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The Dual AngII/AVP Receptor Gene N119S/C163R Variant Exhibits Sodium-Induced Dysfunction and Cosegregates With Salt-Sensitive Hypertension in the Dahl Salt-Sensitive Hypertensive Rat Model

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Abstract

Background: Essential hypertension is a prevalent complex polygenic disease and a major risk factor for cardiovascular disease, the leading cause of death in developed countries. Because of its complex and multifactorial nature, its genetic determinants still remain largely unknown. The Dahl salt-sensitive hypertensive rat model exhibits impaired sodium handling, which is hypothesized to play a key role in the pathophysiology of polygenic hypertension. Thus, genes associated with renal regulation of salt and water balance are a priori likely candidates for a causative role in hypertension pathogenesis. The functional properties and renal-specific expression of the recently characterized AngII/AVP receptor suggest a putative modulator role in tubular sodium and fluid reabsorption. Based on these observations, we investigated the potential involvement of the AngII/AVP receptor in salt-sensitive hypertension.
Materials and Methods: We performed cosegregation analysis of the AngII/AVP receptor locus with salt-sensitive hypertension in an F2 (Dahl S × Dahl salt-resistant [R]) hybrid male cohort characterized for blood pressure by radiotelemetry after 8 weeks of high salt challenge. Further molecular analysis was done to identify putative AngII/AVP receptor molecular variants that could account for the AngII/AVP receptor involvement in salt-sensitive hypertension pathogenesis.
Results: The AngII/AVP receptor was mapped to rat chromosome 1, 1.7 cM centromeric to the *D1Rat188* marker by radiation hybrid mapping analysis. Quantitative trait locus (QTL) analysis detected a highly significant linkage of the AngII/AVP receptor locus with high blood pressure (LRS = 13.8, $p = 0.0002$). Molecular characterization of the Dahl S and Dahl R AngII/AVP receptor cDNAs revealed two amino acid substitutions in the Dahl S AngII/AVP receptor (N119S, C163R) when compared to the Dahl R AngII/AVP receptor. These mutations are associated with an increased receptor affinity for both ligands (AVP and AngII) and an enhanced G_s-coupling by the receptor resulting in increased activation of adenylate cyclase with concomitant increase in cAMP production.
Conclusions: The observed molecular dysfunction in the Dahl S AngII/AVP receptor is consistent with increased tubular sodium and fluid reabsorption observed in Dahl S rats. Interestingly, the AngII/AVPr locus is within the narrowed chromosome 1 QTL region for blood pressure detected in different rat intercross linkage analyses. Altogether, the data strongly suggest that the AngII/AVP receptor is a hypertension susceptibility gene in the Dahl S rat model, as well as raises the hypothesis that it too underlies the chromosome 1 blood pressure QTL identified in other hypertension rat models.

Introduction

The kidney plays a primary role in hypertension pathogenesis in the Dahl salt-sensitive hypertensive rat model (1–3). Transplant studies have shown that the donor kidney determines the blood pressure phenotype in the bilaterally nephrectomized recipient (3). A similar situation has also been described in human renal transplant patients (3). These facts validate the analysis of genes expressed preferentially within the kidney as logical candidates for hypertension-susceptibility genes. Because the AngII/AVP receptor is prominently expressed in renal epithelial cells (4,5), and both AngII and AVP hormones are both known to modulate tubular sodium and fluid reabsorption (6–14), we investigated whether this receptor could have a potential role in hypertension pathogenesis. The AngII/AVP receptor is coupled to adenylate cyclase and responds with equal sensitivity to AngII and AVP (4). Pharmacologic characterization defines this dual receptor as a novel AT_1/V_2 type of receptor (5). These receptor properties, in conjunction with its renal immunocytochemical distribution to the outer medullary thick ascending limb tubules and inner medullary collecting ducts (5), suggest that the AngII/AVP receptor could play a prominent role in renal tubular sodium and fluid reabsorption. Thus, it is intriguing to hypothesize that this dual receptor could mediate the coordination of two distinct blood pressure regulatory systems.

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To assess the putative role of the AngII/AVP receptor in hypertension pathogenesis, we performed genetic and molecular analyses in the Dahl S/Dahl R rat genetic model of salt-sensitive hypertension. The results reported here strongly suggest that the AngII/AVP receptor is a hypertension-susceptibility gene in the Dahl S rat model, thus providing impetus for the future investigation of its potential role in human essential hypertension.

Materials and Methods

Chromosomal Localization of the AngII/AVP Receptor Gene

To localize the AngII/AVP receptor gene within the rat genome, we conducted a radiation hybrid panel screening on a rat panel (Rat/Hamster RH panel RH07) obtained from Research Genetics (Huntsville, AL, USA). A 479-bp polymerase chain reaction (PCR) fragment (upstream primer: 5′-gtt-aca-gag-ctg-agg-gcc-tg-3′; downstream primer: 5′-cct-gga-aac-cac-act-cac-ct-3′) from the 5′ regulatory region of the rat AngII/AVP receptor gene was utilized as indicator product. The data were then tested against the existing framework maps. The results assign the AngII/AVP receptor gene to chromosome 1, 14.6 cR from *D1Rat111* with a LOD = 24.673. This places the AngII/AVP receptor gene approximately 1.7 cM centromeric to *D1Rat188* (Table 1).

