**α₁- and α₂-Adrenoceptor Control of Sodium Transport Reverses in Developing Hypertension**

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**Abstract**—α-Adrenergic receptor (AR) activation enhances sodium retention in certain forms of hypertension. The objective of the present study was to understand the role of α-ARs in regulating sodium transport by distal tubules (DT). DT cells were isolated from kidneys of spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats at 6 weeks, when hypertension is developing, or at 12 weeks, when hypertension is established. The α₁-AR agonist phenylephrine increased ²³Na uptake by 50% into DT cells of 6-week SHR; no effect was observed with WKY cells. The α₂-AR agonist B-HT 933 increased uptake by only 10%. At 12 weeks, the pattern of α-AR regulation was reversed: α₁-AR–induced sodium uptake was only 15%, whereas α₂-AR activation increased sodium uptake by 35% in SHR and WKY cells. α₁-AR–induced sodium uptake in 6-week SHR cells was abolished by prazosin; α₂-AR–stimulated sodium uptake was blocked by yohimbine in 12-week SHR and WKY. Competitive binding studies were performed with [³H]prazosin and α₁A-, α₁B-, and α₁D-selective antagonists with DT cell membranes from 6- and 12-week SHR and WKY. α₂-AR subtypes were determined with [³H]rauwolscine and α₂A- and α₂B-selective antagonists. Expression of α₁B-ARs was increased 4-fold in DT cells during the developing phase of hypertension in SHR. No change was detected in α₂-AR expression. DT cells transiently increase [Ca²⁺], in response to α₁-AR agonists from 6-week but not 12-week SHR. Conversely, α₂-AR agonists increase [Ca²⁺], at 12 weeks. In summary, during developing hypertension, α₁-ARs increase sodium uptake and [Ca²⁺], in SHR cells. Expression of α₁B-ARs is selectively upregulated during developing hypertension. In established hypertension (and normotension), α₂-ARs regulate sodium transport and [Ca²⁺] in DT cells. We conclude that a molecular switch of α₁-AR and α₂-AR signaling occurs in DT cells during the development of hypertension. *(Hypertension. 1999;33[part II]:524-529.)*

**Key Words:** receptors, adrenergic ■ blood pressure ■ catecholamines ■ epinephrine ■ hypertension ■ calcium ■ norepinephrine

One proposal for the initiation and maintenance of hypertension centers on a reduced capacity of the kidneys to excrete salt and water in proper relation to intake.¹ Sodium retention could initiate or contribute to the development of hypertension by activating mechanisms in vascular smooth muscle, the sympathetic nervous system, or expansion of effective extracellular fluid or plasma volume.²,³ Chronic stimulation of renal nerves or intrarenal infusion of norepinephrine increases sodium retention and is capable of producing hypertension.⁴,⁵ Conversely, renal denervation attenuates sodium retention and the onset of hypertension⁶ but exerts only minimal effects in adult animals with established hypertension.⁷,⁸ Metabolic balance studies examining the intake and excretion of sodium and water document that spontaneously hypertensive rats (SHR) between 4 to 7 weeks of age retain more sodium than do age-matched Wistar-Kyoto (WKY) rats.⁹ During this period, urinary excretion was less in SHR than WKY rats and attributed to renal mechanisms. As the animals matured and systolic arterial pressure increased, renal sodium excretion was normalized in 8- to 13-week SHR.⁹

These findings suggest that altered regulation of renal sodium absorption parallels the increase in pressure observed in SHR.

