Upregulation of V₁ receptors in renal resistance vessels of rats developing genetic hypertension

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Vågnes, Øyvind, J Ian J. Feng, Bjarne M. Iversen, and William J. Arendshorst. Upregulation of V₁ receptors in renal resistance vessels of rats developing genetic hypertension. Am J Physiol Renal Physiol 278: F940–F948, 2000.—Previous studies have demonstrated that arginine vasopressin (AVP) produces exaggerated renal vasoconstriction in young spontaneously hypertensive rats (SHR) relative to normotensive rats. The exaggerated renal vascular reactivity does not appear to be due to a primary defect in postreceptor calcium signal transduction. Although the magnitudes of vascular responses differ, the relative proportions of calcium entry and mobilization pathways evoked by AVP in renal resistance vessels are similar in these rat strains. The purpose of the present study was to evaluate possible differences in V₁ mRNA and receptor density and affinity in preglomerular resistance vessels (<50 μm) obtained from young Wistar-Kyoto (WKY) and SHR. Quantitative RT-PCR analysis revealed twofold greater expression of the V₁a receptor gene in preglomerular arterioles of 7-wk-old SHR compared with WKY. In vitro radiolabeled ligand binding studies were performed under equilibrium conditions on preglomerular resistance arterioles freshly isolated from kidneys of 7-wk-old rats. The results indicate that AVP receptor density (Bₘₐₓ) is twofold greater in SHR than in WKY (248 ± 24 vs. 91 ± 1 fmol/mg protein, P < 0.001). The affinity does not differ between strains (Kₘₐₓ = 0.5 nM). Displacement studies yielded similar results for SHR and WKY. Unlabeled AVP competitively displaced [³H]AVP binding, with an IC₅₀ of 2.5 × 10⁻¹⁰ M. Expression of AVP receptor types in afferent arterioles was evaluated using the V₁ receptor agonist, [Phe₁, Ile₈,Org³]vasopressin, the V₁ receptor antagonist, [d(CH₂)₅, Tyr(Me)₂, Tyr(NH₂)₉]Arg⁸ vasopressin, and the V₂ receptor agonist, desamino-[α-Arg₈]vasopressin. Both the V₁ agonist and antagonist displaced up to 90% of the AVP binding with IC₅₀ values of 4 × 10⁻⁸ and 8 × 10⁻⁷ M, respectively. The V₂ receptor agonist was a weak inhibitor, displacing less than 15% of AVP binding at a high concentration of 10⁻⁴ M. These results demonstrate that virtually all AVP receptors in the preglomerular arterioles are of the V₁ type. Collectively, our results provide evidence that the enhanced renal reactivity to AVP is mediated by a higher density of V₁ receptors associated with increased gene expression in renal resistance vessels of SHR developing genetic hypertension.

ARGinine Vasopressin (AVP) exerts important physiological and pathophysiological effects because of its vasconstriction and antidiuretic properties. Its diverse physiological actions include stimulation of water reabsorption in renal epithelial cells, contraction of smooth muscle, stimulation of liver glycogenolysis, and modulation of corticotropin release from the pituitary gland. Such effects are mediated through the binding of AVP to one of two types of membrane-bound receptors of target cells, which have been classified V₁ and V₂ (38, 40, 42, 47, 48). These receptors differ in regard to function, tissue localization and mediation by second messenger systems as well as being characterized by selective pharmacological receptor agonists and antagonists. The V₁ "vascular" receptors are coupled to phosphatidylinositol hydrolysis to increase cytosolic calcium concentration through mobilization and L-type channel entry pathways, thereby mediating the vasoconstrictor and glycogenolytic effects. V₁a and V₁b subtypes are primarily found in vascular and hepatic tissue or in the anterior pituitary gland, respectively. Vascular V₁ receptors are found in conduit and resistance arteries and arterioles (37, 38, 42, 47, 48). In the kidney, microdissected renal cortical arteries and arterioles and medullary vasa recta capillaries express V₁a, but not V₂, receptor mRNA (36). They are also found in glomerular mesangial cells (1). It is via the V₁a receptor that relatively small amounts of circulating AVP elicit systemic and renal vasoconstriction. "Tubular" V₂ receptors are localized mainly in renal epithelial cells and are known to activate adenylate cyclase and the production of cAMP to stimulate aquaporins and to elicit an antidiuretic response in late portions of the nephron. Recent studies also suggest the presence of a V₁a receptor in renal cortical and medullary collecting duct cells (1).

