Age-dependent regulation of vasopressin \(V_{1a}\) receptors in preglomerular vessels from the spontaneously hypertensive rat

Öyvind B. Vågnes, Frank H. Hansen, Rolf E. F. Christiansen, Camilla Gjerstad, and Bjarne M. Iversen. Age-dependent regulation of vasopressin \(V_{1a}\) receptors in preglomerular vessels from the spontaneously hypertensive rat. Am J Physiol Renal Physiol 286: F997–F1003, 2004. First published December 30, 2003; 10.1152/ajprenal.00399.2003.—Experiments were performed to get insight into the role of AVP receptor \(V_{1a}\) regulation with age, i.e., during development and maintenance of high blood pressure. Previous studies showed an increased gene expression and renal vascular response to AVP in young spontaneously hypertensive rats (SHR). The age regulation of the \(V_{1a}\) receptor was examined in preglomerular vessels from 5-, 10-, 20-, and 70-wk-old SHR using normotensive Wistar-Kyoto rats (WKY) as controls. Real-time PCR and ligand binding were used for analysis of receptor expression, and the change in cytosolic calcium concentration during stimulation of isolated preglomerular vessels with AVP was studied. Studies showed an increase of the \(V_{1a}\) receptor protein and mRNA from 5- and 10-wk-old SHR compared with vessels from 20- and 70-wk-old SHR. In 5-wk-old SHR receptor density was \(84 \pm 13\) fmol/mg protein, and \(38 \pm 11\) fmol/mg protein in 70-wk-old SHR \((P < 0.05)\). mRNA in the 5- and 70-wk-old SHR was \(15,854 \pm 629\) and \(3,181 \pm 224\) \(V_{1a}\) mRNA/108 18S ribosomal mRNA, respectively \((P < 0.001)\). Values from WKY at all ages were similar to 20- and 70-wk-old SHR. During stimulation with AVP, the change in cytosolic calcium in vessels from 5-wk-old SHR increased \(234 \pm 59\) nM, whereas the increase was \(89 \pm 9\) nM in 70-wk-old SHR \((P = 0.03)\). These results indicate that the \(V_{1a}\) receptor is increased at protein and mRNA level during development of hypertension in SHR but is normalized when hypertension is established.

hypertension; calcium signaling; renal circulation

ARGININE VASOPRESSIN (AVP) is a nonapeptidic hormone exerting various biological effects in the central nervous tissue and in peripheral organs. These effects include vascular constriction and tone, water retention, baroreceptor modulation, regulation of liver gluconeogenesis, platelet aggregation and adrenocorticotropic release, and central nervous tissue neurotransmitter (25, 42). AVP signaling is mediated through three types of G protein-coupled receptors, \(V_{1a}\), \(V_{1b}\), and \(V_{2}\). \(V_{1a}\) is localized to vascular-coupled muscle cells, hepatocytes, and in the brain (35, 38). \(V_{1b}\) receptors are found exclusively in the brain and stimulate the release of adrenocorticotropic (1). Renal epithelial \(V_{2}\) receptors are involved in regulation of water reabsorption through activation of aquaporins in the collecting ducts of the kidneys (5, 36, 38). The three AVP receptors differ with regard to signal transduction. \(V_{1a}\) and \(V_{1b}\) signaling is mediated through phosphatidylinositol hydrolysis with increased cytosolic calcium concentration ([Ca\(^{2+}\)]\_i) as the effector. The \(V_{2}\) signal transduction is mediated through adenylyl cyclase with increased cytosolic cAMP and the aquaporins as the effector in the collecting ducts.

Previous studies showed that AVP may be a contributor in the pathogenesis of hypertension in the spontaneously hypertensive rat (SHR) (4, 33) and in DOCA salt hypertension (13). The AVP contribution in genetic hypertension seems to be mediated through the \(V_{1a}\) receptors. Intrarenal administration of \(V_{1a}\) agonist produces vasoconstriction, salt and water retention, and hypertension (12). Blocking this receptor with a \(V_{1a}\)-specific antagonist delays the hypertensive development in SHR (4, 33). Water retention through \(V_{2}\) stimulation does not contribute in this process, and treatment with \(V_{2}\) antagonist has been shown to enhance the hypertensive development (33).

