Perturbation of D₁ Dopamine and AT₁ Receptor Interaction in Spontaneously Hypertensive Rats

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Abstract—The dopaminergic and renin-angiotensin systems interact to regulate blood pressure. Because this interaction may be perturbed in genetic hypertension, we studied D₁ dopamine and AT₁ angiotensin receptors in immortalized renal proximal tubule (RPT) and A10 aortic vascular smooth muscle cells. In normotensive Wistar-Kyoto (WKY) rats, the D₁-like agonist fenoldopam increased D₁ receptors but decreased AT₁ receptors. These effects were blocked by the D₁-like antagonist SCH 23390 (10⁻⁷ mol/L per 24 hours). In spontaneously hypertensive rat (SHR) RPT cells, fenoldopam also decreased AT₁ receptors but no longer stimulated D₁ receptor expression. Basal levels of AT₁/D₁ receptor coimmunoprecipitation were greater in WKY RPT cells (29±2 density units, DU) than in SHR RPT cells (21±2 DU, n=7 per group, P<0.05). The coimmunoprecipitation of D₁ and AT₁ receptors was increased by fenoldopam (10⁻⁷ mol/L per 24 hours) in WKY RPT cells but decreased in SHR RPT cells. The effects of fenoldopam in RPT cells from WKY rats were similar in aortic vascular smooth muscle cells from normotensive BD IX rats, that is, fenoldopam decreased AT₁ receptors and increased D₁ receptors. Our studies show differential regulation of the expression of D₁ and AT₁ receptors in RPT cells from WKY and SHR. This regulation and D₁/AT₁ receptor interaction are different in RPT cells of WKY and SHR. An altered interaction of D₁ and AT₁ receptors may play a role in the impaired sodium excretion and enhanced vasconstriction in hypertension. (Hypertension. 2003;42[part 2]:787-792.)

Key Words: hypertension, essential ■ receptors, angiotensin II ■ dopamine ■ kidney ■ muscle, smooth, vascular

Dopamine, a neurotransmitter in neural tissue, acts as an autocrine/paracrine substance in nonneural tissues. Dopamine produced in neural and nonneural tissues is now recognized to serve an important role in the regulation of blood pressure and sodium balance by direct actions on renal and intestinal epithelial ion transport, by interaction with other receptors, by modulation of the secretion of hormonal/paracrine substances such as aldosterone, catecholamines, renin, and vasopressin, and by actions on brain appetite centers. Dopamine receptors are classified into D₁-like (D₁, D₅) and D₂-like (D₂, D₃, and D₄) subtypes, based on their structure and pharmacology. Whereas the D₁-like receptors couple to the stimulatory G protein, Goα, and thus, activate adenylyl cyclases, the receptors of the D₂-like subfamily couple to the inhibitory G protein, Ga/Gαi and inhibit adenylyl cyclases and calcium channels and modulate potassium channels. Under euvolemic conditions and magnified during moderate volume expansion, dopamine, through D₁-like and D₂ receptors, acts to increase sodium excretion and decrease blood pressure. Dopamine exerts its action through several receptor subtypes. AT₁ receptors are linked to Gq/11 and Gα₁Gα₂, whereas AT₂ receptors are linked to Gqα2/3-7; the G protein linkage of AT₁ receptors is not clear. Although angiotensin, through AT₂ and AT₁ receptors, can decrease blood pressure by vasodilation and increase in sodium excretion, the major effect of angiotensin II is increasing sodium reabsorption and blood pressure, counteracting the effect of dopamine.

