Activation of D₃ Dopamine Receptor Decreases Angiotensin II Type 1 Receptor Expression in Rat Renal Proximal Tubule Cells


**Abstract**—The dopaminergic and renin angiotensin systems interact to regulate blood pressure. Disruption of the D₃ dopamine receptor gene in mice produces renin-dependent hypertension. In rats, D₂-like receptors reduce angiotensin II binding sites in renal proximal tubules (RPTs). Because the major D₂-like receptor in RPTs is the D₃ receptor, we examined whether D₃ receptors regulate angiotensin II type 1 (AT₁) receptors in rat RPT cells. The effect of D₃ receptors on AT₁ receptors was studied in vitro and in vivo. The D₃ receptor agonist PD128907 decreased AT₁ receptor protein and mRNA in WKY RPT cells and increased it in SHR cells. PD128907 increased D₃ receptors in WKY cells but had no effect in SHR cells. D₃/AT₁ receptors colocalized in RPT cells; D₃ receptor stimulation decreased the percent amount of D₃ receptors that coimmunoprecipitated with AT₁ receptors in WKY cells and markedly decreased the coimmunoprecipitation in SHR cells. The D₃ receptor also regulated the AT₁ receptor in vivo because AT₁ receptor expression was increased in kidneys of D₃ receptor–null mice compared with wild type littermates. D₃ receptors may regulate AT₁ receptor function by direct interaction with and regulation of AT₁ receptor expression. One mechanism of hypertension may be related to increased renal expression of AT₁ receptors due to decreased D₃ receptor regulation. (Circ Res. 2006;99:494-500.)

**Key Words:** AT₁ receptor ■ D₃ receptor ■ renal proximal tubule cells ■ hypertension

The proximal tubule is the major site of sodium and water reabsorption in the mammalian nephron. Paracrine regulation of sodium reabsorption in the proximal tubule by the renin/angiotensin system occurs via several angiotensin receptor subtypes (AT₁, AT₂, and AT₄). The activation of angiotensin II type 1 (AT₁) receptors by angiotensin II increases sodium transport in this nephron segment. However, in physiological conditions, the major effect of angiotensin II on sodium transport is stimulatory, via AT₁ receptors. The dopaminergic system also exerts a paracrine regulatory role on renal sodium transport in the proximal tubule. Dopamine receptors, like the angiotensin II receptors, are expressed in brush border and basolateral membranes of RPTs. In contrast to the stimulatory effect of angiotensin II on sodium transport in RPTs, the major consequence of the activation of dopamine receptors is an inhibition of sodium transport.

Inhibition of renal proximal tubular angiotensin II production or blockade of AT₁ receptors increases the natriuretic effect of the D₂-like agonist, fenoldopam. D₃-like receptor agonists also antagonize the stimulatory effect of angiotensin II, acting via AT₁ receptors, on renal proximal tubular luminal sodium transport. The 2 D₂-like (D₂ and D₃) and the 3 D₂-like (D₂, D₃, and D₄) receptors are expressed in specific segments of the mammalian kidney. Whereas the D₂ receptor is expressed mainly in collecting ducts, the D₃ receptor, the major D₂-like receptor, like the D₂ and D₃ receptors, is expressed in the proximal tubule. The distribution of D₃ receptor protein along the nephron is still uncertain. The effect of D₂ receptors on renal sodium transport is also not clear because of the lack of agonists that are highly selective to the D₂ over the D₃ receptor. However, 7-OH-DPAT, a ligand with a 50-fold selectivity to the D₃ over the D₂ receptor, increases sodium excretion in rats. Moreover, D₃ receptor–null mice have a decreased ability to excrete an acute sodium load, whereas no such limitation is found in D₂ receptor–null mice. We surmise that the D₃ receptor may be the D₂-like subtype receptor that interacts with the AT₁ receptor in rat RPTs.