Young SHR has an increased renal vascular sensitivity to vasconstrictor agents (8, 15). The increased vasoconstrictive response to ANG II in young SHR seems to be due to a defective G protein activation in the cAMP pathway in the kidney. Due to this, there is a defective buffering activity of dopamine and prostanoid to the renal vasoconstrictive effector of ANG II in SHR (7, 9). In contrast, the exaggerated renal vascular response to AVP is independent of prostanoids (15). In a previous publication, we found that ANG II stimulation of smooth muscle cells isolated from preglomerular resistance vessels from SHR and Wistar-Kyoto (WKY) rats produces an equal rise in [Ca\(^{2+}\)]\_i (23). In contrast, stimulation with AVP induced an almost doubled increase in [Ca\(^{2+}\)]\_i in young SHR compared with age-matched WKY (23). This points toward different mechanisms for the increased renal vascular response to ANG II and AVP in SHR. We found an increased expression of the \(V_{1a}\) receptor in young SHR compared with age-matched WKY (23). This points toward different mechanisms for the increased renal vascular response to ANG II and AVP in SHR. We found an increased expression of the \(V_{1a}\) receptor in young SHR compared with age-matched WKY, whereas AT\(_{1}\) receptor density is similar (6, 46). This is probably the reason for the exaggerated renal vascular response to AVP in young SHR. In 40-wk-old SHR, the vascular AVP response becomes equal to that of age-matched WKY (10).

In the present study, we wanted to test the hypothesis that increased \(V_{1a}\) response may play a role during development of hypertension. The receptor density was examined when the blood pressure rises and in the later stage of hypertension when the blood pressure is stabilized. The isolated cells were submitted to [\(^3\)H]AVP binding assays and real-time PCR to investigate the \(V_{1a}\) expression and to fura 2 fluorescence to study [Ca\(^{2+}\)]\_i after AVP stimulation.

MATERIALS AND METHODS

Animals. Male SHR (Mol) and WKY (Mol) rats were obtained from the Møllegaard colony in Denmark (now Taconic M&B). Ani-
mals at 5, 10, 20, and 70 wk of age were studied, and the experiments were performed in a total number of 66 WKY and 66 SHR. Body weight varied between 160 and 180 g in the youngest group and 450 to 580 g in the oldest animals. Three animals were kept in each cage and were fed ordinary rat chow containing 0.25% sodium, 0.66% potassium, 0.71% calcium, and 14.7% crude protein and had free access to tap water. The experiments were performed with the approval of the Norwegian State Board for Biological Experiments with Living Animals.

**Hemodynamic measurements.** Animals were anesthetized by intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt). A polyethylene catheter was inserted into the left femoral artery for measurement of arterial blood pressure using a Hewlett-Packard pressure transducer connected to a Gould TA 4000 recorder. Renal blood flow (RBF) was recorded by a 2-mm flow probe on the left renal artery connected to a transit time flowmeter (Transonic) and a Gould recorder.

**Isolation of preglomerular resistance vessels for ligand binding and real-time PCR.** After anesthesia, a midline abdominal incision was made, and the abdominal aorta was cannulated below the renal arteries. The aorta was ligated above the renal arteries, the left renal vein was cut, and the kidneys were perfused with ice-cold PBS and thereafter with magnetic iron oxide re-suspended in PBS (1% wt/vol). The kidneys were excised and placed in ice-cold PBS. All subsequent steps of vessel isolation were performed on ice. Kidneys were decapsulated, and the cortex was isolated by dissection. Cortical tissue was minced with a razor blade and homogenized in a glass homogenizer with a loose-fist pestle (Kontes). Iron oxide-containing vessels were removed from the homogenate with a magnet, and the magnetic tissue was passed through 21- and 23-gauge needles and then filtered through a 125-μm mesh sieve. For RNA purification, vessels were passed through 25-gauge needles to get rid of tubular tissue. Microvessels recovered on the top of the sieve were resuspended in PBS, and iron oxide-containing vessels were extracted with a magnet, resuspended in collagenase-PBS (55 U/ml), and incubated for 30 min at 37°C. Vessels were isolated with a magnet, resuspended in ice-cold PBS, and passed through a 23-gauge needle. The tissues were resuspended in PBS and shaken to detach iron oxide from tissue. Iron oxide was removed with a magnet, and the cells were sonicated and centrifuged at 10,000 g for 30 min. Protein concentration in the recovered cell pellet was determined by a colorimetric method (3). Vessel isolation for real-time V_1a mRNA quantification was performed as described above. (To avoid RNase degradation of the mRNA, the isolation was stopped before the collagenase treatment.)