The focus of this study was to test the hypothesis that α-adrenergic receptors (α-ARs) contribute to the increased sodium absorption in distal tubule (DT) cells of SHR during developing hypertension (4 to 7 weeks). Renal nerves impinge directly on proximal tubules (PT) and DT.¹⁰ Previous studies showed that α₁- and α₂-AR agonists stimulate Na⁺/H⁺ exchange to a similar level in PT cells from SHR and WKY rats from 4 to 16 weeks of age.¹¹ It is predicted that α-ARs increase sodium absorption in DT cells. The mammalian DT is composed of 3 segments: distal convoluted tubule, connecting tubules, and initial cortical collecting duct.¹² In aggregate, these segments critically regulate the absorption of up to 15% of the filtered sodium load.¹³ Activation of α-ARs on DT segments may significantly increase sodium retention during the developing phase of hypertension in SHR. The enhanced sodium retention observed during this phase may be due to increased expression of receptor subtypes or alternative receptor signaling.
It is proposed that a reversal of α1- and α2-AR regulation occurs during the developing and established hypertension. During the phase of increasing blood pressure in SHR, α1-ARs increase sodium transport in DT cells. The increase in α1-AR–stimulated sodium uptake correlates with increased α1B-AR binding on DT cells during developing hypertension. In established hypertension in SHR and in normotensive WKY rats, α2-ARs, but not α1-ARs, increase sodium transport. These observations indicate increases in specific α1-AR subtypes enhance sodium transport in DT cells during developing hypertension and α2-ARs regulate sodium absorption in DT cells during established hypertension and normotension.

Methods

Preparation of Freshly Isolated and Primary Cultures of Tubule Cells From SHR and WKY Rats
Salt-insensitive SHR and WKY rats of 6 and 12 weeks of age were obtained (Taconic) and maintained on normal rat chow and water ad libitum. The weights of 6- and 12-week-old SHR were similar to those of WKY rats. Systolic blood pressures were measured in awake animals as previously reported.11 The blood pressures of 6-week-old SHR averaged 139 mm Hg compared with 106 mm Hg in 6-week-old WKY rats (P<0.05). Blood pressures of 12-week-old SHR averaged 175 mm Hg, significantly greater than those observed with WKY rats (110 mm Hg), and agree with our previous observations11 and those of others14,15 that correspond to developing hypertension and established hypertension phases. DT and PT cells were prepared from age-matched SHR and WKY rats of 6 weeks (developing hypertension) and 12 weeks (established hypertension)16 with isolation of DT cells using a double-antibody isolation technique.17 Primary cultures of DT and PT cells were prepared from freshly isolated cells.18

Isotopic Sodium Uptake Measurements
A rapid filtration technique described in previous reports19 was used to measure uptake of 22Na into DCT cells. Briefly, WKY and SHR cells were placed in a sodium-containing buffer, with or without α1- or α2-AR agonists or antagonists for 1 minute before the addition and vortexing of 1 aliquot of 22Na (Amersham) to initiate isotope uptake. In all experiments reported, tracer uptake was terminated after 1 minute by the rapid addition of ice-cold isosmotic Li2SO4–HEPES rinse buffer and filtered onto Whatman GF/C filters using a Millipore 12-port manifold.

Receptor Binding Assays
Saturation binding experiments were performed using [3H]prazosin and [3H]rauwolscine (New England Nuclear) for α1- and α2-ARs,
respectively. Concentrations ranging from 0.01 to 5 nmol/L labeled ligand with 100 μg SHR and WKY cell membrane protein were used per reaction tube. Nonspecific binding was assessed with 10 μmol/L phentolamine or yohimbine.\(^\text{20,21}\) Membrane binding of \([3\text{ H}]\text{prazosin}\) or \([3\text{ H}]\text{rauwolscine}\) was performed at 37°C for 30 minutes, and incubations were terminated by ice-cold buffer addition and rapid filtration. Specific binding was defined as total binding minus nonspecific binding; nonspecific binding averaged \(\sim 21\%\) of total binding. Low concentrations (10 nmol/L) of the subtype-selective receptor antagonists WB4101 (α₁), spiperone (α₂), and BMY7378 (α₁) were used to define the relative proportions of α₁-AR subtypes with \([3\text{ H}]\text{prazosin}\) binding to SHR and WKY DT and PT cell membranes.\(^\text{22}\) For determining the relative proportions of α₁a- and α₁b-ARs in SHR and WKY DT and PT cell membranes, oxymetazoline (α₁a selective) and ARC239 (α₁b selective) were used.\(^\text{23}\) Binding constants (\(B_{max}, K_d\)) were calculated using nonlinear regression analysis with Prism software (GraphPad Software).