Angiotensin and dopamine receptors are expressed in immortalized rat RPT cells. These RPT cells have char-
acteristics similar to freshly obtained RPTs and renal brush border membranes, at least, with regard to the D3 receptor expression in kidneys of D3 null mice. We now report that D3 and AT1 receptors colocalize in rat RPT cells. Furthermore, D3 receptor occupancy decreases expression of AT1 receptors and their immunoprecipitation with D3 receptors. The interaction between D3 and AT1 receptors also occurs in vivo because AT1 receptor expression is increased in the kidneys of D3 receptor–null mice.

Materials and Methods

D3 Dopamine Receptor–Null Mice

Mice homozygous for the D3 null receptor were bred from heterozygous mice in C57BL/6 background (The Jackson Laboratory, Bar Harbor, Me); nontransgenic mice served as controls.18 The mice were anesthetized with pentobarbital (50 mg/kg IP), tracheotomized, and blood pressure determined from the femoral artery. The renal cortices were prepared for immunoblotting as described.9,10,19,21 All experiments were approved by the Georgetown University Animal Use and Care Committee.

Cell Culture

Immortalized RPT cells from Wistar–Kyoto and spontaneously hypertensive rats (SHRs)9,10,20,21 were cultured at 37°C in 95% air/5% CO2 atmosphere in DMEM/F-12. Embryonic thoracic aortic smooth muscle cells22,23 (passage 10 to 20) from normotensive Berlin–Druckrey IX (A10; CRL 1476, American Type Culture Collection, Manassas, Va) were cultured at 37°C in 95% air/5% CO2 atmosphere in DMEM. The cells (80% confluence) were extracted in ice-cold lysis buffer, sonicated, kept on ice for 1 hour, and centrifuged at 16,000g for 30 minutes. All samples were stored at −70°C until use.

Immunoblotting

Polyclonal rabbit anti-human AT1 receptor antibodies (QDDCPK-AGRHC, 15 to 24 position on AT1 receptor) (Santa Cruz Biotechnology Inc, Santa Cruz, Calif) and rabbit antirat D3 receptor antibodies (CHVSPELYR, 405 to 413 position on the D3 receptor) (Alpha Diagnostic International, San Antonio, Tex) were used. The specificity of these antibodies has been reported.9,10,19,21

Rat RPT cells were treated with vehicle, D3 receptor agonist (PD128907) (Sigma Co, St Louis, Mo)16 or the D3 receptor antagonist (U99194A) (Research Biochemicals International, Natick, Mass)27 at the indicated concentrations and times. To prevent the oxidation of reagents, sodium metabisulfite28 was used with a final concentration of 200 μmol/L in the medium. We designed our experiments so that a time control was not needed for each treatment period. Twenty-six hours before cell lysis for immunoblotting, the cells were starved. Cells were treated with PD128907 for 24, 16, 8, or 2 hours or vehicle, as indicated. At 0 hour, the drug-treated and vehicle-treated cells were prepared for immunoblotting. All cells were incubated for 26 hours; the control cells were incubated with vehicle for 26 hours.

Immunoblotting was performed as previously reported except that the transblots were probed with the D3 (1:250) or the AT1 receptor antibody (1:200).21,25,26,29 The amount of protein transferred onto the membranes was determined by Poncet-S staining and immunoblotting for α-actin (Santa Cruz Biotechnology Inc). The receptor densities were normalized by α-actin.