**[^3H]AVP binding assay.** Ligand binding was done in all groups. All reactions were performed in duplicate. Isolated microvessels (50 μg) were incubated in 250-μl incubation medium (50 mM Tris-HCl (pH 7.4), 5 mM MgCl_2, 0.3% bovine serum albumin and [^3H]AVP) at room temperature for 90 min. Bound and unbound ligand was then separated by centrifugation in a sucrose gradient; 0.2 ml of the incubated samples was layered over 0.2 ml assay-binding buffer containing 20% sucrose in 0.5 polyethylene microcentrifuge tubes. The tubes were centrifuged in a fixed angle rotor at 5,000 g for 30 min at 0–4°C. The tubes were then rapidly frozen in dry ice and the bound ligand-containing tips were cut with a guillotine. The isolated fractions were then assayed in a Packard Tri Car 4550 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. Specific binding of [^3H]AVP (5 nM) was linear with increasing amounts of protein added up to 50–100 μg protein. Incubations were performed at room temperature and in the presence of aprotinin (700 U/ml) to minimize the potential degradation by peptides. Specific [^3H]AVP (5 nM) to 50 μg protein took 30–45 min to reach equilibrium at 25°C.

Equilibrium saturation binding experiments were performed with increasing concentrations of [^3H]AVP between 0.125 and 4.0 nM. Specific binding was calculated as total binding minus nonspecific binding measured in the presence of 2.5 μM unlabeled AVP. The maximum specific binding (B_max) and dissociation constant (K_d) were calculated by using the Ligand software program (Biosoft). Each group of experiments consisted of at least three determinations each using fresh tissue preparations.

**Real-time PCR.** Quantitation of V_1a mRNA was done by real-time PCR in all groups except 20-wk-old WKY. All amplifications were done at the same time. First-strand cDNA was synthesized from isolated total RNA using a First-Strand cDNA Synthesis Kit (American Biosciences) and primed by pdN_6 primers. Primers for amplification of V_1a were selected for a 114-bp fragment containing the splicing site of the two V_1a exons. The forward primer was 5’-atgggtgcctggtggta-3’. The reverse primer was 5’-ctgattatccacaggctgg-3’. The Taqman probe was 5’-caactacggtgtctgtc-3’, marked with FAM and 3’ TAMRA. The amplified V_1a cDNA was normalized against amplified 18S ribosomal RNA to compensate for any changes due to RNA degradation, reverse transcriptase efficiency, or amplification success. The primers were made for a 68-bp fragment. The forward primer was 5’-agctgccctggcctgca-3’. The reverse primer was 5’-gggttgctgctgatggta-3’. The Taqman probe was 5’-aggacctgccttgagc-3’, marked with 5’ Yakima Yellow and 3’ TAMRA.

The amounts of V_1a and 18S were quantified using a standard curve for known quantities of V_1a or 18S DNA. The V_1a standard curve was done by amplifying a 1,125-bp region of the V_1a cDNA with the primers cctcggtggtcaatac (forward) and ctgcttggctctgcctgta (reverse). For the 18S standard curve, a 396-bp region of the rat 18S RNA cDNA was amplified using primers tcaagccccaggtgag (forward) and cggagctcatcttctgagaa (reverse). The amplification products were then cloned into pBAD TOPO TA vectors and transfected into TOP10 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, 10^9, 10^8, 10^7, and 10^6 molecules/μl for 18 S, and 10^10, 10^9, 10^8, and 10^7 molecules/μl for V_1a. 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 V_1a and 18S were used in a final concentration of 0.3 μM. Reverse primers for both V_1a 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 of cDNA solution was used as a template. All amplifications of both V_1a and 18S RNA were done using three parallel amplification reactions under standard ABI conditions using 19 μl of reaction volumes.

**[Ca^{2+}]_i measurements.** [Ca^{2+}]_i measurements were done in 5- and 70-wk-old WKY and SHR. The animals were anesthetized (50–70 mg/kg pentobarbital sodium), and the left kidney was perfused in vivo with 1.5 ml agarse solution (2% Seaprep agarose in RPMI, 37°C) after being washed with 5–10 ml warmed RPMI. The kidney was chilled (4°C for 10 min) after excision for the agar to solidify. Cortical slices of 100 μm were incubated for 30–60 min at 37°C in Ca^{2+}-free RPMI with collagenase IV, dispase II, and trypsin inhibitor to dissociate the microvessels from the renal tissue (29). Trypsin inhibitor was used to counteract V_1a cleavage from clostripain contamination of the collagenase IV (30, 40). The vessels were adhered to a microscope perfusion chamber and loaded in 5 μmol/l fura 2-acetoxymethyl ester (45 min) at room temperature.