There are several lines of evidence suggesting the AVP peptide plays an important role in the development and maintenance of hypertension. Previous studies have demonstrated the contribution of AVP to the pathogenesis of several forms of hypertension in spontaneously hypertensive rats (SHR) and in deoxycorticosterone acetate (DOCA)-salt hypertension. In these models, plasma AVP is elevated (11, 17, 27), AVP receptor agonists reduce arterial pressure, and there is an increased vascular responsiveness to exogenous AVP (3, 25, 28, 41, 52). The ability of AVP to produce
hypertension appears to be related more to its vasoconstrictor effects than to its influence on water balance (10, 40). Vasoconstrictor agents are thought to contribute significantly to the genesis and maintenance of hypertension by virtue of exaggerated vascular reactivity. A variety of mechanisms have been proposed to explain abnormal reactions to various receptor ligands (29, 40). In general terms, functional differences in vascular responsiveness may result from receptor density and/or postreceptor signal transduction as well as differences in vessel structure and increased wall-to-lumen ratio.

The SHR has been used to investigate mechanisms involved in the pathogenesis and maintenance of essential hypertension in humans. It is well established that the development of hypertension in young SHR is associated with abnormalities in renal function (2, 6, 8, 12, 16, 23, 51). At 6–8 wk of age, SHR have a reduced renal blood flow and glomerular filtration rate with slightly elevated arterial pressure, suggesting participation of vasoconstriction factor(s) (6, 8, 12, 16, 23, 51). This notion is supported by observations that the renal vasculature of young SHR exhibits exaggerated responses to both angiotensin II (ANG II) and AVP compared with age-matched normotensive Wistar-Kyoto rats (WKY) (6–8, 13, 14, 23). Subsequent studies revealed that this strain difference of ANG II–induced renal response was probably not due to alterations in ANG II receptor density or affinity of ANG II receptors (7) but is more likely due to the decreased offsetting activity of vasodilator agents such as prostaglandins or dopamine in SHR (6, 9, 23). In contrast to the normalization in reactivity to ANG II in SHR during indomethacin treatment, the strain difference in the renal vascular response to AVP persists after cylooxygenase inhibition (13). More recent studies demonstrate that renal vasoconstrictor responses to AVP are mediated by a combination of calcium entry through a dihydropyridine-sensitive channel and calcium mobilization mediated by inositol trisphosphate (IP3) (14). The fact that the relative contributions of these pathways are similar in SHR and WKY suggests that a mechanism(s) other than postreceptor calcium signal transduction is responsible for the exaggerated vascular reactivity to AVP in SHR. This notion is supported further by recent observations that AVP elicits greater than normal changes in cytosolic calcium concentration in preglomerular vasculature of normotensive rats (20).

The present studies were conducted to determine whether there is a difference in AVP V1 receptor gene expression and receptor protein density and/or affinity in renal preglomerular resistance vessels freshly isolated from 7-wk-old WKY and SHR. Gene expression studies for the V1 receptor were conducted using a quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Saturation radioligand binding studies were also performed during equilibrium conditions, and the relative proportions of AVP receptor types were assessed by displacement of AVP binding by selective V1 and V2 receptor agonists.