Intercross Linkage Analysis

The development of the (Dahl S/Hsd × Dahl R/Hsd) F2 male cohort and phenotypic characterization has been described previously (15). Blood pressure measurements were obtained by radiotelemetry after 8 weeks of high salt (8% NaCl) challenge. Genotyping was performed using the following microsatellite markers: *D1Rat68, D1Rat129, D1Rat188, D1Mgh11, D1Rat298, D1Rat371, D1Rat77, D1Rat304,* and *D1Rat122* obtained from Research Genetics. Linkage maps and QTL analysis were done with the Map Manager QTXb08 (MMQTX08) program for windows (16), which generates a likelihood ratio statistic (LRS) as a measure of the significance of a possible QTL. Genetic distances were calculated using Kosambi mapping function (genetic distances are expressed in cM) and were as follows: *D1Rat68* – (7.7, LOD = 25.0) – *D1Rat129* – (6.6, LOD = 27.3) – *D1Rat188* – (0.5, LOD = 44.2) – *D1Mgh11* – (10.5, LOD = 21.8) – *D1Rat298* – (7.2, LOD = 28.3) – *D1Rat371* – (4.5, LOD = 34.2) – *D1Rat77* – (4.0, LOD = 33.8) – *D1Rat304* – (11.5, LOD = 20.4) – *D1Rat122.* Critical significance (LRS) values for interval mapping were determined by a permutation test (2000 permutations at 5-cM intervals) on our 105 informative progeny using Kosambi mapping function and a constrained additive regression model. Thus, the minimum LRS values were 1.4 for suggestive, 6.6 for significant, and 12.2 for highly significant.

cDNA Library Screening and Nucleotide Sequencing

Dahl S/JR and Dahl R/JR rat kidney λgt11 cDNA libraries (17) were screened with the full-length Sprague-Dawley AngII/AVP receptor cDNA (4). Several Dahl S and Dahl R AngII/AVP receptor

Table 1. QTL analysis of rat chromosome 1 for blood pressure in the F2 (Dahl S × Dahl R) intercross

	SBP			DBP			MAP		
Locus (Distance, cM)	**LRS**	**%**	***p***	**LRS**	**%**	***p***	**LRS**	**%**	***p***
D1Rat68 (0.0)	7.1	6	0.00756	5.5	4	0.01926	7.0	6	0.00802
D1Rat129 (7.7)	11.0	9	0.00090	6.9	6	0.00839	10.2	9	0.00138
AngII/AVPr **(4.9)**									
D1Rat188 (1.7)	13.8	12	0.00020	8.6	7	0.00336	12.4	10	0.00043
D1Mgh11 (0.5)	13.6	11	0.00023	8.8	7	0.00298	12.4	10	0.00042
D1Rat298 (10.5)	5.4	4	0.02047	3.1	2	0.07786	4.8	4	0.02921
D1Rat371 (7.2)	3.1	2	0.07920	1.8	1	0.18290	2.9	2	0.08596
D1Rat77 (4.5)	2.7	2	0.09800	1.4	0	0.23236	2.5	1	0.11497
D1Rat304 (4.0)	2.2	1	0.13659	0.6	0	0.43679	1.6	1	0.20576
D1Rat122 (11.5)	1.1	0	0.30434	0.2	0	0.67527	0.6	0	0.44267

BP, blood pressure; SBP, systolic; DBP, diastolic; MAP, mean arterial pressure; LRS, likelihood ratio statistic for the association of the trait with loci; %, the amount of the total trait variance that would be explained by a QTL at these loci, as a percent; *p*, probability value for the association.

Statistical values presented are derived from regression analysis based on an additive model of inheritance using the MapManager QTXb08 program. The additive model fitted best, thus defining the dominance properties of the QTL. Genetic distances are presented in parenthesis in cM. The localization of the AngII/AVP receptor gene. (1.7 cM from *D1Rat188,* as determined by radiation hybrid mapping) is highlighted.

cDNAs were isolated. A Dahl S cDNA (~1.9 kb) and a Dahl R cDNA (~1.6 kb) were subcloned into psp73 transcription vector (Promega, Madison, WI, USA) and sequenced in their entirety by the Sanger dideoxy-chain termination sequencing method using Sequenase (U.S. Biochemical, Cleveland, OH, USA) as described previously (17). All sequences were confirmed on both strands.

