that the levels of receptor protein and mRNA, albeit initially set higher in hypertensive-prone animals during euolemic, are directly related to the hydration status and inversely coupled to the predicted level of circulating AVP. Changes in renal vascular V\textsubscript{1a} mRNA were compared with those in the liver to ascertain if changes in the former are unique or represent a more generalized phenomenon. The results indicate that V\textsubscript{1a} receptor mRNA and protein abundance in both strains are downregulated during dehydration and administration of AVP and upregulated during water loading and infusion of V\textsubscript{1a} receptor antagonist, but always at a higher level in SHR compared with WKY. Changes in receptor expression translate to functional changes in calcium responsiveness in preglomerular arterioles.

**METHODS**

All experiments were performed with the approval of the Norwegian State Board for Biological Experiments with Living Animals and the University of North Carolina-Chapel Hill Institutional Animal Care and Use Committees.

**Experimental groups.** Male SHR (n = 78) and WKY rats (n = 78) averaging 8 wk of age were obtained from our Chapel Hill breeding colony. In three groups of six animals, the effects of AVP on V\textsubscript{1a} receptor density and affinity in the renal vasculature were assessed by radioligand binding under equilibrium conditions. The control group was maintained on a standard rat chow diet (Bemis, Madison, WI) and tap water ad libitum. Other animals were given a water load to suppress plasma AVP; 6% dextrose in water was given ad libitum for 3 days before an experiment. Dehydration was achieved by restriction of fluid intake to 5 ml/day (compared with a normal intake of ~30ml) for 3 days before tissue isolation. Similar binding studies were conducted in Bergen on 8-wk-old male SHR and WKY using identical protocols except for the standard rat chow diet (B&K Universal).

**Hemodynamic measurements.** For comparison, hemodynamic measurements were performed in WKY rats and SHR from Chapel Hill and Bergen laboratories. The arterial pressure was measured with a Hewlett-Packard pressure transducer connected to a Gould TA recorder, and a polyethylene catheter in the left femoral artery after anesthesia was induced by intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt). The kidneys were exposed through a renal artery by a 2-mm-diameter flow probe connected to a transit-time flowmeter (Transonic) and a Gould recorder. The probe was calibrated in vitro. The same system was used in both laboratories.

**Additional studies for [\textsuperscript{3}H]AVP binding assays.** Experiments were done in both laboratories to verify strain differences in V\textsubscript{1a} receptor density between animals obtained for different sources. The incubation medium (final volume 250 ml) contained 50 mM Tris-HCl (pH 7.4), 5 mM MgCl\textsubscript{2}, 0.3% BSA albumin, and [\textsuperscript{3}H]AVP. All determinations were performed in duplicate with incubation at room temperature for 90 min. The reaction was started by the addition of the purified isolated microvessels (50 \mu g protein) and stopped by separating bound ligand from free ligand with centrifugation in a sucrose gradient. Portions (0.2 ml) of the samples were gently layered over 0.2 ml of assay-binding buffer containing 20% sucrose in 0.5 ml polyethylene microcentrifuge tubes. The tubes were centrifuged at 5,000 g for 30 min at 0–4°C in a free-angle rotor. The tubes were then frozen rapidly by insertion in dry ice, and the tips, containing the bound ligand, and the tops, containing the free unbound ligand, were separating by cutting the tube and assayed in a Packard Tri-Carb 4550 scintillation counter.

The protein concentration, binding conditions, and time to attain equilibrium binding of [\textsuperscript{3}H]AVP (Amersham-Pharmacia) to isolated segments of rat afferent arterioles were determined in preliminary experiments. Specific binding of [\textsuperscript{3}H]AVP (5 nM) to the microvessels was linear, with increasing amounts of protein added up to at least 75 \mu g protein. Nonlinear binding was apparent in excess of 100 \mu g protein. Incubations were performed at room temperature in the presence of aprotinin (700 U/ml) to minimize potential degradation by peptidases. Specific binding of [\textsuperscript{3}H]AVP (5 nM) to 50 \mu g protein took 30–45 min to reach equilibrium at 25°C. Nonspecific binding in our radioligand binding studies, in the presence of 2.5 \mu M unlabeled AVP, increased linearly, with 15 ± 3% of the total binding at the lowest concentration of (0.125 nM) [\textsuperscript{3}H]AVP and 34 ± 5% at the highest concentration (4 nM) of [\textsuperscript{3}H]AVP tested.