METHODS

Radioligand Binding Studies

Isolation of preglomerular resistance vessels. Male SHR and WKY averaging 7 wk of age were obtained from our Chapel Hill breeding colony. The animals were maintained on a standard rat chow diet (Bemis, Madison, WI) and tap water ad libitum. The methodology was as described previously for radioligand binding studies of preglomerular resistance arterioles (7). Anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt). A midline abdominal incision was made, and the abdominal aorta was cannulated below the renal arteries. The aorta above the right kidney was ligated, the left renal vein was cut, and kidneys were perfused with iced heparinized isotonic saline solution until the effluent was blood free. Thereafter, the kidneys were perfused with a magnetized iron oxide suspension (1% Fe3O4 in isotonic saline) at constant pressure (120 and 140 mmHg for WKY and SHR, respectively, with an approximate rate of 10 ml for 30 s). The kidneys were excised and placed in a phosphate buffer solution (PBS: in mM, 125 NaCl, 17 K2HPO4, 3 NaH2PO4, and 5 MgCl2, 4°C, pH 7.3). All subsequent steps of vessel isolation were performed at 4°C. The kidneys were decapsulated, and the cortex was dissected from the medulla. Cortical tissue was gently minced with a razor blade and transferred to a tube containing 5 ml of PBS. The tissue was homogenized with a Polytron homogenizer at a moderate speed (3 times for 10 s each time, setting 3, rotor 7-mm diameter). Vascular tissues 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. The latter step was repeated with a smaller-sized needle (22-gauge) until the supernatant was free of nonvascular tissue. Repetitive injections in this fashion mechanically detached most of the connective tissue, removed the iron oxide from the large vessels, and separated arterioles from glomeruli. The vascular suspension was filtered through a 100-µm mesh sieve and thoroughly washed with iced PBS. The microvessels were recovered from the top of the sieve, transferred in PBS, and digested with collagenase (35 U/ml) for 30 min at 37°C. Thereafter, the vascular suspension was resuspended from the collagenase-PBS with the aid of a magnet, resuspended in PBS, passed through a 27-gauge needle, and sonicated for 30 s. Free iron oxide particles were removed with a magnet. 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 remaining vessels were >95% pure based on visual inspection and previous studies on antibody staining against smooth-muscle-specific a-actin and myosin heavy chain (20). Small fragments of proximal tubules were rare; segments of distal nephrons devoid of brush border were never observed. The protein concentration was determined by a colorimetric method as previously described (7).

[3H]AVP binding assays. The incubation medium (final volume 250 µl) contained 50 mM Tris-HCl (pH 7.4), 5 mM MgCl2, 0.3% bovine serum albumin, and [3H]AVP (Amersham-Pharmacia, Arlington Heights, IL). 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 µg protein) and stopped by separating bound ligand from free ligand with centrifugation in a sucrose gradient (7). 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 injection into dry ice, and the tips, containing the bound ligand, and the tops, containing the free, unbound ligand, were assayed in a Packard Tri-Carb 4500 scintillation counter.

The protein concentration, binding conditions, and time to attain equilibrium binding of [3H]AVP to isolated segments of rat afferent arterioles were determined in preliminary experiments. Binding of [3H]AVP to 50 µg protein reached saturation at concentrations greater than 2 nM. Specific binding of [3H]AVP (4 nM) to the microvessels was linear with increasing amounts of protein added up to at least 75 µg protein. Binding became nonlinear when protein concentration exceeded 100 µg. Specific binding of [3H]AVP (5 nM) to 50 µg protein took 30–60 min to reach equilibrium at room temperature.

Saturation binding experiments were performed with increasing concentrations of [3H]AVP between 0.125 and 4.0 nM. Competition displacement studies were performed with a fixed concentration of [3H]AVP (2 nM) and increasing concentrations (10⁻¹ to 10⁻⁴ M) of unlabeled AVP or V₂ or V₁a receptor agonist or antagonist. In all cases, specific binding was calculated as total binding minus nonspecific binding measured in the presence of 2.5 µM unlabeled AVP. The maximum specific binding (Bₘₐₓ) and dissociation constant (Kᵣ) were calculated using the Ligand software program (Biosoft, Cambridge, UK). Each group of experiments consisted of at least three determinations; each utilizing fresh tissue preparations from four animals. Results between strains of rat were analyzed by unpaired t-test. P < 0.05 was considered statistically significant. All values are reported as means ± SE.