Detection of the N119S and C163R Mutations by Using the Amplification Refractory Mutation System (ARMS)

The ARMS was performed using genomic DNA isolated from three Dahl S/Hsd and two Dahl R/Hsd rats essentially as described (18). The downstream primer (5′-tgc-aca-ctt-cct-gcg-ga-**AC**-3′, Dahl S specific) specific for the N119S mutation was "destabilized" by introducing a G/A transition 1 nucleotide upstream of the 3′-OH mismatched C residue. Detection of allele-specific amplified products (102 bp) was done by ^{32}P-end labeling the upstream primer (5′-cac-cta-cca-gtt-cat-tga-cc-3′) and subsequent size fractionation on a 6% denaturing polyacrylamide gel. The optimal stringent PCR cycling conditions experimentally determined for the N119S mutation detection were as follows: 95°C for 10 min; 35 cycles of 94°C × 30 sec, 57°C × 30 sec, 72°C × 1 min; and extension at 72°C × 7 min. The downstream primer (5′-cac-gcc-ctg-gca-cca-ca-**TG**-3′, Dahl S specific) specific for the C163R mutation was "destabilized" by introducing a C/T transition 1 nucleotide upstream of the 3′-OH mismatched G residue. Detection of allele-specific amplified products (131 bp) was done by ^{32}P-end labeling the upstream primer (5′-atg-ctc-ctg-aac-tct-gac-g-3′) and subsequent size fractionation on a 6% denaturing polyacrylamide gel. The optimal stringent PCR cycling conditions experimentally determined for the C163R mutation detection were as follows: 95°C for 10 min; 35 cycles of 94°C × 30 sec, 55°C × 30 sec, 72°C × 1 min; and extension at 72°C × 7 min. Reaction volumes were 10 μL, using 0.05 μmol/L of each primer for the C163R substitution and 0.6 μmol/L of each primer for the N119S substitution, 0.5 U/10 μL of AmpliTaq Gold (Perkin-Elmer, Branchburg, NJ, USA), 1.5 mM $MgCl_2$, and 0.2 μg of genomic DNA. As control, the *D1Mit13* marker (320 bp, not polymorphic between Dahl S and Dahl R strains) was used to assess equivalent amounts of genomic DNA in the different samples. To demonstrate equivalent amounts of cloned Dahl S and Dahl R AngII/AVPr cDNAs, we used a 260-bp PCR product encompassing nucleotides 378-638 (forward primer: 5′-gtt-cat-tga-cca-gag-ctt-cc-3′; reverse primer: 5′-ctt-tgt-cct-tgt-acc-cac-c-3′) of the published Sprague-Dawley AngII/AVPr cDNA (4).

Functional Analysis

The Dahl S and Dahl R AngII/AVP receptor cDNAs were subcloned directionally (5′ to 3′) into the NheI site of the pMAMNeo expression vector (Clontech, Palo Alto, CA, USA). Stable Cos1 neomycin-resistant transfectants were developed as described (4). ^{125}I-AngII and ^{3}H-AVP binding experiments were preformed essentially as described (4) on intact Cos1 pMAM-Dahl S AngII/ AVPr and Cos1 pMAM-Dahl R AngII/AVPr cell transfectants. Specific binding was determined as the difference between the total radioactivity bound to cells and the radioactivity bound to blanks containing 1 μM of AngII or 10 μM of AVP. AngII- and AVP-dependent stimulation of cAMP accumulation was assayed on C127 cell membranes after transient transfection of C127 cells (mouse mammary tumor cell line, ATCC) with the pMAM-Dahl S AngII/AVPr and the pMAM-Dahl R AngII/AVPr expression vectors. For this purpose, the pMAM-Dahl S AngII/AVP receptor and the pMAM-Dahl R AngII/AVP receptor expression vectors (60 μg each) were introduced into C127 cells (10^7 cells) via lipofectin-mediated transfection. After 5 days in culture, cells were harvested and a crude membrane preparation was obtained as described (19). 10 μg of Dahl S and Dahl R AngII/AVP receptor expressing membranes were exposed to 0.1 μM of AVP and 0.1 μM of AngII at 25°C for 20 min with 0, 50, and 150 mM of NaCl, in comparison to basal control (no hormone added) in an incubation buffer containing 40 mM of Tris-HCl (pH 7.6), 5 mM of $MgCl_2$, 1 mM of EDTA, 2 mM of isobutylmethylxanthine, 3 mM of ATP, 20 mM of phosphocreatine, 5 U/ml of creatinephosphokinase, 0.1 μM of GTP, and 0.1% BSA. The level of cAMP was determined by radioimmunoassay according to manufacturer's specifications (Amersham, Piscataway, NJ, USA).

Results

Intercross Linkage Analysis

To assess the potential genetic contribution of the AngII/AVP receptor gene to salt-sensitive hypertension susceptibility, we performed an intercross linkage analysis on 105 F2 (Dahl S ♂ × Dahl R ♀) hybrid male rats phenotyped for BP by radiotelemetry after 8 weeks of high salt (8% NaCl) challenge (15). We localized the AngII/AVP receptor gene to rat chromosome 1, 1.7 cM centromeric to the *D1Rat188* marker (see Table 1) by radiation hybrid mapping analysis. Thus, 105 F2 male hybrids were genotyped at nine informative markers that spanned the AngII/AVP receptor locus on chromosome 1. As seen in Table 1, LRS and p values peaked at *D1Rat188* (the closest marker, 1.7 cM to the AngII/AVP receptor locus) for SBP (LRS = 13.8, $p = 0.00020$), DBP (LRS = 8.6, $p = 0.00336$), and MAP (LRS = 12.4, $p = 0.00043$). These results fulfill the first of four criteria needed to identify a hypertension susceptibility gene (15,20): 1) association of the putative hypertension susceptibility gene with hypertension in validated genetic animal models or human hypertensive patients, 2) identification of a functionally significant structural mutation in the relevant gene, 3) concordance of the observed molecular dysfunction with a pathophysiologic mechanism logical to the