RT-PCR of AT1 Receptors

A total of 2 to 3 μg of total RNA extracted from both WKY and SHR cells was used to synthesize cDNA and served as a template for amplification of AT1, and for β-actin sequences as an endogenous standard.19 For β-actin, the forward primer was 5'-GTGGTATGGGTCAAGAGGA-3' and the reverse primer was 5'-AGCCGCTAACCCTCATAGAT-3' (GenBank accession no. BC 063166). The amplification was performed with the following conditions: 35 cycles of denaturation at 94°C for 2 minutes, annealing for 30 seconds at 52.5°C, and extension for 45 seconds at 72°C. For AT1 receptor, the forward primer was 5'-CCAAAGTCACCGTCTCATC-3' and the reverse primer was 5'-CACAATGCGCTAAATTGCTTA-3' (GenBank accession no. NM-030985). The amplification was performed with the following conditions: 35 cycles of denaturation at 94°C for 2 minutes, annealing for 30 seconds at 52.5°C, and extension for 45 seconds at 72°C.30 The AT1 receptor mRNA expression was normalized by β-actin mRNA.

Immunoprecipitation

RPT cells were incubated with vehicle, or a D3 receptor agonist, PD128907 (10−7 mol/L), for 24 hours as described above. PD128907 has a 10- to 20-fold greater affinity for the D3 than the D1 receptor.36 Cell lysates (800 μg protein/mL supernatant) were incubated with D3 receptor or AT1 receptor antibody (1 μg/mL) for 1 hour and protein-G agarose at 4°C for 12 hours. The immunoprecipitates were suspended in Laemmli buffer, boiled for 10 minutes, divided into 2 aliquots, electrophoresed, transferred, and subjected to immunoblotting with the AT1 and D3 receptor antibodies.

In 1 series of experiments, the sample immunoprecipitated with the D3 receptor antibody was divided into 2 aliquots, 1 immunoblotted with the AT1 receptor antibody and the other immunoblotted with the D3 receptor antibody. The data are shown as the ratio of D3 receptor immunoprecipitate/AT1 receptor immunoblot and D3 receptor immunoprecipitate/D3 receptor immunoblot. To determine the specificity of the bands, normal rabbit IgG (negative control) and AT1 receptor antibody (positive control) were used as the immunoprecipitates instead of the D3 receptor antibody.21

In another series of experiments, we reversed the antibodies used for immunoprecipitation and immunoblotting. The samples were immunoprecipitated with the AT1 receptor antibody and divided into 2 aliquots. One was immunoblotted with the D3 receptor antibody and the other was immunoblotted with the AT1 receptor antibody. The data are shown as the ratio of AT1 receptor immunoprecipitate/D3 receptor immunoblot and AT1 receptor immunoprecipitate/AT1 receptor immunoblot. To determine the specificity of the bands, normal rabbit IgG (negative control) and D3 receptor antibody (positive control) were used as the immunoprecipitates instead of the AT1 receptor antibody.21

Immunofluorescence Microscopy

RPT cells, treated with vehicle or PD128907 (10−7 mol/L) for 24 hours at 37°C, as above, were fixed and permeabilized as reported.31 The D3 receptor was visualized using an IgG affinity-purified polyclonal rabbit anti-rat D3 receptor antibody followed by Alexa 488–goat anti–rabbit IgG antibody (Molecular Probes, Eugene, Ore). The AT1 receptor was visualized using a mouse anti–AT1 receptor monoclonal antibody (Abcam Limited, Cambridgehire, UK), followed by an Alexa Fluor 568–goat anti–mouse IgG antibody (Molecular Probes). Immunofluorescence images were acquired (Olympus AX70 laser confocal microscopy) at an excitation wavelength of 480 nm and 560 nm; emission was detected at 535 and 645 nm. Cells that were treated with only Alexa 488–goat anti–rabbit IgG or Alexa Fluor 568–goat anti–mouse IgG antibodies revealed no immunofluorescence, and omission of the anti-AT1 antibody showed no red or yellow color after merging (data not shown).