**[Ca^{2+}]_o** was measured on isolated preglomerular vessels in a perfused renal chamber using a small window of the optical field of ×40 oil-immersion fluorescence objective of an inverted microscope (Olympus IX-70). The vessels were excited alternately with lights of 340- and 360-nm wavelengths from a dual-excitation wavelength.
system (Delta-Ram) from Photon Technologies International (PTI) with dual-monochrometers and a chopper (PTI). After signals were passed through a barrier filter (510 nm), fluorescence was detected by a photomultiplier tube. Signal intensity was stored and processed using Felix 1.21 software (PTI) on a PC. Calibration of free calcium concentration was based on the ratio of 340/380 nm (20). The calibration of \([Ca^{2+}]_o\), was based on the signal ratio at 340/380 nm and known concentration of calcium (23).

The microscope chamber 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. The solution switching between AVP and medium was done with a Valvebank8 II (AutoMate Scientific). \([Ca^{2+}]_i\), response was recorded when stimulating with \(10^{-7}\) M AVP from six to nine animals for each data point.

Chemicals. All chemicals used in this experiment were from Sigma, except fura-2 AM, which came from Molecular Probes. The RPMI media contained (in g/l) 17.65 NaCl, 0.40 KCl, 0.203 MgCl₂, 0.20 NaH₂PO₄, 1.34 HEPES, 1.0 glucose, 0.11 Na Pyruvat, 0.35 CaHCO₃, 0.22 CaCl₂, RPMI vitamins (Sigma R7256), and aminoacids (Sigma R7131).

Statistics. Data were presented as means ± SE. Sets of data were tested by ANOVA. \(P\) values of ≤0.05 were considered statistically significant.

RESULTS

The mean arterial blood pressure (MAP) increased from 120 ± 3 in 5-wk-old SHR to 184 ± 9 mmHg in 20-wk-old SHR (\(P < 0.01\)); thereafter, the blood pressure did not increase further (Fig. 1). In the 5-wk-old WKY animals, the MAP was 80 ± 2 and increased to 108 ± 6 mmHg in 20-wk-old animals (\(P < 0.01\)). Thereafter, the blood pressure was stable in the normotensive rats (Fig. 1).

RBF was 5.6 ± 0.2 in 5-wk-old SHR and 5.5 ± 0.3 ml·min⁻¹·g tissue⁻¹ in 5-wk-old WKY (\(P > 0.9\)). It increased to 8.2 ± 0.3 in 10-wk-old SHR (\(P < 0.01\)) and to 7.8 ± 0.4 ml·min⁻¹·g tissue⁻¹ (\(P < 0.05\)) in 10-wk-old WKY. There was no strain-specific difference (\(P > 0.4\)). In 20-wk-old SHR, RBF was 6.2 ± 0.8 and declined to 4.4 ± 0.4 ml·min⁻¹·g tissue⁻¹ in 70-wk-old SHR (\(P < 0.01\)). In 20-wk-old WKY, RBF was 7.0 ± 0.4 and declined to 4.6 ± 0.3 ml·min⁻¹·g tissue⁻¹ in 70-wk-old WKY (\(P < 0.05\)). The RBF was not significantly different between the two strains at 20 and 70 wk of age.

Age-related regulation of \(V_{1a}\) receptors was studied in WKY and SHR. The AVP/V₁a dissociation constant (\(K_d\)) was identical in all groups.