hypertension pathogenesis, and 4) delineation of the mechanistic role in an *in vivo* model. Based on a permutation test performed on the 105 informative progeny that established the minimum LRS value for highly significant as equal to 12.2, the AngII/AVPr locus exhibits a highly significant linkage with SBP (LRS = 13.8). Analysis for interaction with α1 Na,K-ATPase, bumetanide-sensitive Na,K,2Cl-cotransporter and thiazide-sensitive Na, Cl-cotransporter loci were negative. As shown in Table 1, the AngII/AVP receptor locus accounts for 12% of the SBP variance.

Molecular Analysis

To identify molecular variants of the AngII/AVP receptor that could account for the cosegregation of the AngII/AVP receptor locus with salt-sensitive hypertension in the Dahl S rat model, we screened Dahl S and Dahl R rat kidney λgt11 cDNA libraries with the full-length Sprague-Dawley AngII/AVP receptor cDNA (4) as probe. Several cDNA clones were isolated and characterized. The entire amino acid coding region for both Dahl R and Dahl S receptors were obtained, revealing three nucleotide differences between the Dahl R and Dahl S AngII/AVP receptor cDNAs (Fig. 1). Two of the nucleotide changes resulted in amino acid substitutions: A^{356} (Dahl R) $\rightarrow$ G^{356} (Dahl S) nucleotide transition resulting in N119S substitution localized in the first intracellular loop (Fig. 1b); T^{487} (Dahl R) $\rightarrow$ C^{487} (Dahl S) nucleotide transition resulting in C163R substitution localized to the second extracellular loop (Fig. 1b). The third A^{627} (Dahl R) $\rightarrow$ G^{627} (Dahl S) nucleotide transition did not change the encoded amino acid. The nucleotide sequence encompassing the entire amino acid coding region of the Dahl R cDNA was identical to the previously reported Sprague-Dawley AngII/AVP receptor cDNA (4). The presence of N119S and C163R mutations in Dahl S and its absence in Dahl R rat genomic DNA was corroborated by error-independent amplification refractory mutation system (ARMS) detecting G^{356} and C^{487} in Dahl S in contrast to non-G^{356} and non-C^{487} in Dahl R rat genomic DNA, respectively (Fig. 1c).

To elucidate potential functional differences between the Dahl S and Dahl R AngII/AVP receptors, both molecular variants were expressed in tissue culture and assayed in ligand binding experiments and hormone-dependent activation of adenylate cyclase. For this purpose, the Dahl S and Dahl R cDNA clones containing the full-length amino acid coding region of the AngII/AVP receptor were subcloned directionally (5′→3′) into the NheI site of the pMAM expression vector (Clontech). Both expression vectors (pMAM-Dahl R AngII/AVPr and pMAM-Dahl S AngII/AVPr) were then permanently transfected into Cos1 cells, and subsequently assayed for receptor function. Saturation binding studies using ^{125}I-AngII and ^{3}H-AVP on intact pMAM-Dahl S and pMAM-Dahl R AngII/AVPr cell transfectants revealed significant differences in affinities for ^{3}H-AVP and ^{125}I-AngII binding between the Dahl S and Dahl R receptors (Fig. 2 and Table 2). Scatchard analysis of ^{125}I-AngII and ^{3}H-AVP saturation binding revealed 4.9-fold and 2.3-fold higher affinity of the Dahl S receptor for ^{125}I-AngII and ^{3}H-AVP, respectively, when compared with the Dahl R AngII/AVP receptor (Table 2). We notice that the Dahl S receptor shows two distinct AngII binding sites, a high-affinity binding site ($K_H = 0.88 \pm 0.38$ nM), presumably reflecting binding sites of receptors couple to G-protein, and a low-affinity binding site ($K_L = 8.90 \pm 2.31$ nM) representing binding sites of receptors not coupled to G-protein. In contrast, the Dahl R receptors have a single AngII binding site ($K = 4.30 \pm 0.99$ nM), further implicating a differential coupling of the Dahl R receptor to G protein.