Statistical Analysis

The data were expressed as mean ± SEM. Comparison within groups was made by ANOVA for repeated measures (or paired t test when only 2 groups were compared), and comparison among groups (or t test when only 2 groups were compared) was made by ANOVA with Duncan’s test. Corresponding periods between 2 different groups were analyzed by independent t test. A value of P < 0.05 was considered significant.
Results

D3 Receptors Decrease AT1 Receptor Expression in WKY RPT Cells and Increase It in SHR RPT Cells

The D3 receptor agonist PD128907 decreased AT1 receptor expression in a concentration- and time-dependent manner. The inhibitory effect was evident at 10^{-7} mol/L with a 50% decrease (EC_{50}) in AT1 receptor expression at 7.3 × 10^{-10} mol/L (Figure 1A). The inhibitory effect of PD128907 (10^{-7} mol/L) was noted as early as 2 hours and maintained for at least 24 hours; a 50% decrease (t_{1/2}) in AT1 receptor expression occurred at 7.1 hours (Figure 1B). In contrast, in RPT cells from SHRs, PD128907 (10^{-7} mol/L per 24 hours) increased AT1 receptor expression (WKY: control = 0.1 density units [DU]; PD128907 = 0.5 ± 0.1 DU; SHR: control = 0.9 ± 0.1 DU; PD128907 = 1.6 ± 0.2 DU; n = 8) (Figure 1C).

The specificity of PD128907 as a D3 receptor agonist was also determined by studying the effect of the D3 receptor antagonist U99194.

To investigate a mechanism for D3 receptor downregulation of AT1 receptor, the RPT cells from WKY and SHRs were treated with PD128907 for 24 hours. Activation of the
D₃ receptor decreased AT₁ receptor mRNA expression in WKY cells, but increased it in SHR cells (WKY: control/H₁₁₀₀₅ 1.1/H₁₁₀₀₆ 0.1 DU, PD₁₂₈₉₀₇/H₁₁₀₀₅ 0.8/H₁₁₀₀₆ 0.03 DU; SHR: control/H₁₁₀₀₅ 0.9/H₁₁₀₀₆ 0.1 DU, PD₁₂₈₉₀₇/H₁₁₀₀₅ 1.3/H₁₁₀₀₆ 0.1 DU; P<0.05, n=6) (Figure 1E).

**D₃ Receptor Agonist Increases D₃ Receptor Expression in WKY RPT Cells, Not in SHR Cells**

To investigate the effect of a D₃ receptor agonist on its own receptor, RPT cells from SHR and WKY rats were incubated with the D₃ receptor agonist, PD₁₂₈₉₀₇ (10⁻⁷ mol/L), for 24 hours. Immunoblots showed that the D₃ receptor agonist increased its own receptor expression in WKY RPT cells, not in SHR cells (WKY: control/H₁₁₀₀₅ 1.1/H₁₁₀₀₆ 0.2, PD₁₂₈₉₀₇/H₁₁₀₀₅ 1.8/H₁₁₀₀₆ 0.2; SHR: control/H₁₁₀₀₅ 0.7/H₁₁₀₀₆ 0.15, PD₁₂₈₉₀₇/H₁₁₀₀₅ 0.6/H₁₁₀₀₆ 0.2; P<0.05, n=13), and the basal level of D₃ receptor protein expression was higher in WKY cells than in SHR cells (Figure 2).

**D₃ Receptor Colocalizes With the AT₁ Receptor in Rat RPT Cells**

To determine whether there is a potential for a direct or an indirect interaction between D₃ and AT₁ receptors, we studied the colocalization of D₃ and AT₁ receptors in rat RPT cells using immunofluorescence laser confocal microscopy. Both D₃ receptor and AT₁ receptor were found throughout the whole cell. It was also evident that the D₃ receptor and AT₁ receptor colocalized in these cells, especially in the cellular membrane (Figure 3).

To determine whether there is a physical interaction between the D₃ and the AT₁ receptors, additional experiments were performed. When D₃ receptors were immunoprecipitated with the D₃ receptor antibody and immunoblotted with the AT₁ receptor antibody, and the data were normalized by D₃ receptor immunoprecipitated and immunoblotted with D₃ receptor antibody (Figure 4A), basal coimmunoprecipitation levels were higher in WKY than in SHR cells. However, D₃

---

**Figure 2.** Differential effects of PD₁₂₈₉₀₇ (10⁻⁷ mol/L per 24 hours) on D₃ receptor expression in RPT cells from WKY and SHRs. The cells were incubated at the indicated times and concentrations. Results are expressed as the ratio of D₃ receptor and α-actin densities (n=13, *P<0.05 vs control, #P<0.05 vs WKY, ANOVA, Duncan’s test).