In 5- and 10-wk-old animals, receptor density was higher in SHR compared with age-matched WKY. Five- and 10-wk-old SHR had a significantly higher \(V_{1a}\) receptor density than WKY of all age groups (\(P < 0.05\)). In 5-wk-old SHR, receptor density was 84 ± 13, and it was 87 ± 6 fmol \(V_{1a}\)/mg protein in 10-wk-old SHR rats (\(P > 0.5\)). In 5-wk-old WKY, receptor density was 47 ± 3 and 52 ± 9 fmol/mg protein in 10-wk-old animals (\(P > 0.4\)). In 20- and 70-wk-old animals, there were no significant differences between SHR and WKY. It was 49 ± 1 in 20-wk-old SHR and 38 ± 11 fmol/mg protein in 70-wk-old SHR (\(P > 0.5\)). In WKY, the corresponding values were 51 ± 4 in 20- and 63 ± 8 fmol/mg proteins in 70-wk-old animals (\(P > 0.1\)). \(V_{1a}\) receptor density was similar at all ages of WKY. The receptor density was significantly higher in 5- and 10-wk-old SHR compared with older SHR (5 vs. 70 wk, \(P = 0.05\); 5 vs. 20 wk, \(P = 0.05\); 10 vs. 70 wk, \(P < 0.05\); Fig. 2).

The number of \(V_{1a}\) mRNA showed similar pattern of results as was seen in the ligand binding studies with significantly increased numbers of \(V_{1a}\) mRNA in 5- and 10-wk-old SHR compared with age-matched WKY rats (\(P < 0.01\)). In 5-wk-old SHR, mRNA for \(V_{1a}\) was 15,854 ± 447 and in 10-wk-old SHR it was 13,991 ± 881 \(V_{1a}\) mRNA/10⁸ ribosomal RNA (\(P > 0.2\)). The corresponding values for 5- and 10-wk-old WKY were 2,903 ± 447 and 3,108 ± 291 \(V_{1a}\) mRNA/10⁸ ribosomal RNA (\(P > 0.2\)). In 70-wk-old animals, there were no significant differences between SHR and WKY (\(P > 0.2\)). In 70-wk-old SHR, it was 3,181 ± 447 and it was 2,710 ± 447 \(V_{1a}\) mRNA/10⁸ ribosomal RNA in 70-wk-old WKY. There was no significant difference in mRNA for \(V_{1a}\) among WKY groups. mRNA for the \(V_{1a}\) receptor was significantly
higher in 5- and 10-wk-old SHR compared with 70-wk-old SHR (5 vs. 70 wk, \( P < 0.001 \); 5 vs. 20 wk, \( P < 0.001 \); 10 vs. 70 wk, \( P < 0.001 \); 10 vs. 20 wk, \( P < 0.001 \); Fig. 3).

Studies on the change in \([\text{Ca}^{2+}]_i\) during stimulation with AVP were performed on 5- and 70-wk-old animals. Figure 4 shows three typical responses in isolated preglomerular vessels after stimulation with AVP in all four groups. The baseline in \([\text{Ca}^{2+}]_i\), was not significantly different, and the \([\text{Ca}^{2+}]_i\) response showed a sharp initial peak followed by a stable plateau that was maintained until the agonist was removed. The initial peak response was significantly higher in 5-wk-old SHR compared with old SHR as well as young and old WKY (Fig. 5). The increase was 234 ± 59 nM \([\text{Ca}^{2+}]_i\) in young SHR, whereas...
it was 89 ± 9 nM Ca\(^{2+}\) in 70-wk-old SHR (P = 0.03). The [Ca\(^{2+}\)] response was higher in 5-wk-old SHR than in 5-wk-old WKY (115 ± 10 nM) and 70-wk-old WKY (82 ± 14 nM; P < 0.03). There was no difference in the [Ca\(^{2+}\)] response in 5- and 70-wk-old WKY (P = 0.09), and there was no difference in [Ca\(^{2+}\)] response in 70-wk-old WKY and SHR (P > 0.2).

The plateau level of [Ca\(^{2+}\)] recorded 200 s after stimulation was not significantly different among the groups (SHR 5 wk: 58 ± 16 nM, SHR 70 wk: 25 ± 7 nM, WKY 5 wk: 57 ± 7 nM, WKY 70 wk: 45 ± 10 nM; SHR 5 wk vs. SHR 70 wk, P > 0.11; SHR 5 wk vs. WKY all ages, P > 0.5).

**DISCUSSION**

The main new observation that emerges from the present study is that mRNA and receptor proteins for the V\(_1\alpha\) receptor are increased until the age of 10 wk in SHR. At higher ages, the receptor is present in the same amount as in WKY rats where the density of the V\(_1\alpha\) receptor stays stable throughout life. A new finding is also the decrease in V\(_1\alpha\) mRNA after 10 wk of age in SHR. A striking observation in the present study is the parallel change in V\(_1\alpha\) mRNA and receptor proteins in SHR throughout life. A new finding is also that the exaggerated intracellular calcium response after stimulation with AVP found in 5-wk-old SHR is normalized in 70-wk-old SHR. In WKY rats, the magnitude of the intracellular calcium response was similar in both young and old animals.