Reverse Transcriptase-Polymerase Chain Reaction

Smooth muscle cell isolation and RNA purification. Measurements of mRNA were performed on 7-wk-old SHR and WKY obtained from Møllegaard and maintained on standard rat chow diet (B & K Universal). The rats were anesthetized with pentobarbital sodium, and preglomerular resistance vessels were isolated as described above. Care was taken to prepare the vessels as quickly as possible to minimize the period of RNA degradation. Briefly, vessel segments were isolated from the renal cortex with the aid of a magnet and injected through stepwise progressively smaller needles (21, 23, and 25 gauge) to mechanically detach interlobular arteries and afferent arterioles from glomeruli and larger vessels. As noted above, the remaining vessels were >95% pure and devoid of tubular fragments and glomeruli; no collecting ducts were observed. Tissue recovered from the top of the 120-µm sieve was enriched again with a magnet and then homogenized in TRIzol (GIBCO) 1:10 wt/vol. Total RNA was extracted according to the product manual (GIBCO). The RNA was resuspended in 30 µl H₂O and digested for 15 min at 37°C with 20 units of RNase-free DNase (Promega). The RNA was resuspended in 0.5 ml TRIzol and extracted again to remove the DNase. The extracted RNA was finally resuspended in 25 µl H₂O. The amount of RNA was measured by 260-nm absorbance on a Gene Quant DNA/RNA Calculator (Pharmacia).

Amplification for cloning. Primers for RT-PCR amplification were selected for a 293-bp-long region (bp 550–842) of the V₂ receptor gene. The forward primer chosen was GCC TTC TTC CAA GTA TTA CCG CA; the reverse primer was TTT TTC ACC TCG ATT TCG ATC AC. The assay was done using an Access RT-PCR kit (Promega) on a PTC-200 Peltier thermal cycler (MJ Research). The Access RT-PCR kit is based on avian myeloblastosis virus reverse transcriptase (AMV-RT) and Tfl DNA polymerase. Preliminary studies confirmed that the optimal concentration of MgSO₄ was 1 mM. The AMV-RT was specifically primed and the RT process done at 48°C for 45 min, followed by 94°C for 4 min to destroy the AMV-RT. Amplification for cloning of the RT-PCR product was done using 5 cycles at 94°C for 1 min, a stepwise decrease of annealing temperature of 1 degree/cycle from 55–51°C for 1 min and a elongation period of 68°C for 1 min. Then 35 cycles at 94°C for 1 min, 50°C for 1 min, and 68°C for 1 min followed by a elongation period of 68°C for 20 min to ensure terminal transferase addition of a terminal deoxynucleosine used in the cloning. For the quantitative RT-PCR, we used 24 cycles at 94°C for 1 min, 50°C for 1 min, and 68°C for 1 min. This was followed by an elongation period of 68°C for 10 min.

Construction of the reference nucleic acid. The 293-bp-long fragment of the V₂a mRNA was amplified as described above and cloned into a pCRII-TOPO dual promoter vector (Invitrogen). When mapping the endonuclease restriction sites of the fragment, two BsrBI restriction sites separated by 30 bp were identified. The plasmid with the V₂a PCR fragment was cut by BsrBI (New England Biolabs) and the products were run on a 2% agarose gel. The cutting pattern was used to verify the identity and direction of the cloned RT-PCR product. The two restriction product fragments containing parts of the vector and the RT-PCR product, except the 30 bp between the BsrBI restriction sites, were isolated from the gel and ligated with the use of T4 ligase (Promega). The ligation product was amplified using the primers mentioned above to yield a PCR product V₂a short form (V₂aS) 30 bp less than the original. The V₂aS was then cloned into a pCRII-TOPO vector and used as an internal standard. The plasmid was linearized by cutting with EcoRV (Promega) in the vector’s poly linker. V₂aS was then transcribed using a Ribomax in vitro transcription kit with a SP6 RNA polymerase (Promega).

Quantitative RT-PCR. Total RNA was purified as described above and amplified for 20–27 cycles to establish linearity. Based on linear results in the midrange we chose to use 24 cycles with three concentrations (0.25, 0.5, and 1 µg) total RNA of each sample. The RT-PCR was prepared as one master mix. A volume of 25 µl master mix and 7.3 pg V₂aS mRNA reference template were used for each test. A total of 31 assays were performed, which included a negative control containing water instead of RNA. The RT-PCR products were then separated on 2% agarose gels and analyzed densitometrically (Bio-Rad Multi-Analyser).

The ratios of PCR products of sample V₂a mRNA/reference V₂aS RNA were then estimated for each of the three concentrations of total RNA. The relation between the ratio and total RNA was found to be linear, with a slope less than one. Gene expression was calculated using the average ratio for different concentrations of total RNA, SHR values are expressed as a percentage of the ratio for WKY.