To assess putative additional functional differences between the Dahl S and Dahl R AngII/AVP receptors, both expression vectors (pMAM-Dahl S AngII/AVPr and pMAM-Dahl R AngII/AVPr) were transfected into C127 cells (mouse mammary tumor cell line [ATCC]), respectively, and membranes were isolated from transient transfectants and assayed for AVP- and AngII-induced cAMP accumulation. In earlier xenopus oocyte microinjection expression experiments, we noticed a pronounced effect of NaCl on the AngII-induced cAMP accumulation in isolated oocyte membrane preparations (unpublished results). Thus, we evaluated the effect of different NaCl concentrations on the AVP- and AngII-induced cAMP accumulation by the Dahl S and Dahl R receptors. As shown in Figure 3, striking differences can be observed between the Dahl S and Dahl R expressed AngII/AVP receptors. First, upon addition of 50 mM of NaCl, the AVP-induced cAMP accumulation increased by 14% over control levels (at 0 mM of NaCl) in Dahl R AngII/AVP receptor expressing membranes (from 150 ± 7 pmol/mg Mb to 171 ± 8 pmol/mg Mb). In contrast, the addition of 50 mM of NaCl produced an exaggerated response with a far greater increase (182%) in AVP-induced cAMP accumulation in Dahl S AngII/AVP receptor expressing membranes over control levels (0 mM of NaCl; from 123 ± 6 pmol/mg Mb to 347 ± 12 pmol/mg Mb, Fig. 3a). Similarly, a greater response (209%) to AngII was also observed in Dahl S versus Dahl R AngII/AVP receptor expressing membranes in the presence of 50 mM of NaCl (170 ± 11 pmol/mg Mb versus 55 ± 4 pmol/mg Mb, Fig. 3b), respectively. This differential response is not due to differences in receptor density; similar, if not higher, AVP response (123 ± 6 pmol/mg Mb in Dahl S versus 150 ± 7 pmol/mg Mb in Dahl R membranes) can be observed in Dahl R AngII/AVP receptor expressing membranes under control conditions (at 0 mM NaCl; Fig. 3).