Dopamine and angiotensin receptors counteract each other in the paracrine regulation of renal sodium transport. Thus, the natriuretic effect of D₁-like receptors is enhanced when angiotensin II production is decreased or when AT₁ receptors are blocked. Dopamine, through D₂-like and D₁-like receptors, by themselves or in concert, antagonizes the stimulatory actions of angiotensin II on proximal tubular luminal sodium transport. Dopamine, through D₂-like receptors, also decreases AT₁ receptor expression and angiotensin II binding sites in renal proximal tubules, and more specifically, the D₁ receptor, also decreases AT₁ receptor expression in immortalized renal proximal tubule (RPT) cells from normotensive Wistar-Kyoto (WKY) rats. Immortal-
ized RPT cells have characteristics similar to freshly obtained RPTs and renal brush border membranes. 17–20 Similar to the counterregulatory effects of these systems on renal sodium transport, the renal vasoconstrictor effect of angiotensin II is countered by the vasodilator action of dopamine, through D1-like receptors, in normotensive WKY rats. 21,22 In spontaneously hypertensive rats (SHR), the renal vasodilatory effect of D1-like receptors is decreased and there is a lack of D1-like receptor antagonism of the vasoconstrictor effect of angiotensin II. 21,22 Our previous study showed that angiotensin II increases D1 receptor expression in RPT cells from WKY rats but not from SHR. 23 We hypothesize that there is a differential interaction of D1 and AT1 receptors not only in RPT cells but also in vascular smooth muscle cells of WKY and SHR. We now report that fenoldopam, an agonist for D1-like receptors (D1 and D5), increases expression of D1 receptors and decreases expression of AT1 receptors in RPT cells from WKY rats and A10 cells. 24,25 A primary culture of vascular smooth muscle cells from the embryonic thoracic aorta of normotensive BD IX rats. The actions of fenoldopam are exerted through D1-like receptors because the effects are blocked by SCH23390, an antagonist for D1-like receptors (D1 and D5). Although D1/AT1 receptors physically interact in both cell lines, fenoldopam increases D1/AT1 receptor immunoprecipitation in RPT cells from WKY rats but decreases it in SHR. In SHR, fenoldopam no longer affects D1 receptor expression but its ability to decrease AT1 receptors is preserved; the effect of fenoldopam on AT1 receptor expression may be exerted through another D1-like receptor. 1–3

Methods

Cell Culture

Immortalized RPT cells from WKY and SHR and primary cultures of embryonic thoracic aortic smooth muscle cells (A10) (passage 10 to 20) from normotensive Berlin-Druckrey IX (BD IX) rats were cultured at 37°C in 95% air/5% CO2 atmosphere in DMEM/F-12 or DMEM, respectively. 17,24,25 The cells (80% confluence) were extracted in ice-cold lysis buffer, 17 sonicated, kept on ice for 1 hour, centrifuged at 16,000g for 30 minutes. The supernatants were stored at −70°C until use.

Immunoblotting

The antibodies are polyclonal, purified antipeptides. The human AT1 receptor immunogenic peptide is QDDCPKAGRHC, amino acids 15 to 20 of the AT1 receptor. The rat D1 receptor antibody corresponds to positions 299 to 307 (GSEETQPFC) of the D1 receptor. The specificities of these antibodies have been reported. 17–19,26–30 The tests were treated with vehicle (dH2O), fenoldopam, or a D1-like receptor antagonist (SCH23390) at the indicated concentrations and times. Immunoblotting was performed as reported 17–20,26 except that the transblots were probed with the D1 (1:800) or the AT1 receptor antibody (1:400). Equality of total amount of protein per sample (50 μg) transferred onto the membranes was ascertained by Ponceau-S staining and by immunoblotting for α-actin.

Immunoprecipitation

RPT cells were incubated with vehicle or fenoldopam (10−7 mol/L) for 24 hours, as described above. The cells were lysed with ice-cold lysis buffer for 1 hour and centrifuged at 16,000g for 30 minutes. Supernatant (500 μg protein/mL) was incubated with affinity-purified rabbit anti-rat D1 receptor antibody (2 μL/mL) for 1 hour and protein-G agarose at 4°C for 12 hours. The immunoprecipitates were pelleted and washed 4 times with lysis buffer. The pellets were suspended in sample buffer, boiled for 10 minutes, and subjected to immunoblotting with the AT1 receptor antibody. To determine the specificity of the bands, preimmune serum of D1 receptor antibody (negative control) and AT1 receptor antibody (positive control) were used as immunoprecipitants instead of the D1 receptor antibody. The bands were quantified by densitometry. 17–20,26

Materials

Rabbit anti-human AT1 receptor antibodies were purchased from Santa Cruz Biotechnology, Inc; D1 receptor antibodies were custom-produced (Research Genetics). Fenoldopam and SCH23390 were from Sigma. A10 cells were from ATCC. Other reagents were of the highest purity available (Sigma or Gibco).