The following chemicals were used in the present study: AVP, bovine serum albumin, and indomethacin (Sigma Chemical); the V₁ receptor agonist, [Phe₂, Ile⁹]vasopressin, the V₁ receptor antagonist, [d(CH₂)₅, Tyr(Me)₂,Tyr(NH₂)⁹]Arg₈-vasopressin, and the V₂ receptor agonist, desamino-[o-Arg]vasopressin (Peninsula Laboratories); and [3H]AVP (sp act 64.2 Ci/mM, New England Nuclear-Du Pont).

RESULTS

Total binding of [3H]AVP to microvessels was directly related to increasing concentrations of [3H]AVP. Nonspecific binding (in the presence of 2.5 µM unlabeled AVP) increased linearly, averaging 15 ± 3% of the total binding at the lowest concentration of 0.125 nM [3H]AVP.
and 34 ± 5% at the highest concentration (4 nM) of [3H]AVP tested.

Representative examples of AVP binding to arteriolar smooth muscle cells under equilibrium conditions are presented in Fig. 1. In this experiment, specific binding of [3H]AVP increased with increasing concentration of [3H]AVP until binding reached saturation, at ~2 nM (Fig. 1A). Transformation of this experiment's data for Scatchard analysis is presented in Fig. 1B. Comparisons of AVP binding were made between three different preparations of vessels from four SHR or WKY. The summarized group averages in Fig. 2 clearly demonstrate that AVP receptor density (Bmax) was about two- to threefold greater in SHR than in WKY vessels (248 ± 24 vs. 91 ± 11 fmol/mg, P < 0.001). There was no strain difference in AVP receptor affinity (Kd) (0.5 ± 0.1 vs. 0.5 ± 0.1 nM). Analysis of these binding data by the Hill equation gives a coefficient of 0.96 ± 0.04 (n = 8), indicating [3H]AVP binding in this vascular preparation is noncooperative, which is consistent with the model of a single type of binding site.

To identify AVP receptor type(s) expressed in these renal vascular protein preparations, unlabeled AVP, a V1 receptor agonist, [Phe²,Ile³,Org⁶]vasopressin, a V1 receptor antagonist, [d(CH₂)₅,Tyr(Me)²,Tyr(NH₂)⁹]Arg⁸-vasopressin, and the V2 receptor agonist desamino[D-Arg⁸]vasopressin were used to displace the binding of [3H]AVP (2 nM). Figure 3 shows the displacement ability of these agents expressed as a percentage of control specific binding without a competitor. No difference was observed in these displacement studies between SHR and WKY; thus the data were pooled. The order of displacement potency for [3H]AVP binding is AVP > V1 agonist > V1 antagonist > V2 agonist. These displacement data were analyzed using the iterative model-fitting program, Ligand, to calculate the best-fit regression for each analog. The results indicate a one-site model for the displacement of [3H]AVP by each analog. Unlabeled AVP completely displaced [3H]AVP binding, with a half-maximal inhibitory concentration (IC₅₀) of 0.24 nM (Table 1). The V1 agonist and antagonist tested were potent inhibitors of [3H]AVP binding.
with IC_{50} values <1 µM. These V_1 receptor agents displaced up to 90% of the \[^{[3]H}\]AVP binding between concentrations of 10^{-5} and 10^{-4} M. The values of the inhibition constant (K_i) for AVP, V_1 agonist, and V_1 antagonist ranged between 0.05 and 180 nM (Table 1). The V_2 receptor agonist was a weak inhibitor, displacing \sim 10–15% of \[^{[3]H}\]AVP binding at concentrations between 10^{-10} M and 10^{-4} M (Fig. 3).

V_1 receptor gene expression was analyzed by quantitative RT-PCR. As Fig. 4 shows, the reaction utilizing 24 and 25 cycles provides densitometric estimates that are linear as a function of amount of total RNA. The relation has a slope less than unity. Figure 5 presents results of representative densitometric determinations of RT-PCR products establishing the relationship between the internal standard V_{1a}S and V_1a receptor mRNA and total RNA for renal preglomerular vascular tissue of euvolectric SHR and WKY. Gene expression of the V_1 receptor was estimated from three different concentrations of protein relative to a constant amount of standard V_{1a}S mRNA. The density of V_{1a}S bands differed due to competition between V_{1a}S and V_1 mRNA for the enzymatic site of Tfl polymerase. On the average, 0.50 µg of total RNA from vessels of WKY yielded a V_{1a}/V_{1a}S ratio equal to that provided by 0.25 µg total RNA from SHR vessels. Accordingly, the V_1a receptor gene expression was almost twofold greater in SHR compared with the age-matched WKY (Fig. 6, P < 0.001). The magnitude of the strain difference in gene expression was almost identical for two different concentrations of total RNA (192 vs. 197%, P > 0.5), verifying linearity of reaction product.