**Saturation binding experiments** were performed with increasing concentrations of [\textsuperscript{3}H]AVP between 0.125 and 4.0 nM (58). In all cases, specific binding was calculated as total binding minus nonspecific binding measured in the presence of 2.5 \mu M unlabeled AVP. The maximum specific binding and dissociation constant (Kd) were calculated by using the Ligand software program ( Biosoft). Each group of experiments consisted of at least three determinations, each utilizing fresh tissue preparations.

**Isolation of preglomerular resistance vessels for ligand binding and mRNA measurements.** Preglomerular resistance arterioles were isolated using a previously described iron oxide-sieving method (7, 20, 46). Briefly, the aorta above the right kidney was ligated, the left renal vein was cut, and kidneys were perfused with ice-cold heparinized isotonic saline solution until the effluent was blood free. Magnetized iron oxide suspension (1% Fe\textsubscript{2}O\textsubscript{3} in isotonic saline) was then perfused at constant pressure (120 and 140 mmHg for WKY and SHR, respectively, at roughly 10 ml for 30 s). The kidneys were excised and placed in PBS (in mM: 125 NaCl, 20 K\textsubscript{2}HPO\textsubscript{4}/Na\textsubscript{2}HPO\textsubscript{4}, and 1 MgCl\textsubscript{2}, 4°C, pH 7.3). All subsequent steps of vessel isolation were performed at 4°C. Cortical tissue was gently minced with a razor blade and transferred to PBS for homogenization with a Polytron homogenizer at a moderate speed. Renal vessels, glomeruli, and the surrounding connective tissue were removed from the crude homogenate with the aid of a magnet. The iron oxide-loaded tissue was resuspended in PBS and injected through a curved 20-gauge needle and then a smaller-sized needle (22 gauge) until the supernatant was free of nonvascular tissue. Such repetitive injections mechanically detached most of the connective tissue, removed the iron oxide from the large vessels, and separated afferent arterioles from glomeruli. The vascular suspension was filtered through a 120- \mu m mesh sieve and washed with ice-cold PBS. The microvessels were recovered from the top of the sieve and digested with collagenase (55 U/ml) for 30 min at 37°C. Thereafter, the vascular suspension was removed with the aid of a magnetic field, resuspended in PBS, passed through a 27-gauge needle, and sonicated for 30 s. Free iron oxide particles were removed by applying a magnetic field. This procedure was repeated until no more iron oxide particles were attached on the magnet. The remaining suspension was centrifuged (10,000 g for 30 min), and the pellet was recovered. The protein concentration was determined by a colorimetric method, as previously described (7, 50).

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RT-PCR, VSMC isolation, and RNA purification. These experiments were done in Bergen. Isolation of renal resistance vessels was performed using the iron oxide/sieving method similar to that previously described for radioligand binding studies. Exceptions were that the cortical tissue was homogenized in a 1.5-ml centrifuge tube using a Teflon pellet pestle mixer (Kontes) and that iron oxide-containing vessels segments were separated from the homogenate when the magnet remained outside the tube wall (to minimize contamination). Tissue recovered from the top of the 120-μm sieves was enriched with a magnet and transferred to RNA later (Ambion). The isolated vessel segments were then homogenized in TRIZol (GIBCO), and total RNA was purified according to the GIBCO manual. Liver tissue was excised and incubated in RNA later, and total RNA was purified using an RNAeasy Midi Kit (Qiagen).