**Figure 3.** D₃ and AT₁ receptor colocalization in rat RPT cells from WKY rats. The cells grown on coverslips were washed, then fixed and double-immunostained for D₃ and AT₁ receptors, as described in Materials and Methods. Colocalization appears as yellow after merging the images of Alexa 488–tagged D₃ receptor (green) and Alexa 568–tagged AT₁ receptor (red).

**Figure 4.** Effect of the D₃ receptor agonist PD₁₂₈₉₀₇ on AT₁/D₃ receptor coimmunoprecipitation in RPT cells from WKY and SHRs. The cells were incubated with PD₁₂₈₉₀₇ (10⁻⁷ mol/L) for 24 hours. Thereafter, the samples were immunoprecipitated with anti–D₃ receptor antibodies and immunoblotted with anti–AT₁ receptor antibodies, normalized by D₃ receptor immunoprecipitated and immunoblotted with anti–AT₁ receptor antibody. One immunoblot (45 kDa) is depicted in the inset: lane 1, positive control; lane 2, negative control; lane 3, vehicle-treated WKY cells; lane 4, PD₁₂₈₉₀₇-treated WKY cells; lane 5, vehicle-treated SHR cells; lane 6, PD₁₂₈₉₀₇-treated SHR cells (n=5) (A). The coimmunoprecipitation results were also shown after reversing the antibodies used for immunoprecipitation and immunoblotting, using the AT₁ antibody for immunoprecipitation and the D₃ antibody for immunoblotting, and normalized by AT₁ receptor immunoprecipitated and immunoblotted with AT₁ receptor antibody (n=5) (B). *P<0.05 vs respective controls, #P<0.05 vs WKY control (ANOVA, Duncan’s test). One immunoblot (45 kDa) is depicted in the inset: lane 1, negative control; lane 2, positive control; lane 3, vehicle-treated WKY cells; lane 4, PD₁₂₈₉₀₇-treated WKY cells; lane 5, vehicle-treated SHR cells; lane 6, PD₁₂₈₉₀₇-treated SHR cells.
receptor stimulation with PD128907 decreased the percent amount of D3 receptors that coimmunoprecipitated with AT1 receptors to a greater extent in WKY than in SHR cells (Figure 4A: WKY: control=1.4±0.15 DU, PD128907=0.4±0.2 DU; SHR: control=0.5±0.1 DU, PD128907=0.25±0.06 DU, P<0.05, n=5). This occurred because PD128907 increased D3 receptor protein in WKY cells (Figure 4B) (WKY: control=0.15±0.04 DU, PD128907=1.1±0.2 DU; SHR: control=0.4±0.05 DU, PD128907=0.15±0.04 DU; P<0.05, n=5). In the SHR, D3 receptor stimulation with PD128907 markedly decreased the coimmunoprecipitation because PD128907 increased D3 receptor protein in SHR cells.

When AT1 receptors were immunoprecipitated with the AT1 receptor antibody and immunoblotted with the D3 receptor antibody, and the data normalized by AT1 receptor immunoprecipitated and immunoblotted with AT1 receptor antibody (Figure 4B), basal coimmunoprecipitation levels were also higher in WKY than in SHR cells, in agreement with the results shown in Figure 4A. However, D3 receptor stimulation with PD128907 did not change the percent amount of AT1 receptors that coimmunoprecipitated with D3 receptors in WKY cells because PD128907 decreased AT1 receptor protein in WKY cells (Figure 4B) (WKY: control=1.1±0.2 DU, PD128907=1.3±0.1 DU; SHR: control=0.4±0.05 DU, PD128907=0.15±0.04 DU; P<0.05, n=5). In the SHR, D3 receptor stimulation with PD128907 markedly decreased the coimmunoprecipitation because PD128907 increased AT1 receptor protein in SHR cells.