**DISCUSSION**

Our previous in vivo blood flow studies demonstrated that AVP acts through V_1 receptors to elicit renal vasoconstriction that is more pronounced in young SHR relative to normotensive rats (13). Injections of either 2 or 5 ng AVP caused significantly greater renal vasoconstriction in 7-wk-old SHR than in WKY, an

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**Table 1. Summary of competitive binding displacement studies of \[^{[3]H}\]AVP to evaluate AVP receptors in freshly isolated afferent arterioles of 7-wk-old SHR and WKY**

<table>
<thead>
<tr>
<th>Displacement by</th>
<th>Rat Strain</th>
<th>IC_{50}, nM</th>
<th>K_i, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVP</td>
<td>SHR</td>
<td>0.24 ± 0.06</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>0.22 ± 0.03</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>V_1 agonist</td>
<td>SHR</td>
<td>45 ± 12</td>
<td>8.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>47 ± 9</td>
<td>8.2 ± 1.8</td>
</tr>
<tr>
<td>V_1 antagonist</td>
<td>SHR</td>
<td>870 ± 86</td>
<td>179 ± 33</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>836 ± 57</td>
<td>181 ± 41</td>
</tr>
<tr>
<td>V_2 agonist</td>
<td>SHR</td>
<td>&gt;10^{-4} M</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>&gt;10^{-4} M</td>
<td>ND</td>
</tr>
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</table>

Values are means ± SE for 3 determinations on 12 animals per group. K_i, inhibition constant; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyota rat; AVP, arginine vasopressin. ND, cannot be determined.

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**Fig. 3. Dose-dependent inhibition of specific \[^{[3]H}\]AVP binding to preglomerular arteriolar segments by increasing concentrations of AVP, V_1 agonist, V_1 antagonist, and V_2 agonist in 7-wk-old WKY and SHR. Data were not different between strains and were pooled. Values are means ± SE for 6 experiments in each group.**

**Fig. 4.** RT-PCR products at 0.25, 0.50, and 1.0 µg total RNA using 24 and 25 cycles of amplification. Reactions are linear and parallel within this range of RNA.
effect that was not normalized by indomethacin treatment (13). Indeed, the dose of AVP in WKY had to be increased to normalize the amount of AVP-induced renal vasoconstriction, suggestive of a strain difference in receptor density. This view is supported by evidence that the relative contributions of calcium entry and mobilization pathways were similar in normotensive and hypertensive animals (14). These results argue against a primary defect in postreceptor signaling to account for the exaggerated renal vascular reactivity to AVP challenge in SHR. In addition, we recently reported that AVP produces greater changes in cytosolic calcium concentration in cells isolated from preglomerular arterioles from young SHR vs. WKY and Sprague-Dawley rats (19, 43). Such magnified responses may be due to a strain difference in V1 receptor properties or postreceptor signal transmission. Other investigators, however, believe increased vascular reactivity in hypertension-prone animals is primarily due to postreceptor alterations, because AVP receptor density in certain blood vessels is essentially normal in SHR, DOCA-salt hypertension, and Goldblatt hypertension (26).

The current study utilized saturation, radioligand binding studies under equilibrium conditions to investigate the density and affinity of AVP receptors in preglomerular resistance vessels of young SHR and WKY. Afferent arterioles were isolated from 6- to 8-wk-old SHR and WKY according to procedures refined in our laboratory (7, 19). A major new finding in the present study was that density of AVP V1 receptor binding sites is two- to threefold greater in SHR than in age-matched WKY, whereas affinity is similar. These observations provide new information implicating a larger number of V1 receptors as a causative factor in the exaggerated response of renal vascular resistance to AVP stimulation in the 7-wk-old SHR during the development of hypertension.