**Measurement of [Ca\(^{2+}\)]\(_i\)**

Intracellular fluorescence measurements of calcium were performed as previously described in detail.\(^\text{24}\) Primary cultures of SHR and WKY DT and PT cells were grown to near-confluence on 25-mm glass coverslips and incubated for 60 minutes at 37°C with Fura-2 AM (10\(^{-5}\) mol/L; Molecular Probes). Fluorescence excitation and emission intensity were measured with a Nikon Photoscan-2.

**Materials and Preparation of Drug Solutions**

α-AR agonists and antagonists were prepared so that the molar concentration indicated in the text is the final concentration to which cells were exposed. Solutions containing drugs were prepared fresh daily. Rolipram was purchased from BIOMOL Research Laboratories; B-HT 933 was a gift from Boehringer-Ingelheim Pharmaceuticals. Other adrenergic agonists and antagonists were purchased from Research Biochemical International.

**Statistical Evaluation of Data**

All \([3\text{ H}]\text{prazosin}\) and \([3\text{ H}]\text{rauwolscine}\) binding and \(\text{Na}\) uptake measurements were made in triplicate within individual experiments. The data are presented as mean±SEM, where \(n\) indicates the number of separate experiments. Comparisons between control and drug-treated groups were examined by posthoc analysis of multiple comparisons with the Bonferroni or Newman-Keuls multiple comparisons test using the statistical software Instat for MacIntosh (GraphPAD Software). Values of \(P\leq0.05\) were considered significant.

**Results**

**α-AR Effects on Sodium Uptake**

The responses of DT cells to equimolar concentrations of the α₁-selective agonist phenylephrine (PHE), the α₂-selective agonist B-HT 933, and the endogenous mixed norepinephrine (NE) are depicted in Figure 1. The basal (control) rates of uptake were similar among SHR and WKY rats at both 6- and 12-week time points. Only DT cells from 6-week SHR increased sodium uptake when treated with PHE. There was no effect when 6-week WKY DT cells were treated with PHE. By comparison, DT cells from 6-week SHR exhibited no response to the α₁-AR agonist B-HT 933. DT cells from 6-week WKY rats increase sodium uptake in response to the α₂-AR agonist. Both 12-week SHR and WKY DT cells treated with B-HT 933 exhibit significantly increased rates of sodium uptake. The mixed α₁- and α₂-AR agonist NE increases sodium uptake in DT cells from SHR and WKY; this presumably is due to the α₁-AR stimulation and α₂-AR regulation during established hypertension. As depicted in Figure 2, NE-stimulated sodium uptake into 6-week SHR DT cells is abolished by pretreatment with prazosin, an α₁-AR antagonist. At 12 weeks, NE-stimulated sodium uptake into SH DR DT cells is blocked by the α₂-AR antagonist yohimbine. When 6- or 12-week WKY DT cells were stimulated with NE, sodium uptake was blocked only by yohimbine. Pretreatment of 6- or 12-week WKY DT cells with prazosin had no demonstrable effect on NE-stimulated sodium uptake.