**Figure 5.** Immunoreactive AT1 receptors in renal cortex from D3 receptor-null mice (D3−/−) and their wild-type controls (D3+/+). Results are expressed as the ratio of D3 receptor and α-actin densities (P<0.05 vs D3 receptor wild-type, n=5, t test).

**Figure 6.** Concentration response of AT1 receptor expression in A10 cells treated with the D3 agonist PD128907. Immunoreactive AT1 receptors were determined at the indicated concentrations for 24 hours (P>0.05, n=5, ANOVA, Duncan’s test).

**AT1 Receptor Expression Is Increased in the Kidneys of D3 Receptor–Null Mice**

To determine whether the apparent D3 regulation of AT1 receptors in vitro occurred in vivo, we determined AT1 receptor expression in D3 receptor–null mice. We have reported that arterial blood pressure and renin release were increased in D3 receptor–null mice in mixed B129 and C57BL/6 background. The ability of the homozygous D3 receptor–null mice to excrete an acute sodium load was also impaired.

In the current study, we found that pentobarbital-anesthetized homozygous D3 receptor–null mice, in C57BL/6 background, had higher systolic (SBP) and diastolic (DBP) blood pressures (SBP=117±5 and DBP=85±2 mm Hg; n=5) than wild-type C57BL/6 mice (SBP=94±2 and DBP=69±3 mm Hg; n=5; P<0.05, t test). Immunoreactive AT1 receptors were also greater in the kidneys of D3 receptor–null mice than in wild-type C57BL/6 mice (null mice, 1.4±0.1 DU; wild-type mice, 0.7±0.1 DU; n=5) (Figure 5). To determine whether there was any effect of the D3 receptor on vascular AT1 receptor expression, we treated A10 cells with different concentrations of the D3 receptor agonist PD128907 (10−10 mol/L to 10−6 mol/L). We found that stimulation of the D3 receptor had no effect on AT1 receptor expression in A10 cells (Figure 6).

**Discussion**

There are several novel observations in our study. First, we showed that a D3 receptor agonist PD128907 16 decreased AT1 receptor protein and mRNA expression in rat WKY RPT cells; in contrast, the D3 receptor agonist increased AT1 receptor expression in SHR RPT cells. This effect was clearly exerted at the D3 receptor because a D3 receptor antagonist, U99194,27 blocked the effect of the D3 receptor agonist. Second, the D3 receptor agonist increased D3 receptor protein expression in WKY cells but had no effect in SHR cells. Third, AT1 receptor expression was increased in the kidneys of D3 receptor–null mice. Fourth, we demonstrated that D3 receptors colocalized and coimmunoprecipitated with AT1 receptors in rat RPT cells. D3 receptor stimulation with PD128907 decreased the percent amount of D3 receptors that coimmunoprecipitated with AT1 receptors to a greater extent in WKY than in SHR cells. However, D3 receptor stimulation did not change the percent amount of AT1 receptors that coimmunoprecipitated with D3 receptors in WKY cells and markedly decreased the coimmunoprecipitation in SHR cells.

Several studies have shown that the dopaminergic/angiotensin systems interact to regulate renal function. As stated in the introduction, sodium transport in the kidney, especially in the luminal membrane of the proximal tubule, is reciprocally regulated by these systems. For example, dopamine, via D3-like receptors, antagonizes the stimulatory effects of angiotensin II on Na⁺ uptake in rat RPTs and brush border membrane vesicles. Also, angiotensin-converting enzyme inhibition or an AT1 receptor blockade enhances the natriuretic effect of a D3-like agonist. The renal vasoconstrictor effect of angiotensin II can also be antagonized by D3-like receptor agonists.