Scatchard analysis of our binding data reveals the existence of a single class of high-affinity AVP binding site in isolated preglomerular arterioles. These preglomerular resistance vessels have a higher density of binding sites in young SHR relative to WKY, with similar affinity in the nanomolar range. In our displacement studies of radioligand binding, pressor-selective and antidiuretic-selective analogs of AVP were utilized to characterize V1 and V2 receptor types. The results convincingly demonstrate that the major, if not exclusive, type in the afferent arteriole is V1. AVP and the V1 receptor agonist competed for [3H]AVP binding with a high affinity (IC50, 10^{-8}–10^{-10} M). In contrast, the selective V2 receptor agonist desamino-[d-Arg8]-vasopressin competed weakly for receptor binding, with an IC50 in excess of 10^{-4} M. These present in vitro binding results agree well with our in vivo blood flow studies, which showed that V1 receptors mediate more than 90% of AVP-induced constriction (13). This observation is also supported by experiments done on microdissected renal tissue. Using a PCR method on kidneys of Sprague-Dawley rats, cortical and medullary vessels were found to express mRNA for the V1a receptor but not the V2 receptor (36). This finding supports earlier observations using in situ hybridization technique where the V1a receptors are mainly localized to the interlobular and arcuate arteries (35). All of these studies provide evidence to support the notion that the vascular action of vasopressin is mediated through V1 receptors in the rat.

Several binding studies provide support for the view that the Okamoto-Aoki strain difference in vascular reactivity is due to greater V1 receptor number, without any change in receptor affinity. In one study, the density of AVP receptors was found to be five times greater in cultured thoracic aortic smooth muscle cells of 12-wk-old SHR, either in proliferative or quiescent states, compared with those from WKY (34). In a recent study on freshly isolated smooth muscles cells from preglomerular vessels, we observed a more pronounced increase in cytosolic calcium after AVP stimulation in 7-wk-old SHR compared with WKY (20). The increased cytosolic calcium response compared with the receptor
density and gene expression from the present report suggest that an upregulation of the V₁ receptor may explain the increase in vascular reactivity to AVP in young SHR. It is of particular interest to note that the magnitude of the calcium response, the increase in receptor densities, as well as gene expression were strikingly similar and may suggest that the functional aspects of AVP in SHR are mediated by parallel upregulation of V₁a receptor message and protein. It is, however, not clear whether AVP causes larger changes in cytosolic calcium concentration in cultured aortic smooth muscle cells derived from 4- to 6-wk-old adult SHR and WKY (31, 32). Interestingly, AVP caused larger calcium responses in freshly isolated, third-order mesenteric arteries from adult but not young SHR (49). In cultured mesangial cells isolated from glomeruli of adult rats, the number of AVP binding sites was two- to threefold greater in SHR as was AVP-induced production of IP₃ (18). On the other hand, AVP receptor density in the mesenteric circulation is reported to be similar in 4-, 8-, and 16-wk-old SHR or reduced in SHR at 16 wk of age (25). Further investigation is needed to determine whether renal AVP V₁ receptor upregulation is a primary event, occurring at a younger age than receptor changes in other vascular beds. With regard to V₁ receptors in the liver, studies indicate no difference between 14- and 16-wk-old SHR and WKY or a reduced density in SHR at 6–10 wk of age (3, 15). Although specific types were not identified, an increased number of AVP binding sites was found in renal medullary membranes of 12-wk-old SHR (45). Thus there is no consistent pattern of V₁ receptor regulation among the liver and different vascular beds. A more recent autoradiographic study combined with radioligand binding reported no difference in V₂ receptor density in renal medullary membranes of 3- and 7-wk-old SHR but an increased density in 12-wk-old SHR at a time when plasma AVP is increased (17).

Our radioligand binding and the gene expression studies were performed on rats from different colonies. We believe that the strain differences we have observed in V₁ receptor density and V₂, mRNA are real and not related to our testing of animals from different sources for radioligand binding and RT-PCR studies. The body weights range from 150 to 190 g for 7-wk-old rats. Mean arterial pressure is similar in the animals, averaging 135–150 mmHg for 7-wk-old SHR and 95–125 mmHg in age-matched WKY (13, 14, 21, 22). Most importantly, renal vascular reactivity to AVP administered into the renal artery is almost identical and exaggerated by roughly twofold in 7-wk-old SHR vs. WKY from Chapel Hill (34 vs. 16% of basal renal blood flow) (13, 14) and Møllegaard colonies (43 vs. 25%, R. E. F. Christiansen and B. M. Iversen, unpublished observations). Also, the method used to isolate preglomerular resistance vessels for radioligand binding and RT-PCR studies is virtually identical in the Chapel Hill and Bergen laboratories.