**Expression of α-AR Subtype Protein in Developing and Established Hypertension**

To discern the subtypes of α₁- and α₂-ARs expressed in SHR and WKY DT cells, receptor protein was measured using \([3\text{ H}]\text{prazosin}\) and \([3\text{ H}]\text{rauwolscine}\) in competitive binding experiments. Specific high affinity binding for both α₁- and α₂-ARs was detected in DT and PT membranes from SHR.
and WKY. Specific binding averaged 73% to 90% in all saturation binding experiments. The mean $\alpha_1$-AR density in 6-week SHR DT membranes was $250 \pm 26$ fmol/mg protein and was significantly greater than that of 6-week WKY DT membranes ($84 \pm 11$ fmol/mg protein). The $\alpha_1$-AR density of 12-week SHR DT membranes was $113 \pm 16$ fmol/mg protein and comparable to the value detected for 12-week WKY DT membranes ($92 \pm 14$ fmol/mg protein). As depicted in Figure 4, there is significantly greater $\alpha_{1B}$-AR binding with 6-week SHR DT membranes than with those of 12-week SHR or WKY DT membranes. The significant increase in total $\alpha_1$-AR density of a 6-week SHR DT membranes is in large part attributable to the increased expression of the $\alpha_{1B}$-AR subtype. Compared with expression of $\alpha_{1B}$-ARs in 12-week SHR and WKY DT cells, there is nearly 4-fold greater expression of this subtype in 6-week SHR DT cells. Expression of $\alpha_{1A}$-AR subtypes was comparable in SHR and WKY 6- and 12-week DT membranes. Although message for $\alpha_{1D}$-AR was detected by RT-PCR, competitive binding studies indicate <6% of the total binding in DT membranes from SHR and WKY rats was attributable to this particular subtype. Conflicting results relating to the expression of the $\alpha_{1D}$-AR subtype indicate the absence of this subtype in kidney cortex, whereas $\alpha_{1D}$-AR mRNA is present in DT cells; protein expression of this subtype is very low in DT cells.

To determine whether protein expression of $\alpha_1$-ARs is increased in other tubule segments, we measured $\alpha_1$-AR binding in PT membranes from 6- and 12-week SHR and WKY rats (Figure 5). The increased expression of the $\alpha_{1D}$-AR subtype is specific for DT membranes from 6-week SHR. The $\alpha_{1D}$- and $\alpha_{1A}$-AR subtype expression is not increased in PT membranes from SHR or WKY membranes at 6 or 12 weeks. Again, consistent with other reports, $\alpha_{1A}$- and $\alpha_{1B}$-AR subtypes comprise ~50% each of the $\alpha_1$-ARs expressed on the PT. Expression of the $\alpha_{1D}$-AR averaged <7% of the total $\alpha_1$-AR binding in WKY and SHR PT membranes.

We theorized that with a reduction in $\alpha_1$-AR expression at 12 weeks in SHR DT cells, there may be an accompanying increase in expression of a particular $\alpha_2$-AR subtype. To test this theory, competitive binding studies were performed with DT membranes from SHR and WKY rats of 6 and 12 weeks and summarized in Figure 6. Similar levels of $\alpha_2$-AR binding were observed in DT and PT cell membranes from 6- and 12-week SHR and WKY. Total specific binding of $\alpha_2$-ARs was 93 fmol/mg protein in membranes from 6-week SHR compared with 84 fmol/mg protein in WKY. $\alpha_2A$-AR binding was 61 versus 47 fmol/mg protein in SHR compared with WKY, whereas $\alpha_2D$-AR binding was 44 versus 36 fmol/mg protein in SHR compared with WKY. With membranes from 12-week SHR, total specific binding was 106 versus 97 fmol/mg protein in SHR compared with WKY, respectively. $\alpha_2A$-AR binding was 68 fmol/mg protein compared with 57 fmol/mg protein in WKY. $\alpha_2B$-AR binding was ~50 fmol/mg protein for both SHR and WKY with membrane from 12-week DT cells. An equal ratio of $\alpha_2A/\alpha_2B$ sites in PT and DT membranes from SHR and WKY was consistent with densities reported by others. Although 6-week SHR express significantly greater numbers of $\alpha_2A$-ARs during developing hypertension, the densities of $\alpha_2$-ARs remain constant during developing and established hypertension.