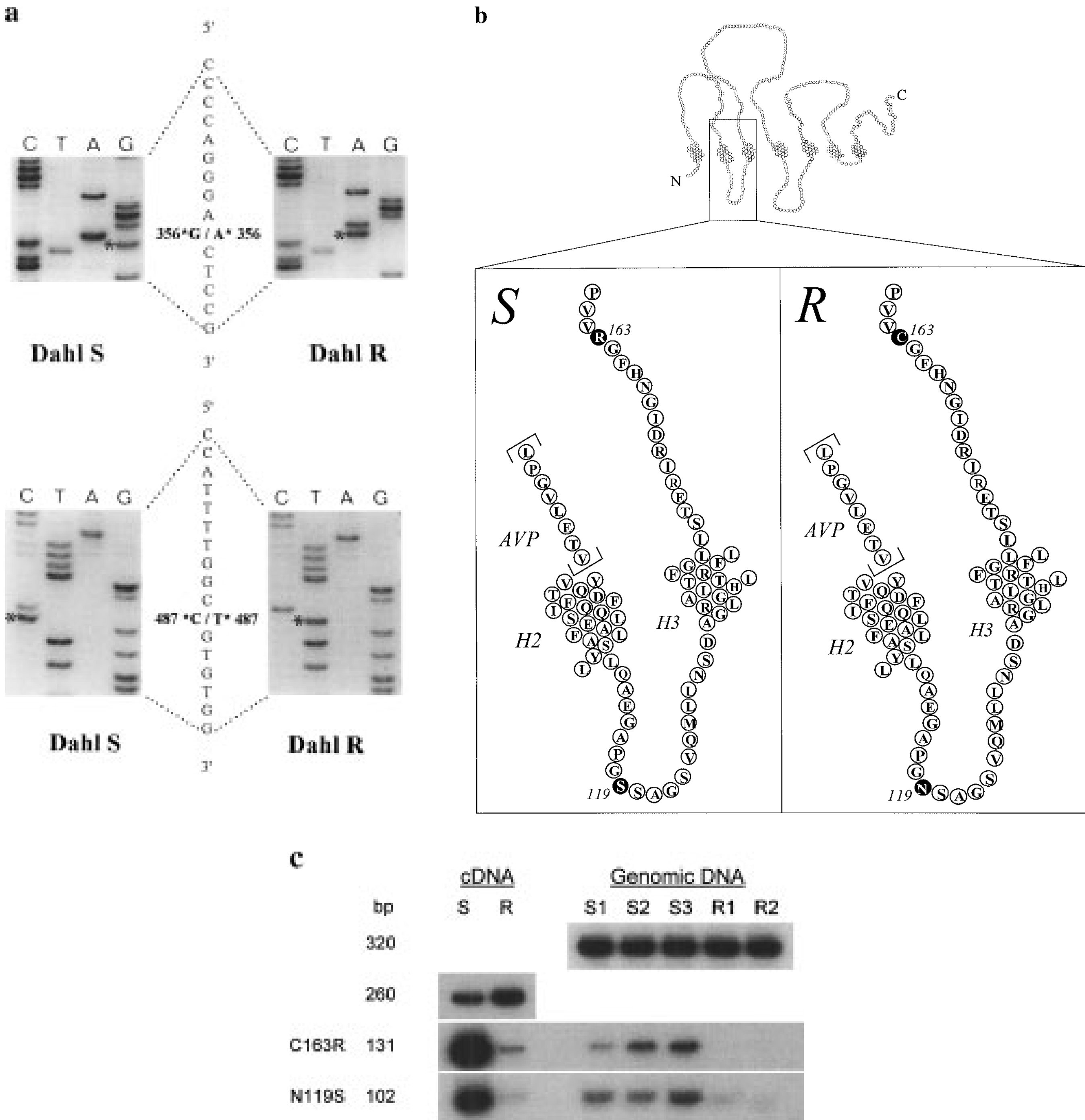

Fig. 1. Comparative analysis of nucleotide and deduced amino acid sequences of Dahl S and Dahl R AngII/AVP receptor cDNAs. **(a)** Sequencing gels of Dahl S and Dahl R cDNAs spanning the $A^{356} \rightarrow G^{356}$ (Dahl R → Dahl S) nucleotide transition that results in a N119 substitution in Dahl S AngII/AVP receptor for S119 and $T^{487} \rightarrow C^{487}$ (Dahl R → Dahl S) nucleotide transition that results in a C163 substitution in Dahl S AngII/AVP receptor for R163. Nucleotide and amino acid numbering as per Ruiz-Opazo et al (4). Specific nucleotides (C, T, A, G) and 5′ to 3′ directions are indicated. **(b)** Schematic structure of the AngII/AVP receptor in the region around transmembrane domains H2 and H3. Open circles indicate amino acids common to both Dahl S and Dahl R AngII/AVP receptors. Black circles highlight the amino acids involved in the substitutions. **(c)** Detection of the N119S and C163R mutations in genomic DNA by using the amplification refractory mutation system (ARMS). ARMS analysis was designed to detect the Dahl S AngII/AVP receptor gene variant, when present, by the production of an expected 102-bp (for the N119S substitution) and a 131-bp (for the C163R substitution) product, respectively. Analysis was done on three Dahl S (S1, S2, and S3) and two Dahl R (R1 and R2) genomic DNAs. Controls for the ARMS analysis were cloned Dahl S (S) and cloned Dahl R (R) AngII/AVPr cDNAs at four copies per genome equivalent. The 260-bp fragment corresponds to control PCR product showing equivalent amounts of cloned Dahl S and Dahl R AngII/AVPr cDNAs. The 320-bp fragment corresponds to control PCR product ascertaining that all genomic DNA samples amplify equivalently. The ARMS test readily detects G^{356} and C^{487} in Dahl S genomic DNA and their absence in Dahl R genomic DNA.