Statistical Analysis

The data are expressed as mean±SEM. Comparisons within and among groups were made by repeated-measures and factorial ANOVA, respectively, with the Duncan test. A value of P<0.05 was considered significant.

Results

Fenoldopam Decreases AT1 Receptor Expression in RPT Cells From WKY and SHR

Fenoldopam decreased AT1 receptor expression (∼45 kDa) in a concentration- and time-dependent manner in WKY RPT cells. The inhibitory effect was evident at 10−8 mol/L, with a 50% decrease at 1.4×10−7 mol/L (Figure 1A). The inhibitory effect of fenoldopam (10−7 mol/L) was noted as early as 8 hours and maintained for at least 30 hours (t1/2=15.5 hours) (Figure 1B). In SHR RPT cells, fenoldopam also decreased AT1 receptor expression (WKY: control=31±1, fenoldopam=19±2 density units, [DU]; SHR: control=27±2, fenoldopam=10−7 mol/L=20±2 DU; n=8/group), (Figure 1C). The specificity of fenoldopam as a D1-like receptor agonist was determined by studying the effect of the D1-like receptor antagonist SCH23390. In WKY RPT cells, consistent with the study shown in Figures 1A and 1B, fenoldopam (10−7 mol/L per 24 hours) decreased AT1 receptor expression (control=26±2 DU, fenoldopam=19±2 DU; n=9, P<0.05). SCH23390 (10−7 mol/L) by itself had no effect (28±2 DU) but reversed the inhibitory effect of fenoldopam on AT1 receptor expression (28±3 DU) (Figure 1D). In SHR RPT cells, SCH23390 also blocked the inhibitory effect of fenoldopam on AT1 receptor expression (control=29±3 DU, fenoldopam=16±3 DU, SCH23390=28±4, SCH23390+f enoldopam=29±3; n=12, P<0.05).

Fenoldopam Increases D1 Receptor Expression in RPT Cells From WKY Rats But Not From SHR

To investigate the effect of fenoldopam on the D1 receptor, RPT cells were incubated with fenoldopam (10−7 mol/L) for 24 hours. Fenoldopam increased D1 receptor expression (∼80 kDa) in RPT cells from WKY rats (control=25±3 DU, fenoldopam=37±3 DU; P<0.05, n=8) but had no significant effect in cells from SHR (control=22±2 DU, fenoldopam=17±4 DU; n=8/group) (Figure 2A).

The specificity of fenoldopam as a D1-like receptor agonist also was determined by studying the effect of the D1-like receptor antagonist SCH23390 on D1 receptor protein expression. In WKY RPT cells, consistent with the study shown in
Fenoldopam Increases D1 Receptors and Decreases AT1 Receptors in A10 Cells

To test our hypothesis that the effects of fenoldopam on AT1 and D1 receptors happens not only in RPT cells but also in vascular smooth muscle cells, A10 cells were treated with fenoldopam at the indicated concentrations and times. In A10 cells, as in RPT cells, fenoldopam decreased AT1 receptors and increased D1 receptors (D1: EC50 = 1.0 × 10^{-9} mol/L, t1/2 = 16.0 hours; AT1: EC50 = 5.1 × 10^{-10} mol/L, t1/2 = 16.1 hours) in a time-dependent (2 to 30 hours) and concentration-dependent (10^{-11} to 10^{-5} mol/L) manner (D1 receptor: Figures 3A and 3B; AT1 receptor: data not shown). In A10 cells, as in RPT cells, fenoldopam exerted its effects on D1-like receptors; SCH23390 completely blocked the effect of fenoldopam on AT1 receptor expression (Figure 4).