**Signaling of $\alpha_1$- and $\alpha_2$-ARs During Developing and Established Hypertension**

The $\alpha_1$- and $\alpha_2$-ARs activate a number of signaling pathways, including phosphoinositide-sensitive phospholipase C (PI-
PLC), phospholipase D (PLD), phosphatidylinositol-sensitized PLC (PC-PLC), phospholipase A₂ (PLA₂),²⁷,²⁸ and mitogen-activated protein kinase (MAPK).²⁷,²⁹ Studies with PT and MDCK cells demonstrate α₁-ARs activate PI-PLC and lead to formation of IP₃ and diacylglycerol, transient increases in intracellular Ca²⁺ ([Ca²⁺]ᵢ), and subsequent activation of PKC.²⁷,²⁸,³⁰ α₂-ARs couple to pertussis toxin–sensitive Gi proteins in PT to inhibit adenyl cyclase but couple to PI-PLC in DT cells.²⁴ To examine the signal pathways activated by α₁- and α₂-ARs during developing and established hypertension, we measured agonist-induced increases of [Ca²⁺]ᵢ in DT cells of SHR and WKY rats with the fluorescent dye, Fura-2 AM. A representative experiment is depicted in Figure 6. After measurement of resting levels of [Ca²⁺]ᵢ, in DT cells from 6-week SHR or WKY, cells were treated with the α₁-agonist PHE. SHR DT cells responded with a prompt and significant increase in [Ca²⁺]ᵢ from a basal level of 107 nmol/L to 248 nmol/L, whereas WKY DT cells displayed negligible changes in [Ca²⁺]ᵢ, (basal level of 103 nmol/L versus 112 nmol/L with PHE). When cells were treated with the selective α₁-agonist B-HT 933, there was an increase in WKY DT cells (control level of 102±6 nmol/L versus 266±9 nmol/L with B-HT 933) and no response in the SHR DT cells (basal level of 114±5 nmol/L compared with 128±6 nmol/L with B-HT 933). We next sought to determine whether the α₁- and α₂-AR response was different at 12 weeks between SHR and WKY DT cells given the reduction in α₁β-AR expression. DT cells from 12-week SHR increase [Ca²⁺]ᵢ, in response to B-H 933 from 109±3 to 249±8 nmol/L. A similar increase was observed in DT cells from 12-week WKY rats (control level of 119±5 nmol/L and 249±8 nmol/L with B-HT 933 treatment. By comparison, PHE did not significantly increase [Ca²⁺]ᵢ, in DT cells from 12-week SHR or WKY rats (control levels of 112 and 106 nmol/L and PHE levels of 121 and 117 nmol/L for SHR and WKY, respectively). The α₁-AR–stimulated increase of [Ca²⁺]ᵢ, observed with 6-week SHR DT cells is absent in DT cells from 12-week SHR. In contrast, DT cells of 12-week SHR and WKY rats displayed similar increases to an α₁-agonist. These findings suggest that α₁β-ARs mediate the increase of [Ca²⁺]ᵢ, in 6-week SHR DT cells and that α₂-ARs assume this function during established hypertension in SHR or in normotensive WKY DT cells.

Discussion

The objective of this study was to determine the role of α-ARs in the development of hypertension. We propose that renal α₁-ARs are important in the initiation and development of hypertension because they significantly increase sodium absorption in the DT, a segment that critically regulates 10% to 15% of final sodium excretion. As hypertension progresses, α₁-AR expression is downregulated and α₂-ARs regulate sodium absorption in DT cells.

Renal nerve stimulation reduces sodium excretion./dat This effect is abolished by α₁-AR antagonists.²⁵ In rats chronically treated with prazosin, an α₁-AR antagonist is required to fully abolish the increase in sodium retention.²⁵ These findings suggest both α₁- and α₂-AR receptors regulate sodium absorption. In PT cells, α₁- and α₂-ARs exert synergistic effects on Na⁺/H⁺ exchange.³³ However, α₁- and α₂-AR–stimulated Na⁺/H⁺ exchange is similar in WKY and SHR PT cells during developing and established hypertension.¹¹ These findings support the view that α₁-AR regulation of sodium absorption is not altered in PT cells during developing hypertension.