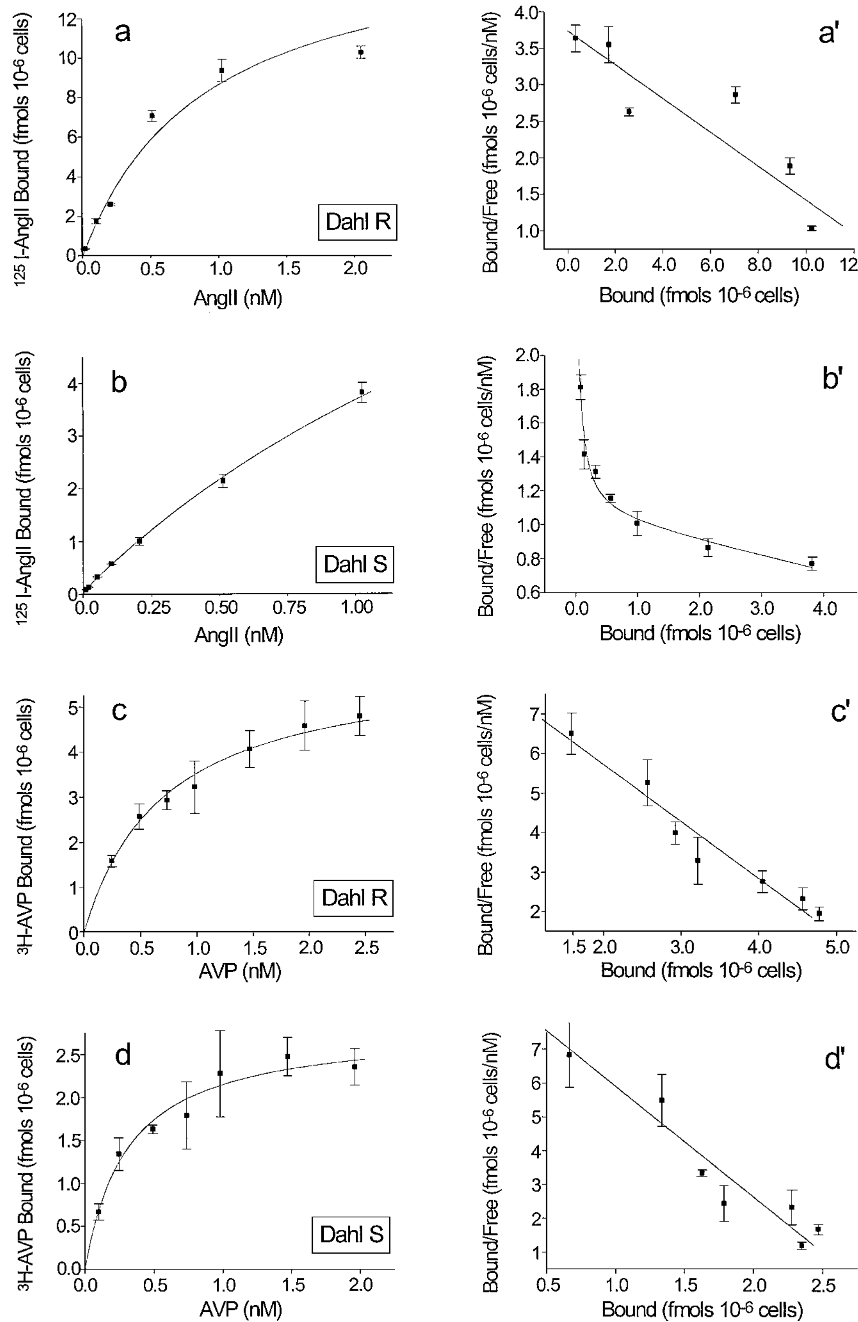

Fig. 2. Ligand binding studies of Dahl R and Dahl S AngII/AVP receptors expressed in Cos1 cells. Saturation binding curves (**a, b, c, d**) and corresponding Scatchard plots (**a′, b′, c′, d′**) with ^{125}I-AngII (**a, b**) and [^{3}H]-AVP (**c, d**) in Dahl R (**a, c**) and Dahl S (**b, d**) AngII/AVP receptors expressed in permanent Cos1 cell transfectants. Each experiment was performed in quadruplicate. Values are presented as mean ± standard deviation.

Discussion

Our studies have detected a modulator effect of sodium on G-protein coupling, a unique feature of the AngII/AVP receptor function that could have a significant impact on modulation of receptor function under physiologic conditions. Whereas AVP-dependent activation of cAMP accumulation (presumably via G_s-receptor coupling) is minimally affected by sodium in the wild-type (Dahl R) receptor, the AngII-dependent activation of cAMP accumulation is dramatically influenced by sodium in both Dahl S and Dahl R receptors resulting in a dichotomous sodium effect. Inhibition of AngII-induced cAMP accumulation (presumably via G_i-receptor coupling) is observed in the absence of sodium, whereas stimulation of AngII-induced cAMP accumulation

Table 2. AngII and AVP binding parameters of Dahl S and Dahl R AngII/AVP receptors

AngII/AVP Receptor	K_H AngII (nM)	B_{MAX}(fmol 10^{-6} cells)	K_L AngII (nM)	B_{MAX} (fmol 10^{-6} cells)	K_H AVP (nM)
Dahl S	0.88 ± 0.38	1.50 ± 0.54	8.90 ± 2.31	10.40 ± 2.18	0.30 ± 0.04
Dahl R	4.30 ± 0.99	16.40 ± 2.72	ND	ND	0.70 ± 0.08

Scatchard analysis (RADLIG, Version 4 Program, McPherson) of the [^{3}H]-AVP and ^{125}I-labeled AngII saturation curves (Fig. 3) was performed. K_H. high affinity (nM ± error); K_L, low affinity (nM ± error); ND, nondetected.

(presumably via G_s-receptor coupling) is observed in the presence of sodium (see Fig. 3).

Sodium modulation of receptor function is not without precedent. Allosteric regulation of α2-adrenergic receptor ligand binding by sodium has been reported (21) in which the allosteric effects of sodium have been found to be maximal at 40 mM (21). This is similar to the maximal effect of sodium on AngII/AVP receptor function detected at 50 mM (see Fig. 3). Altogether, the data suggest that the sodium modulation of AngII/AVP receptor function is a biologically significant phenomenon that contributes to susceptibility to salt-sensitive hypertension in the Dahl S rat model. Furthermore, we hypothesize that this receptor could be a key component of a sodium-sensing gene network underlying physiologic regulation of sodium homeostasis. It is important to note that the AngII/AVP receptor is broadly expressed in neurons of the central nervous system, including those regions thought to be involved in central control of blood pressure and salt appetite (22,23). However, additional molecular, cellular, and physiologic studies are necessary to test this hypothesis.