The reason for the increased density of V₁ receptors in renal resistance vessels of 7-wk-old SHR is not known. A commonly held view is that the plasma concentration of AVP is a major signal regulating receptor density, with a reduced plasma concentration leading to an increased receptor density. Our data of the V₁ receptor gene expression, determined by quantitative RT-PCR, indicate that the V₁ mRNA is upregulated in the young SHR. The values can be taken as suggestive evidence for duplication of the gene for V₁ in SHR. Although conflicting evidence exists, there is a general tendency to support the view that chronic AVP administration causes downregulation or desensitization of both V₁ vascular and V₂ epithelial receptors (4, 5, 45, 48). However, other studies suggest differences in the regulation of AVP receptor types. For example, low AVP plasma levels in the Brattleboro rat are associated with increased AVP binding to V₁ receptors in liver membranes but not to V₂ receptors in the renal medulla (44). In addition, increased AVP plasma concentration during 72-h water restriction is associated with decreased V₂ receptor mRNA in collecting ducts, whereas V₁a receptor mRNA was essentially unchanged (46). The mediator(s) of this homologous regulation is not known, although several studies point to a role of protein kinase C, perhaps leading to receptor phosphorylation that reduces the coupling efficiency of receptor and GTP-binding protein (5, 33, 42, 47). This, however, does not appear to be the major reason for the change in the renal vasculature of SHR. Plasma AVP concentration is either normal or elevated in young SHR (3, 11, 17, 25, 27). The increased receptor density in the presence of such plasma levels implicates an abnormality in a regulatory mechanism in hypertensive-prone animals. Other factors known to influence AVP receptor expression include glucocorticoids. The elevated plasma concentration of glucocorticoids noted in young SHR may account for some of the stimulation of AVP receptor density (24). Dexamethasone increases V₁ receptor density without affecting affinity; V₁ receptor mRNA may increase two to three times (30). A recent study of AVP receptors reports that DOCA-salt hypertensive rats with increased plasma AVP levels exhibit reduced AVP binding to the liver V₁ receptor and the kidney V₂ receptor, whereas mRNA for V₁ (liver and kidney) and V₂ receptors was unchanged (39). Another study of V₁a receptor regulation suggests that the breakdown of mRNA is decreased with increasing mRNA stability during glucocorticoid treatment (30). However, some investigators report that liver V₁ receptor number is upregulated and renal medullary V₁ receptor density downregulated in association with increased plasma AVP in DOCA-salt hypertension (50). Also reduced were renal medullary V₂ receptors and subsequent cAMP formation (50). Mesenteric arterial AVP receptor density is reduced during increased plasma AVP concentration in DOCA-salt-induced hypertension (26). Future studies are required to more completely characterize AVP receptor responses to changes in hydration state and plasma AVP concentration and to identify critical steps concerning synthesis, internalization, and metabolism in normal animals and in models of hypertension.

In summary, our observations provide new information about the mechanisms that may be responsible for mediating exaggerated renal vascular reactivity to AVP.
in young animals developing genetic hypertension. Quantitative RT-PCR of preglomerular arterioles reveals that V_{1a} receptor mRNA is two- to threefold greater in kidneys of 7-wk-old SHR compared with age-matched WKY. Saturation equilibrium binding data indicate that the receptor density is two to three times greater in preglomerular resistance vessels obtained from 7-wk-old SHR; receptor affinity did not differ from WKY values. Competition binding studies demonstrate that the order of potency for V_{1} receptors was: unlabeled AVP > V_{1} agonist > V_{1} antagonist. The tested V_{2} receptor agonist was a very weak inhibitor of AVP binding to arteriolar smooth muscle cells. Thus the V_{1} receptor is the predominant type in the afferent arteriole. The increased density of V_{2} receptors in renal resistance arterioles of SHR may be responsible for the enhanced renal vascular responsiveness to AVP observed in young SHR in vivo during the development of genetic hypertension.

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