The effect of the D3-like receptor, by itself, on renal sodium transport is controversial: stimulation, no effect, and...
even inhibition have been reported. However, under certain circumstances, such as during moderate sodium loading, D1- and D2-like receptors can synergistically inhibit sodium transport. The inhibitory effect of dopamine on sodium transport can be antagonized by both D1-like and D2-like receptor blockers. A D2-like agonist, bromocriptine, has also been reported to antagonize the stimulatory effect of angiotensin II on Na+/K+-ATPase activity in RPTs.

The mechanism for the antagonistic effect of D2-like receptors on angiotensin II action is not well understood. Aminochrome, a metabolite of the dopamine oxidative pathway, has been reported to decrease AT1 expression in a dopaminergic neuronal cell line RCSN3 of rat substantia nigra. Bromocriptine, a D2-like agonist, has also been reported to decrease AT1 receptor expression in rat RPTs. However, because bromocriptine has equal affinity to D2 and D3 receptors, it is not known which D2-like receptor mediates the inhibitory effect of bromocriptine on AT1 receptor expression in rat RPTs.

The current studies show that the D3 receptor participates in the regulation of AT1 receptor expression in RPT cells. A role for D2 receptors cannot be excluded because D2 receptors are also expressed in rat renal cortex. However, the D2 receptors expressed in rat RPTs are probably prejunctional because D2 receptor mRNA is not detected in the immortalized rat RPT cells (unpublished observations). It is also not clear whether the inhibitory effect of D2 receptors on AT1 receptor expression is direct or indirect. The D3 receptor, but probably not the D2 receptor, is the D2-like receptor that inhibits renin release. Thus, plasma renin levels are elevated in D3 receptor–null mice but not in D2 receptor–null mice. Because RPT cells can also generate angiotensin II, it is possible that a D3 receptor–mediated decrease in angiotensin II formation could have led to the decrease in AT1 receptor expression because angiotensin II has been reported to increase AT1 receptor mRNA in RPTs. However, long-term infusion of angiotensin II in vivo in rats has no effect on AT1 receptor expression in RPTs. We also have preliminary observations indicating that AT1 receptor expression in immortalized RPT cells is not increased by incubation with angiotensin II. We, therefore, suggest that D3 receptors, independent of angiotensin II, can regulate AT1 receptor expression.

The mechanism for the decrease in AT1 receptors caused by D3 receptors was also investigated in this study. Stimulation of the D1 receptor for 24 hours decreases AT1 receptor mRNA expression in WKY cells but increases it in SHR cells. This result is consistent with AT1 receptor protein result and indicates that the regulation of D3 receptors on AT1 receptor expression may occur at the transcriptional/posttranscriptional level. The decrease in AT1 receptor protein expression at 2 hours, however, is probably not attributable to alterations at the transcriptional/posttranscriptional level. In the SHRs, renal G protein–coupled receptor kinase 4 (GRK4) expression and activity are increased and may be responsible for the impaired D3 receptor function in the kidney and hypertension. The GRK4 variant, A142V, but not GRK4 wild-type transgenic mice have increased AT1 receptor protein. We have preliminary data indicating that the D3 dopamine receptor increases the degradation of the AT1 receptor. Therefore, the possibility that the D3 receptor directly or indirectly increases the degradation of the AT1 receptor cannot be ruled out. In SHRs, the decreased expression of D3 receptor, even if constitutively active, may not be sufficient to impair the degradation of AT1 receptor and, thus, the absence of a difference in total cell AT1 receptor expression between WKY and SHRs. Stimulation of D3 receptors would assist in the degradation of AT1 receptors in WKY rats, but this does not occur in SHRs, hence the increase in AT1 receptor protein with D3 receptor stimulation in the latter rat strain. In addition, in the SHRs, stimulation of an altered D3 receptor (secondary to increased GRK4 activity) may be responsible for the increase in AT1 receptor mRNA.