RT-PCR quantitation of V1a mRNA was done by real-time PCR. First-strand cDNA was synthesized from isolated total DNA using a First-Strand cDNA Synthesis Kit (Amersham Biosciences) and primed by pd(N)6 primers. Primers for real-time PCR amplification of V1a were selected for a 114-bp fragment containing the splicing site of the two V1a exons. The forward primer was 5′-ATGGCTGCTGCTCTGGGATGA-3′. The reverse primer was 5′-CATGTATATCC-A CGGTTGTCG-3′. The Taqman probe was 5′-CAATACCGGCC- TTGCTGGCTG-3′ marked with FAM and 3′-TAMRA. The amplified V1a cDNA was normalized against amplified 18S ribosomal RNA to compensate for any results resulting from RNA degradation, RT efficiency, or amplification success. The primers were made for amplifying a 68-bp fragment. The forward primer was 5′-AGT-CCTGGCTCCTTTGTACACA-3′. The reverse primer was 5′- GATCCCGAGGGCTCCTACTAACC-3′. The Taqman probe was 5′-CGCCCGCTGCTACTACCGATTGG-3′ marked with 5′-Yakima yellow and 3′-TAMRA.

The amounts of V1a and ribosomal 18S were quantified using a standard curve for known quantities of DNA. The V1a standard curve was made by amplifying an 1,125-bp region of V1a cDNA with the primers CCGTGGTGGCCTCTAACCAC (forward) and CTGTCTT-CTGGGATGA-3′. The reverse primer was primed by pd(N)6 primers. Primers for real-time PCR amplification of the rat 18S RNA cDNA was amplified using primers TICA GGCACCGAGATTGAGC (forward) and CGCAGGTTCAC-GAGATTGAGC (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 the following concentrations appropriate for the standard curve: 10^6, 10^5, 10^4, 10^3, and 10^2 molecules/μl for 18S, and 10^6, 10^5, 10^4, and 10^3 molecules/μl for V1a. 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 a qPCR Core Kit (Eurogentec). The primer concentrations were optimized before use in quantification. Forward primers for both V1a and 18S were used in a final concentration of 0.3 μM. Reverse primers for both V1a and 18S were used in a final concentration of 0.9 μM. For each sample, 1 μg total RNA in 15 μl was used for cDNA synthesis. In each amplification reaction, 1 μl cDNA solution was used as template. All amplifications of both V1a and 18S RNA were done using three parallel amplification reactions. The amplifications were performed under standard ABI conditions using 19 μl reaction volumes. The amplifications of both V1a and 18S RNA were done using three parallel amplification reactions. The amplifications were performed under standard ABI conditions using 19 μl reaction volumes.

Isolation of renal vessels for Ca^2+ measurements. These experiments on animals anesthetized with pentobarbital sodium (50–70 mg/kg) were performed in Bergen. The left kidney was perfused with 5–10 ml warmed RPMI without calcium to remove blood from the vasculature, followed by 1 ml agaroase solution (2%) Seaprep agarose in RPMI without calcium (37°C). The kidney was chilled in RPMI without calcium (4°C for 10 min) to solidify the agarose. Thick cortical slices (100 μm) were cut with a Thomas slicer and incubated for 30–60 min at 37°C in RPMI with collagenase IV, dispase II, and trypsin inhibitor to dissociate the vessels. Trypsin inhibitor was used to counteract the cleavage of V1a receptor protein from clostripain contamination in the collagenase IV (28, 34). The vascular fragments were aspirated with a small pipette (diameter ~ 100 μm) and transferred to acid-washed cover slips in a perfusion chamber. The arterial segments were loaded in 2.5 μmol/l fura 2-AM in RPMI at room temperature for 45 min. Thereafter, the 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 the chamber. The perfusion chamber had a volume of 400 μl and was gravity fed (2 ml/min) with RPMI containing 2 mM Ca^2+ through a perfusion inline heater (Warner TC344-B) that maintained chamber temperature at 36–37°C. The vessels were first perfused with RPMI for 150 s to obtain a stable baseline and then perfused with AVP (10^-7 M) for 150 s followed by a recovery period of RPMI perfusion for 150 s. The switching between RPMI and normal solutions was done automatically in a programmed sequence with a Valvebank8 (AutoMate Scientific). The peak value was defined as the maximum cytosolic Ca^2+ concentration ([Ca^2+]i) after 5 s of stimulation. The peak response was calculated as the difference between baseline and peak values. Measurements were performed on one to two vascular segments from each of six to eight animals.