AT1 Receptor Coimmunoprecipitates With the D1 Receptor in Rat RPT Cells

To determine whether there is a physical interaction between the D1 and the AT1 receptor, additional experiments were performed. D1 receptors were first immunoprecipitated with anti-D1 receptor antibodies and then probed with anti-AT1.
receptor antibodies. As shown in Figure 5A, basal coimmunoprecipitation was greater in RPT cells from WKY rats than those in SHR (45-kDa bands represented the coimmunoprecipitated D₁ and AT₁ receptors). The 24-hour treatment of fenoldopam (10⁻⁷ mol/L) increased D₁/AT₁ coimmunoprecipitation in RPT cells from WKY rats but decreased it in SHR (WKY: control = 29 ± 2 DU, fenoldopam = 39 ± 2 DU; n = 7, *P < 0.05; SHR: control = 21 ± 2 DU, fenoldopam = 11 ± 1 DU; n = 7, *P < 0.05). The changes in D₁/AT₁ coimmunoprecipitation caused by fenoldopam were blocked by 10⁻⁷ mol/L SCH23390 (n = 2) (Figure 5B).

**Discussion**

There are several novel observations in our study. First, we show that the D₁-like receptor agonist fenoldopam increases D₁ receptor expression but decreases AT₁ receptor expression in RPT cells from WKY rats. This effect is exerted at the D₁ receptor because a D₁-like receptor antagonist, SCH23390, completely blocks the effect of fenoldopam. Second, in SHR, although fenoldopam also decreases AT₁ receptor expression, it has no effect on D₁ receptor expression. Third, AT₁ receptors coimmunoprecipitate with D₁ receptors in rat RPT cells. The basal level of AT₁/D₁ coimmunoprecipitation is greater in WKY than in SHR. Moreover, fenoldopam increases the physical interaction between AT₁ and D₁ receptors in rat RPT cells. The basal level of AT₁/D₁ coimmunoprecipitation in SHR rats but decreases it in SHR.

Fourth, the stimulatory effect of fenoldopam on D₁ receptor expression and inhibitory effect on AT₁ receptor in RPT cells from WKY rats also occur in A10 cells.

Both dopamine and renin-angiotensin systems exist in the RPT. The components of the renin-angiotensin system, including angiotensinogen mRNA, renin, and ACE, have been localized the proximal tubule. The proximal tubule is also the site of local dopamine production. Urinary dopamine and angiotensin II concentrations exceed circulating levels, suggesting that both systems may modulate RPT function in an autocrine or paracrine function. Dopamine and angiotensin II are two important regulators of sodium and water absorption in the kidney serving counteracting functions in the proximal tubule. Low concentrations of angiotensin II stimulate net reabsorption through activation of Na⁺/H⁺ exchanger-3, Na⁺-HCO₃ cotransporter, and Na⁺-K⁺-ATPase whereas dopamine exerts the opposite effect. Salt depletion increases angiotensin II production and AT₁ receptor expression in renal proximal tubules in normotensive rats, resulting in increased reabsorption of sodium and water. Conversely, sodium loading increases the production of dopamine, which by activation of D₁- and D₂-like receptors, promotes renal sodium and water excretion.

The current study shows that long-term D₁-like receptor stimulation (hours) increases D₁ receptors and decreases AT₁ receptors both in RPT from WKY rats and in A10 vascular smooth muscle cells from normotensive BD IX rats. This may be a mechanism by which D₁-like receptors continue to exert their effects long after D₁ receptor desensitization should have occurred. The inhibitory effect of the D₁-like receptor on sodium transport has been consistent, whereas its effect on resistance vessels has not been so. Thus, although most studies have reported a vasorelaxant effect of D₁-like receptors, a vasoconstriction effect has been reported in the rat tail artery. In our study, we found that fenoldopam increased D₁ receptor expression in A10 cells but decreased AT₁ receptor expression. These effects together with a D₁-like receptor-induced increase in cAMP levels should lead to vasodilation in resistance vessels, at least in the normotensive state.