The result in the present paper confirms previous studies showing an increased V\(_1\alpha\) expression at mRNA and receptor protein level in renal preglomerular resistance vessels of young SHR compared with age-matched WKY. The functional consequence of increased receptor density has been demonstrated by an enhanced vasoconstriction in the renal vessels after injection of AVP into the renal artery in 5- to 7-wk-old SHR (15). Specific antagonists are able to block this response. In addition, exaggerated intracellular calcium signaling has been shown in isolated smooth muscle cells from preglomerular vessels in young SHR, a response that can be inhibited by a specific antagonist (23). Observations from older animals are few. In an earlier study, we found that infusion of AVP reduced RBF more in young SHR than in 40-wk-old SHR, whereas there was no difference between young and old WKY (10). Gene expression has not been examined in old animals before and, collectively, our data represent the first comprehensive study where the gene expression and receptor protein for V\(_1\alpha\) are studied throughout life in WKY and SHR and where the results are supported by the functional studies demonstrating responses of intracellular calcium signaling that are strictly dependent on level of V\(_1\alpha\) receptor density.

The role of the V\(_1\alpha\) receptor in preglomerular vessels during development of hypertension is unknown. A striking observation in the present study is the normalization of the receptor level when the blood pressure has stabilized and suggests a relationship between receptor level and blood pressure. This finding explains why long-time treatment with V\(_1\alpha\) receptor antagonists in young SHR ameliorates the development of hypertension, while this effect was not seen in older SHR (4). It has earlier been shown that SHR retains salt and water at 5–6 wk of age and the kidneys of these rats are vasoconstricted. Constriction of the afferent arterioles seems to persist at least 10–12 wk of age (24), whereas the afferent arteriolar diameter increases with age during adaptation to nephron loss in older SHR (24). The total RBF is usually similar in both WKY and SHR as shown in the present study. It is, however, well known that the renal vasculature is highly responsive to both ANG II and AVP and the enhanced vasoactivity that is higher in SHR than in WKY may depend on several mechanisms such as excitation-contraction coupling related to receptor types, but receptor densities may also be of major importance. Structural changes in the vessels may play a larger role in the vascular reactivity in old compared with young animals (17). In our previous work, we demonstrated similar vasoactivity in 40-wk-old WKY and SHR during injection of AVP into the renal artery (10). This finding was unexpected as the structural differences are obvious in these two strains at this age. The normalized vasoactivity to AVP in 40-wk-old SHR may be explained by decreased density of the V\(_1\alpha\) receptor in these animals. Normalization of the V\(_1\alpha\) density between the age of 10 and 20 wk is supported by Gao et al. (18). These authors found the V\(_1\alpha\) receptor to be equally expressed in 16-wk-old SHR and WKY using Western blotting, a finding that supports the data from the present study where decreased density of V\(_1\alpha\) receptor in SHR seems to take place between 10 and 20 wk of age.

Previous work showed that two-thirds of the vasoconstrictive response of AVP is due to IP\(_3\)-mediated Ca\(^{2+}\) store mobilization and one-third is due to Ca\(^{2+}\) influx through L-type calcium channels (14). This is similar for both SHR and normotensive WKY and argues against a defect in the V\(_1\alpha\) signal transduction. It is therefore reasonable to suggest that the increased calcium response in young SHR is due to the increased density of V\(_1\alpha\) receptors. The increased calcium response in young SHR has been demonstrated before, although the shape of the curve in the present study is different from what we showed earlier (23). This might be due to temperature differences as the present study was performed at 37°C while earlier work was done at 4°C. Temperature dependence of the calcium response has been shown before, although the reason is not clear (44). In support of the receptor-dependent response is also the finding of normalized intracellular response in 70-wk-old SHR, where the receptor level was found to be similar as in WKY. The difference in calcium increase was seen only in the initial peak while we were not able to see any difference in the maintained plateau activation in neither the young nor the old animals. The maintained plateau response is mainly dependent on calcium entry mechanisms; one may speculate that entry mechanisms are not changed with age in SHR and may not be different from WKY. It should, however, be added that mechanisms other than receptor density could play a role. Growth factors are known to increase with age, and Bouillier et al. (2) found an increased ANG II-induced Ca\(^{2+}\) response after pretreatment of smooth muscle cells with transforming growth factor-β. This increase was related to tyrosine kinase and not to increased receptor density.