Measurement of [Ca^2+]i. [Ca^2+]i was measured from the 340- to 380 nm ratio using an inverted Olympus IX-70 with an X40 UAPO objective, as described earlier (18, 21). The cells were excited alternatively with light of 340 and 380 nm wavelengths from a dual-excitation wavelength system [Delta-Ram; Photon Technologies (PTI)]. After the signals passed through a barrier filter (510 nm), fluorescence images were recorded by an IC-200 intensified charge-coupled device camera and analyzed with ImageMaster 1.49 software (PTI).

Chemicals. Chemicals used in the present study were as follows: AVP (Sigma Chemical); the V1a receptor agonist [Phe²,Ile³,Org8]vasopressin and the V1a receptor antagonist [d(Ch²)₅,Tyr(Me)²,Tyr(NH₂)⁹]Arg₈-vasopressin (Peninsula Laboratories); and [³H]AVP (sp act 64.2 Ci/mM; New England Nuclear-Du Pont). The RPMI media contained (in g/l) 7.65 New England Nuclear-Du Pont). The RPMI media contained (in g/l) 7.65 NaCl, 0.40 KCl, 0.203 MgCl₂, 0.20 NaH₂PO₄, 1.34 HEPES, 1.0 glucose, 0.11 sodium pyruvate, 0.35 CaHCO₃, 0.22 CaCl₂, RPMI vitamins (Sigma R7256), and amino acids (Sigma R7131).

Results. The data are presented as means ± SE. Sets of data were tested by ANOVA, with the post hoc Student-Newman-Keul’s test to determine differences between rat strain and hydration state. A P value <0.05 was considered statistically significant.

Results. Baseline renal hemodynamic variables in young rats are summarized in Table 1. Group averages for arterial pressure were similar in Bergen and Chapel Hill laboratories, being moderately higher in 8-wk-old SHR, as was the case for renal vascular resistance. Chapel Hill SHR had a significantly lower RBF than WKY. Overall body weight was higher in animals from Chapel Hill.

Regulation of AVP receptors in preglomerular resistance arterioles utilizing fluid restriction and water loading to vary endogenous AVP concentrations were assessed in both Chapel Hill and Bergen laboratories. Using similar methodology, both laboratories observed consistent relative changes, although the absolute values were higher in Chapel Hill rats (Fig. 1). Young euolemic SHR had a 2.0–2.5 times higher V1a receptor density than WKY, a finding verified in both laboratories. Also, the percent changes with hydration status were similar. In response to dehydration, V1a receptor density declined 18–46 fmol/mg protein in WKY compared with 42–58 fmol/mg protein in SHR (Fig. 2). During water loading, V1a receptor density
density increased in WKY by 44–59 fmol/mg protein and in SHR by 40–70 fmol/mg protein. Thus the absolute changes were similar in SHR and WKY. $K_d$ was unchanged during dehydration and water loading (0.51 ± 0.03 vs. 0.53 ± 0.10 nM in Chapel Hill, 0.65 ± 0.11 vs. 0.70 ± 0.12 nM in Bergen).

Regulation of renal V$_{1a}$ receptors was further characterized during subcutaneous infusion of AVP or a V$_{1a}$ receptor antagonist for 6 days in conscious rats. As shown in Fig. 3, AVP infusion downregulated V$_{1a}$ receptors from preglomerular arteries of both SHR and WKY; the changes paralleled each other. On the other hand, infusion of the V$_{1a}$ receptor antagonist upregulated the receptor numbers. Overall, the infusion of AVP and V$_{1a}$ receptor antagonist mimicked the effects of dehydration and water loading on receptor expression, respectively. The magnitude of the response to AVP infusion vs. dehydration was similar (Figs. 2 and 3), as was the case for changes elicited by V$_{1a}$ receptor antagonist vs. water loading (Figs. 2 and 3).

To determine whether the changes in receptor density were mediated by regulations at protein level or mRNA level, V$_{1a}$ receptor gene expression in the renal vasculature was determined during dehydration, euvoolemia, and water loading. Real-time PCR revealed that V$_{1a}$ receptor mRNA (with ribosomal 18S RNA as a reference) in microvessels was two to three times higher in young euvolemic SHR than in WKY (69,569 vs. 25,277 molecules/18S, $P < 0.01$; Fig. 4). Moreover, V$_{1a}$ receptor mRNA in both strains declined with dehydration and increased during water loading (Fig. 4). Thus regulation of V$_{1a}$ receptor was responsive and appropriate for the change in hydration state such that the abnormally high set point in young SHR persisted during all tested conditions.