Numerous studies indicate α₁-ARs enhance sodium retention.²⁵,³⁴,³⁵ SHR excrete significantly less sodium during the onset of hypertension (weeks 4 to 7).³⁶ As hypertension progresses, the tendency to retain sodium is reversed until levels approximate those of normotensives during weeks 10 to 13. Our results with α₁-AR stimulation of sodium uptake are consistent with these observations.

We observe significant differences in α₁- and α₂-AR regulation of sodium absorption in DT cells during developing and established hypertension. As depicted in Figure 1, the selective α₁-AR agonist PHE significantly increases sodium uptake into DT cells during developing hypertension in SHR but has no effect in age-matched WKY. By comparison, an α₂-AR agonist increases sodium uptake in WKY, but not SHR, DT cells. At 12 weeks, both SHR and WKY increase sodium uptake in response to α₂- but not α₁-AR agonists. The increased uptake of sodium in SHR DT cells during developing hypertension coincides with high levels of the α₁β-AR subtype expressed at this phase (Figure 4). Once hypertension is established, the density of the α₁β-ARs in SHR DT cells is similar to that observed in WKY. Finally, the signaling through increased [Ca²⁺]ᵢ, also correlates with enhanced α₁β-AR expression and sodium uptake during this phase. Once expression of the α₁β-ARs is reduced during established hypertension, α₁-AR subtypes no longer signal increases in [Ca²⁺]ᵢ, in DT cells. Although speculative, α₂-ARs may regulate final changes in sodium absorption through activation and signaling of the PI-PLC pathway in DT cells. Because this pathway is present in 6-week WKY rats and these animals do not exhibit increased blood pressure, the α₂-ARs may modulate physiological changes in sodium absorption.

Although the exact mechanisms underlying the upregulation of renal α-ARs in SHR remain unclear, the increased density may represent a genetic abnormality.³⁷ Two lines of evidence support this theory: (1) increased receptor density in SHR occurs before onset of the development of hypertension, and (2) nongenetic models of hypertension do not exhibit increased receptor expression. Several studies show that renal α₁-AR receptor density is increased in hypertension.³⁷⁻³⁹ Sanchez et al.⁴⁰ suggests that α₁-ARs increase during the developing hypertension but that α₂-ARs density is greater during established hypertension. The data in Figure 4 are consistent with reports that α₁-ARs increase during developing hypertension. By comparison, our data do not indicate upregulation of α₂-ARs during developing or established hypertension. Intengan and Smyth⁴¹ suggest a defective modulation of solute excretion in 8-week SHR due to the α₂AR-AR. This defect is not present in acquired models of hypertension. Our results would be consistent with these observations because α₁-ARs in DT cells do not signal an increase in [Ca²⁺]ᵢ, during developing hypertension. During established hypertension and in normotensive animals, the subtype of α₂-ARs that modulates sodium transport possesses the ability to signal through PI-PLC pathways.²⁴ Altered renal sodium balance in SHR may represent a consequence of en-
hanced α₁-AR--stimulated retention and lack of α₂-AR-induced clearance during the developing phase of hypertension.

In summary, we demonstrate that α-ARs stimulate sodium uptake in DT cells. During developing hypertension in SHR, the expression of α₁B-AR protein correlates with a significant increase in sodium uptake from α₁-AR stimulation. In age-matched normotensive WKY DT cells, α₂-ARs are capable of increasing sodium uptake. During established hypertension, the expression of α₁B-ARs is reduced and α₁-ARs no longer stimulate sodium uptake. In DT cells from SHR with established hypertension and normotensive WKY rats, regulation of sodium transport is mediated by α₂-ARs. Transient increases in [Ca²⁺]i occur with α₂-ARs during developing hypertension but switch to α₁-ARs during established hypertension. The switch in receptor expression and signaling does not occur in PT cells. We propose that the enhancement of α₁-AR expression that occurs during developing hypertension contributes to the initiation of hypertension, whereas during established hypertension or in normotensive animals, α₂-ARs modulate sodium transport in DT cells.

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