Sequencing the V\(_1\alpha\) gene of rat and sheep displayed the V\(_1\alpha\) promoter to have a structure typical of housekeeping genes with the lack of both TATA and CCAAT boxes and a high GC contents (28, 31). The density of the receptor is, however, tightly regulated to specific tissue types and organs. The renal and hepatic V\(_1\alpha\) receptor density in Sprague-Dawley rats is also found to be developmentally regulated (36). The reason why
the V1α receptor density is changed with age is not known. We recently showed the hepatic and renal V1α receptors to be regulated by the systemic AVP hormone concentration, with high AVP concentrations eliciting V1α decreased receptor density and low concentrations of AVP eliciting V1α an increase in numbers of receptor (unpublished observations). This is common for G protein-coupled receptors. In Sprague-Dawley rats, AVP-mediated vasoconstriction in peripheral nerve blood vessels is shown to have an age-related decline (26). Aging Sprague-Dawley rats had a significantly higher systemic AVP concentration compared with young animals (26). The decreased vasoresponsiveness in aged Sprague-Dawley rats could therefore be due to the raised AVP concentration. Risvanis et al. (41) found a reduced density of hepatic V1α receptors in DOCA-salt-hypertensive rats compared with salt- and water-controlled rats. The mRNA level was unchanged compared with the control animals. The changes in hepatic V1α receptors were associated by an increased plasma vasopressin concentration. This regulation may be at the protein level, probably by internalization due to the increased receptor stimulation. Renal tubular tissue from old female Wag/Rij rats is shown to have a reduced cAMP response compared with adult animals after AVP stimulation. The V2 receptor density is age dependent decreased even though the plasma AVP concentration is unchanged (27). Plasma AVP is difficult to measure due to the fact that only minor treatment may stress the animals to raise the hormonal level. Studies presenting plasma AVP concentrations are therefore contradictory. Hosoya et al. (22) found the plasma AVP concentration in young WKY and SHR to be almost equal, 1.5 ± 0.09 and 1.0 ± 0.08 pg/ml SHR and WKY, respectively. In 12-wk-old rats, the SHR plasma AVP concentration was increased compared with age-matched WKY, 21.3 ± 8.8 and 2.8 ± 0.2 pg/ml in SHR and WKY, respectively. This is different from Ghaemmaghami et al. (19), who found an equal plasma AVP concentration in adult SHR and WKY. The increased V1α density in young SHR therefore seems to be unrelated to the level of plasma AVP. One may speculate that events taken place during pregnancy could explain the increased density of V1α in early life of SHR. It has, for example, been shown that hypoxia in the uterus is linked to increase in receptor numbers of endothelin receptors that induce pulmonary hypertension in adulthood (21, 39, 43). The fact that the V1α receptor density is increased in SHR at a very young age and before the establishment of hypertension makes it possible that this receptor increase might be due to in utero conditions.

In addition to regulation of receptor protein through transcription and translation, G protein-coupled receptors can also be regulated at the protein level by receptor desensitization and internalization. The stimulated receptor is desensitized through PKC phosphorylation and uncoupling from G proteins by the intracellular protein β-arrestins (16, 47). This protein will also target the receptor to clathrin-coated pits. Internalized receptors will either be recycled or degraded (16, 34). Our results show V1α mRNA and protein to be regulated in the same manner in both young and old rats. This is in accordance with the results from our previous study with water-loaded and dehydrated animals (unpublished observations). Other studies with dehydrated rats showed the same pattern of regulation for both V1α and V2 receptor regulation (37, 45). The regulation seems to be done at the mRNA level. This could be done through direct transcription regulations and mRNA stability. Both cyclosporin A treatment and glucocorticoids are known to increase V1α density (11, 32) with an increase in both V1α receptor protein and mRNA numbers. The mRNA increase in both of these occasions is shown to be due to an increased mRNA stabilization and not increased transcription.

In conclusion, the present study shows that receptor protein and mRNA for the V1α receptor are increased until the age of 10 wk in SHR, but later on the levels of receptor are normalized. Intracellular calcium signaling is strictly dependent on receptor level. The density of V1α receptors is increased during development of increased blood pressure and normalized when hypertension is established, and these findings suggest a link between development of hypertension and numbers of V1α receptors in SHR.

REFERENCES