We found a direct interaction between AT₁ and D₁ receptors by immunoprecipitation study. The basal level of AT₁/D₁ coimmunoprecipitation is much higher in WKY rats as compared with SHR. D₁ receptor stimulation with fenoldopam results in an increase in the coimmunoprecipitation of AT₁ and D₁ receptors in WKY rats, whereas a decrease occurs in SHR. The increase in AT₁/D₁ coimmunoprecipitation in
WKY rats have been caused by the increase in D1 receptor expression per se or an increased physical interaction between AT1 and D1 receptors. We favor the latter because D1 receptor expression in SHR RPT cells is not affected by fenoldopam, yet the AT1/D1 receptor coinmunoprecipitation is still decreased. It is possible that the D1 receptors antagonize AT1 receptor function in WKY rats by sequestering AT1 receptors. In SHR, the defective D1 receptor is unable to perform this function, allowing more AT1 receptors to respond to angiotensin II. Whether this differential D1/AT1 interaction in WKY and SHR has functional consequences remains to be determined. Further studies are also needed to determine whether the increased interaction between these two receptors is a direct or an indirect mechanism, possibly by the alteration of an adaptor gene or adaptor proteins. Others have shown that several G protein–coupled receptors are known to interact directly with each other by homo- or hetero-oligomerization. Thus, an adaptor protein for D2 and D3 receptors (protein 4.1N) has been recently identified that is important in their localization in plasma membranes.

It is known that the D1-like receptor function is impaired in the renal proximal tubule and medullary thick ascending limb of genetically hypertensive rats, such as the SHR. The impaired D1-like receptor function is not caused by abnormalities in G proteins or effectors proteins but rather the D1-like receptor is uncoupled from its G protein effector complex because of increased activity of the G protein–coupled receptor kinase type 4. Therefore, it is not unexpected that the ability of the D1-like receptor agonist to increase D1 receptor expression in WKY rats is no longer present in SHR. However, the persistence of the ability of D1-like receptor stimulation to inhibit AT1 receptor expression in RPT cells of SHR is unexpected. Because there are two D1-like receptors expressed in RPT cells, we hypothesize that the effect of D1-like agonist stimulation may have been exerted at the other D1-like receptor, the D3 receptor, which remains functional in the SHR. Our preliminary data show that in D3 knockout mice, AT1 receptor expression is higher in the renal cortical membrane as compared with its wild-type control animals (C. Zeng, J.E. Jones, L.D. Asico, and P.A. Jose, unpublished data, 2003). Because the increase in cAMP levels after D1-like receptor stimulation is due mainly to D1 receptor rather than D3 receptor effect, we presume that the inhibitory effect of D1 receptors on AT1 receptor expression may not have a significant impact on overall D1-like receptor function mediated by cAMP in the kidney of normotensive animals.

In mesangial, renal proximal tubular, vascular smooth muscle, and adrenocortical cells, increasing intracellular cAMP levels decreases steady state AT1 receptor expression. Since the 5′ promoter region of rat AT1 receptor expression has a cAMP response element, further studies will be required to determine whether cAMP decreases receptor expression by transcriptional and/or posttranscriptional mechanisms. We have reported that D1-like receptors can inhibit NHE3 activity independent of cAMP, through GoS. The linkage between D1 and D3 receptors to G protein subunits are different. Thus, the D1 receptor (but not the D3 receptor) is linked to Gq12 and Gq13, Gβ1 and Gγ7 transduce D1 (but not D3) receptor function. It is therefore possible that the regulation of AT1 receptors by D1-like receptors can occur by cAMP-independent and G protein subunit–dependent mechanisms.

In summary, we have demonstrated that the D1-like agonist fenoldopam positively regulates the expression of D1 receptors and negatively regulates AT1 receptors in vascular smooth muscle and RPT cells from normotensive rats. Whereas fenoldopam does not stimulate D1 receptor expression in SHR RPT cells, the inhibitory effect on AT1 receptor expression is seen. AT1 and D1 receptors physically interact with each other; fenoldopam increases the interaction between these two G protein–coupled receptors in WKY RPT cells but decreases it in SHR RPT cells.

Perspectives

The dopaminergic and renin-angiotensin systems are two important systems that regulate blood pressure. Dopamine causes natriuresis and vasorelaxation, whereas angiotensin II leads to opposite effects. The major D1-like receptor subtype mediating the increase in sodium excretion is probably the D1 receptor, whereas the major angiotensin II receptor mediating the decrease in renal sodium excretion is the AT1 receptor. In SHR, renal proximal tubular D1 receptor function is impaired. It is possible that the increased renal vascular resistance and increased renal sodium transport in SHR are caused by a defective interaction between D1 and AT1 receptors. However, which is primary and which is secondary remains to be determined.

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References


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