Other experiments evaluated whether V$_{1a}$ receptor message in a nonrenal tissue exhibited similar regulation. As was the case for the renal vasculature, mRNA values for SHR were increased during water loading ($P < 0.01$) and reduced during dehydration ($P < 0.01$). There were similar tendencies in WKY (data not shown). Thus, in response to different hydration states, changes in gene expression for liver V$_{1a}$ receptor showed a pattern similar to that of the preglomerular vasculature.

To study the functional consequences of changed receptor densities, calcium signaling in isolated preglomerular vessels was evaluated to gain insight into the functional consequences of change in V$_{1a}$ receptor densities during dehydration, euvoolemia, and hydration. As seen in Fig. 4, the responses to AVP stimulation were significantly greater in SHR at all states of fluid balance. In dehydrated animals, AVP increased the peak $[\text{Ca}^{2+}]_i$ by 60 ± 5 nM in WKY and 112 ± 13 nM in SHR ($P < 0.01$). In euvolemic animals, the increase in $[\text{Ca}^{2+}]_i$ was 115 ± 10 nM in WKY and 157 ± 12 nM in SHR ($P < 0.01$). The responses to AVP in hydrated animals were 169 ± 10 nM in WKY and 220 ± 18 nM in SHR ($P < 0.05$). An important observation was that the responses in dehydrated WKY and SHR were significantly attenuated relative to the respective euvolemic animals ($P < 0.05$ for both). In hydrated WKY and...
SHR, the responses were significantly greater than in euvolemic rats (P < 0.01 for both).

DISCUSSION

Our study provides new information about regulation of [Ca^{2+}]_i in renal resistance vessels as it relates to V1a receptor mRNA and protein in young SHR developing genetic hypertension. The results indicate exaggerated [Ca^{2+}]_i responses to AVP in preglomerular arterioles during water loading and reduced reactivity during dehydration. To our knowledge, such correlations with fluid balance have not been reported before for renal resistance arterioles. During all hydration states, the [Ca^{2+}]_i response was exaggerated in young SHR compared with WKY. Similarly, the change in V1a receptor density as measured by ligand binding and mRNA for the V1a receptor increased in parallel to experimentally induced water loading and in an inverse manner to the predicted in vivo alterations in AVP plasma concentration. Similar changes were seen employing receptor stimulation/inactivation with pharmacological agonist and antagonist, respectively. Such parallel changes in receptor mRNA and protein implicate primary regulation at the mRNA level rather than receptor protein stability. Interestingly, the change in mRNA levels was not unique to the renal vasculature, as similar changes were noted for V1a receptors in hepatocytes. The present results extend our previous findings that V1a receptor mRNA and protein are set at an abnormally high level in the preglomerular resistance arterioles of young SHR vs. WKY by providing evidence that these observations for strain differences are present during a wide range of hydration states as well as euvolemia. Although set high in SHR, the responses to changes in plasma AVP concentration associated with volume state or receptor stimulation/antagonism are normal and regulated appropriately in that they parallel changes in normotensive animals. Both restricted fluid intake and chronic administration of AVP resulted in parallel decreases in vascular V1a receptor message and number. The converse was also observed. Water loading or pharmacological blockade of V1a receptors led to parallel increases in V1a receptor mRNA and protein in preglomerular vessels of young SHR and WKY. mRNA for hepatocyte V1a receptors responded in a manner similar to those in the renal vasculature. Such in vivo regulation is as would be expected with long-term homologous regulation mediated by the circulating level of AVP. Insight into regulation by a transcriptional mechanism is provided by the consistent parallel changes in receptor mRNA and protein expression and the functional consequences evidenced as changes in [Ca^{2+}]_i, that parallel changes in receptor mRNA and protein. Our collective findings advance our knowledge about receptor regulation and function in the renal microcirculation in vivo and in young rats prone to develop genetic SHR hypertension.