We found by both morphological (confocal microscopy) and biochemical (immunoprecipitation) methods that D3 and AT1 receptors can directly interact with each other. When D3/AT1 receptor coimmunoprecipitation is normalized by D3 receptor or AT1 receptor, the basal levels of D3/AT1 receptor coimmunoprecipitation are higher in WKY cells as compared with SHR cells. However, when data of D3/AT1 receptor coimmunoprecipitation are normalized by D3 or AT1 receptor, the ratios are different. Normalization by D3 receptor shows that stimulation with PD128907 decreases the percent amount of D3 receptors that coimmunoprecipitate with AT1 receptors to a greater extent in WKY than in SHR cells. This occurs because PD128907 increases D3 receptor expression in WKY but not in SHR cells. Normalization of the data with AT1 receptor shows that PD128907 does not change the D3/AT1 receptor coimmunoprecipitation in WKY cells but markedly decreases it in SHR cells.

Taking into account that PD128907 increases D3 receptor expression but decreases AT1 receptor expression in WKY cells, we suggest that in WKY cells, PD128907 decreases the amount of D3 receptors bound to AT1 receptors while keeping the same amount of AT1 receptors bound to the D3 receptor. Therefore, more D3 receptors can exert their effects while keeping AT1 receptors in check; D3 receptors are available to inhibit sodium reabsorption, and fewer AT1 receptors are available to increase sodium reabsorption in RPTs in WKY rats. In SHR cells, PD128907 increases AT1 receptor expression but does not affect D3 receptor expression. The data in Figure 4A and 4B suggest that D3 receptor stimulation with PD128907 makes more AT1 receptors than D3 receptors available to exert their effects, more sodium reabsorption is consequence.

D3 receptor–null mice in a C57BL/6 background have higher blood pressures than wild type C57BL/6 mice, confirming our previous study of D3 receptor–deficient mice with mixed B129 and C57BL/618 background. The D3 receptor–deficient mice in the previous study have increased renal renin activity, and their blood pressures are normalized by AT1 receptor blockade with losartan. It was not determined in the previous study whether the hypertension in the D3 knockout mice is caused by an increase in angiotensin II levels and/or an increase in AT1 receptors. Consistent with the ability of D3 receptors to inhibit AT1 receptor expression in immortalized RPT cells, AT1 receptor expression is increased in the kidneys of D3 receptor–null mice. It is, therefore, likely that the hypertension in D3 receptor–null mice is also related to an increase in AT1 receptor expression.
In summary, we have demonstrated that the D₃ receptor regulates AT₁ receptor expression in vivo and in vitro. It increases its own expression but decreases AT₁ receptor expression in RPT cells from normotensive WKY rats. In contrast, in RPT cells from SHRs, the D₃ receptor no longer regulates its own expression and actually increases AT₁ receptor expression. D₃ and AT₁ receptors colocalize and coimmunoprecipitate in RPT cells. Basal D₃ and AT₁ receptor coimmunoprecipitation is greater in WKY than SHR cells, but PD128907 had different effects on the percent amount of D₃ or AT₁ receptor that coimmunoprecipitated with the other receptor. It is possible that an impaired interaction between D₃ and AT₁ receptors play a role in the pathogenesis of genetic hypertension.

**Sources of Funding**

These studies were supported in part by the NIH (grants HL 23081, DK 39308, HL68866, DK52612, HL 62211, HL-41618, and HL074940) and the National Natural Science Foundation of China (grant 30470728).

**Disclosures**

None.

**References**


Activation of D3 Dopamine Receptor Decreases Angiotensin II Type 1 Receptor Expression in Rat Renal Proximal Tubule Cells


Circ Res. 2006;99:494-500; originally published online August 10, 2006;
doi: 10.1161/01.RES.0000240500.96746.ec

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/99/5/494

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/