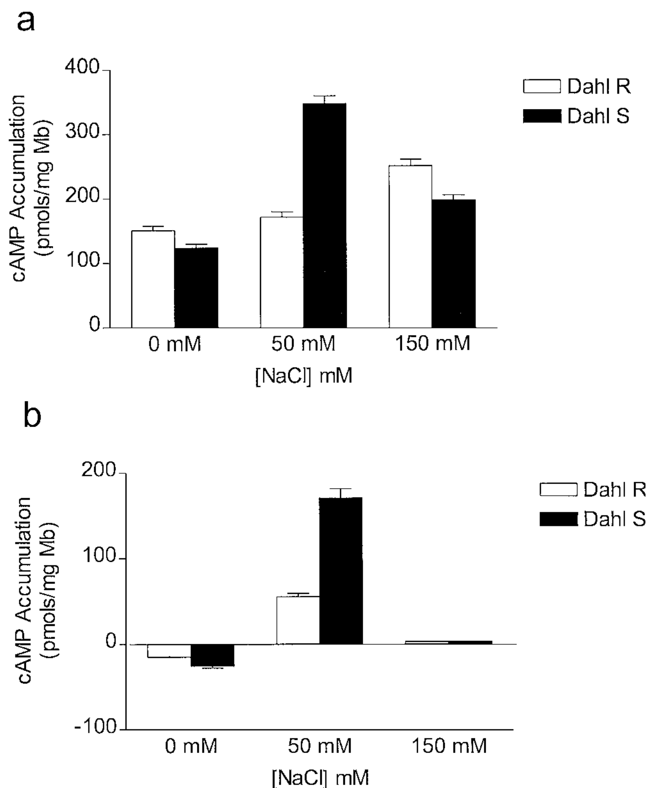

Fig. 3. Hormone-dependent stimulation of cAMP accumulation in Dahl S and Dahl R AngII/AVP receptors expressed in C127 cells. Ten micrograms of Dahl S and Dahl R AngII/AVP receptor expressing membranes were exposed to 0.1 μM of AVP (**a**) or 0.1 μM of AngII (**b**) at 25°C for 20 min in the presence of 0, 50, or 150 mM of NaCl, in comparison to basal control (no hormone added). Each experiment was performed in triplicate. Values are presented as mean ± standard deviation.

The differential effects of sodium on AngII- and AVP-induced cAMP accumulation indicate that the AngII/AVP receptor mediates AngII and AVP stimulation via two distinct effector pathways. Moreover, it also documents alternative use of guanine nucleotide-binding regulatory proteins by a single receptor as a mechanism for diversifying receptor function. The sodium-dependent "switching" of the AngII/AVP receptor from G_i to G_s coupling observed in our studies, resembles the PKA-dependent "switching" from G_s to G_i coupling recently reported in the α2-adrenergic receptor (24), thus corroborating the existence of multiple G-protein coupling "switching" mechanisms.

The localization of the amino acids substitutions within the Dahl S receptor suggests that they may differentially influence receptor function. The N119S substitution in the H2–H3 intracellular loop (see Fig. 1b) could affect G_S coupling because of its proximity to the putative G_S activation domain (H4–H5 intracellular loop, see Fig. 1b). In contrast, the C163R substitution in the H3–H4 extracellular loop (see Fig. 1b) might influence AVP and AngII binding due to its putative effects on the secondary structure of the extracellular receptor domains.

Our results demonstrate the existence of a "hyperfunctioning" AngII/AVP receptor present in Dahl salt-sensitive rats exhibiting increased affinity for both ligands (AVP and AngII) and enhanced G_S coupling resulting in increased activation of adenylate cyclase. Analogous dysfunctional behavior has been reported for the constitutively activated mutation of the luteinizing hormone receptor in familial

male precocious puberty (25) and in thyrotropin receptor involved in thyroid adenomas (26). Thus, for any given level of ligand (AVP or AngII) concentrations, the "hyperfunctional" Dahl S AngII/AVP receptor will effectively produce a higher level of cAMP accumulation compared with the Dahl R receptor. Intuitively, we hypothesize that this AngII-AVP "hyperresponse" could contribute to increased tubular sodium and fluid reabsorption in Dahl S rats, a characteristic pathophysiology observed in this genetic hypertensive rat model (3). Our hypothesis is substantiated by the prominent expression of the AngII/AVP receptor in the thick ascending limb of the loop of Henle and collecting ducts (5).

We note that several genetic studies in different rat models have documented a chromosome 1 quantitative trait locus (QTL) in the region spanning the AngII/AVP receptor locus for high blood pressure (27–37). Reports include intercross linkage analysis of F2 cohorts derived from SHRSP (stroke-prone spontaneously hypertensive rat) × WKY (Wistar-Kyoto normotensive rat) (27,28), SHR (spontaneously hypertensive rat) × WKY (29), SS/JR (Dahl salt-sensitive hypertensive rat) × LEW (Lewis normotensive rat) (32), FHH (Fawn-hooded hypertensive rat) × ACI (ACI normotensive rat) (31) and SBH/y (salt-sensitive Sabra hypertension-prone rat) × SBN/y (salt-resistant Sabra hypertension resistant rat) (37). Based on recent evidence excluding *Sa* as a candidate gene for the blood pressure QTL on rat chromosome 1 (35,36), our results, in conjunction with the available genetic evidence, point to the AngII/AVP receptor as the candidate gene for the chromosome 1 QTL in different rat models of essential hypertension. Strategic transgenic experiments will be necessary to demonstrate the mechanistic role of the AngII/AVP receptor in salt-sensitive hypertension.

Most importantly, the selective AngII-dependent sodium hyperresponsiveness of the dual AngII/AVP receptor is concordant with studies in humans demonstrating the central role of the renin-angiotensin system (RAS) in salt-sensitive blood pressure response (38). Altogether the data provide a compelling basis and strategic focus for investigating the role of the AngII/AVP receptor in human hypertension.

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