We have previously shown that preglomerular arterioles of 8-wk-old SHR express approximately two- to three-times as many V1a Receptors normalized to milligram tissue protein as do vessels of age-matched WKY under euvolemic conditions (50), an observation recently confirmed (41). PCR analysis
reveals that the reason for the elevated receptor number likely results due to twice the amount of V1a mRNA (50). These two lines of evidence serve as the foundation for our functional studies demonstrating that AVP produces greater V1a receptor-mediated in vivo reductions in RBF in young euvoletic SHR (14, 15). Consistent with this notion, although absolute blood flow responses were greater in SHR, analyses based on percentage change indicated that, for both strains, about one-third of the AVP-induced renal vasoconstriction is mediated by voltage-gated, L-type calcium channels, and approximately two-thirds of the vascular response is the result of inositol trisphosphate-mediated Ca2+ mobilization from intracellular sources (13). Thus the relative fractional contributions of these two pathways are similar in normotensive and hypertensive animals, which is at variance with the view of the postreceptor signaling accounting for the exaggerated renal vascular reactivity in SHR.

Similar supportive evidence is provided by the findings that V1a receptor stimulation produces greater increases in [Ca2+]i in VSMC isolated from preglolemular arterioles of young SHR compared with those of age-matched Sprague Dawley rats and WKY (22). Our results clearly show that the [Ca2+]i response is closely related to the level of mRNA and receptor protein for the V1a receptor. Because regulation seems to be appropriate in response to physiological changes in fluid intake and plasma AVP concentration in both strains, it is reasonable to postulate that V1a mRNA and receptor density in SHR and the functional consequence of these changes must have been reset at an earlier age, presumably by a factor other than plasma AVP per se. Early duplication of the gene for V1a receptor is a possibility.

Considerable evidence implicates increased renal vascular reactivity to vasoconstrictor agents in 8-wk-old SHR developing hypertension. Young SHR exhibit exaggerated renal vascular responses to both ANG II and AVP relative to age-matched normotensive WKY and other normotensive strains (7, 8). Subsequent studies revealed that hyperreactivity to ANG II in SHR is probably not the result of alterations in ANG II AT1 receptor density or affinity in the preglolemular vasculature (6, 7) but is more likely because of the impaired offsetting activity of vasodilator prostaglandins and possibly also dopamine (8, 9).

Long-term regulation of V1a receptors varies among organ and strains of rats. The elevated number of renal V1a receptors in SHR afferent arterioles at 8 wk of age decreases to the level seen in WKY at 20 wk of age (49, 50). In contrast, the density of renal vascular V1a receptor is stable throughout life in WKY both at the protein and mRNA levels (49). Other investigators report an equal expression level of V1a receptors in adult WKY and SHR using Western blotting in renal microvessels (17).

Cultured aortic VSMC derived from 12-wk-old SHR have a higher density of V1a receptors than WKY cells (30). A previous report on the density of liver V1a receptors suggests no difference between SHR and WKY and that liver V1a receptors are unresponsive to changes in plasma AVP concentration (33). Our findings do not support this and demonstrate clearly that the regulation of V1a receptors in the liver is similar to that in renal resistance vessels in young rats. In contrast to our recent finding of stable V1a in the renal vasculature of normotensive rats (49), the numbers of vasopressin binding sites of hepatocytes in normotensive Wag/Rij rats is reported to increase nearly twofold between 10 and 30 mo of age. This study found similar changes in calcium signaling and glucose release (44). The underlying age-dependent mechanism(s) for some organs and strains but not others are poorly understood, albeit apparently independent of changes in plasma AVP concentration.

Vasopressin receptor density is regulated by multiple mechanisms (1, 22, 34, 42). Most studies on vascular V1a receptors and their regulation have been performed on cultured aortic VSMC or glomerular mesangial cells. Our results provide valuable information about V1a receptor regulation and function in native resistance arterioles in vivo and in vitro. In general terms, AVP receptors are regulated by the plasma hormone concentration and hydration state such that dehydration and increased endogenous AVP concentration decrease V1a and V2 mRNA expression in renal tissue (33, 40, 43). Homologous desensitization of the V2 receptor is thought to be primarily mediated by cAMP-dependent and -independent phosphorylation in combination with receptor internalization. Dehydration for 48–72 h increases plasma AVP concentration >10 times above basal levels (33, 40, 45), and V2 receptor density in medullary collecting ducts is downregulated to 25–50% of the euvoletic level without a change in affinity (5, 26, 40). Chronic infusion of AVP leads to attenuated responsiveness of cAMP production in membranes isolated from the renal medulla, an effect that can be reversed by administration of a V2 receptor antagonist, which inhibits agonist binding and downstream signaling and results in upregulation of V2 receptor density.

Plasma AVP is elevated in DOCA-salt hypertension at a time when the number of hepatic and renal V1a and renal V2 receptors are reduced (27, 37, 47, 48). On the other hand, there are reports suggesting differences in the regulation of V1a receptors among tissues. For example, in one study, hepatic V1a receptor density was decreased compared with an increase in renal V1a density (47). The changes in that study appear to have occurred independent of a change in the level of mRNA for either hepatic or renal receptors, suggesting changes in mRNA stability or a difference in receptor processing. Other results suggest that steroid hormones improve mRNA stability. Cyclosporin appears to increase V1a receptor mRNA in cultured VSMC by enhancing the stability and half-life of message (10).

It is not clear why vascular V1a receptor mRNA and receptor protein are elevated in young SHR at a time when plasma AVP concentration in SHR younger than 12 wk of age is either in the normal range or slightly elevated (24) or markedly elevated (2, 20). In this regard, there appears to be a paradoxical upregulation of functional renal vascular V1a receptor density rather than the predicted no change or homologous downregulation. One or more causative factors may dominate over the expected AVP effect on receptor expression. Such a non-AVP regulator factor(s) may have been initiated by maternal patterning or imprinting during fetal development that translates to long-lasting effects on protein expression after postnatal maturation (16). Transcripts for renal cortical V1a and medullary V2 receptor mRNA are detected at the gestational ages of 16 and 19 days and tend to be relatively constant throughout life (32). In contrast to the kidney, the liver does not express V2 receptors, and its V1a receptor transcripts do not appear until after birth, reaching adult levels at 8 wk of age (32). An
example of long-lasting maternal patterning is that modest maternal nutrient restriction in early gestation is associated with postnatal hypertension and increased activity of the hypothalamic-pituitary-adrenal axis (17). Chronic in utero plasma hyperosmolality is known to increase hypothalamic AVP synthesis, pituitary AVP content, and plasma AVP in the newborn (36, 51). The reason for the marked upregulation of mRNA and protein for V1a receptors in the renal vasculature (and the liver) requires further investigation.

In summary, our observations provide new information about mechanisms that may be responsible for exaggerated calcium signaling in the renal microcirculation in genetic hypertension as it relates to regulation of the V1a receptor in the kidney and in the liver in young SHR and WKY. The three variables assessed ([Ca^{2+}]], V1a receptor mRNA, and V1a receptor protein density) are all upregulated in the preglomerular vasculature of 8-wk-old SHR compared with WKY. These strain differences persisted during fluid restriction and water loading as well on an ad libitum diet. In both the liver and the renal vasculature, the receptor density and gene expression exhibited homologous desensitization, following the general pattern of responding in an inverse manner to the level of circulating AVP when changes over 3–7 days were produced by dehydration, water loading, AVP infusion, and administration of a V1a receptor antagonist. The factors responsible for the elevated V1a mRNA and receptor protein in SHR vs. WKY during euvoiuma, dehydration, and water loading await further investigation. The apparent normal regulation in young SHR implicates factors other than plasma AVP per se.

ACKNOWLEDGMENTS

We thank Susan Kim and Camilla Gjerstad for excellent technical assistance in the radioligand binding studies.

GRANTS

The Chapel Hill studies were supported by National Heart, Lung, and Blood Institute Research Grant HL-02334. Bergen studies were funded by a grant from the Norwegian Council of Cardiovascular Diseases.

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HYDRATION STATUS AND Ca2+ RESPONSE TO AVP IN RENAL VESSELS FROM SHR

