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 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. 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.

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).1–5 The activation of angiotensin II type 1 (AT1) receptors by angiotensin II increases sodium reabsorption in the proximal tubule.1–5 However, in physiological conditions, the major effect of angiotensin II on sodium transport is stimulatory, via AT1 receptors.1,2,6

The dopaminergic system also exerts a paracrine regulatory role on renal sodium transport in the proximal tubule.7,8 Dopamine receptors, like the angiotensin II receptors, are expressed in brush border and basolateral membranes of RPTs.8–11 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.7,8

Inhibition of renal proximal tubular angiotensin II production or blockade of AT1 receptors increases the natriuretic effect of the D2-like agonist, fenoldopam.11 D2-like receptor agonists also antagonize the stimulatory effect of angiotensin II, acting via AT1 receptors, on renal proximal tubular luminal sodium transport.12,13 The 2 D2-like (D2a and D2b) and the 3 D3-like (D3a, D3b, and D3c) receptors are expressed in specific segments of the mammalian kidney.7,8,14–19 Whereas the D2 receptor is expressed mainly in collecting ducts, the D3 receptor, the major D2-like receptor, like the D2a and D3 receptors, is expressed in the proximal tubule.7–10 The distribution of D3 receptor protein along the nephron is still uncertain.8,19 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.16,17 However, 7-OH-DPAT, a ligand with a 50-fold selectivity to the D2 over the D3 receptor,16 increases sodium excretion in rats.17 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.18,19 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.20,21 These RPT cells have char-
acteristics similar to freshly obtained RPTs and renal brush border membranes, at least, with regard to the D₃ receptor.

In this study, we determined whether D₃ receptors interacted with AT₁ receptors. We also examined AT₁ 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. The mice were anesthetized with pentobarbital (50 mg/kg IP), tracheotomized, and blood pressure determined from the femoral artery. The renal kidneys of D₃ receptor–null mice. A total of 2 to 3 g of total RNA extracted from both WKY and SHR were analyzed by independent t test. A value of P < 0.05 was considered significant.

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. 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 D₁ receptor antibody. The data are shown as the ratio of D₃ receptor immunoprecipitate/AT₁ receptor immunoblot and D₃ receptor immunoprecipitate/D₁ 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.

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 D₁ receptor antibody and the other was immunoblotted with the AT₁ receptor antibody. The data are shown as the ratio of AT₁ receptor immunoprecipitate/D₁ 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.

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. The D₃ receptor was visualized using an IgG affinity-purified polyclonal rabbit anti–rat D₃ receptor antibody (Sigma Co, St Louis, Mo) or the D₃ receptor antagonist (U99194A) (Research Biochemicals International, Natick, Mass) at the indicated concentrations and times. To prevent the oxidation of reagents, sodium metabisulfite 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 D₁ (1:250) or the AT₁ receptor antibody (1:200). The amount of protein transferred onto the membranes was determined by Ponceau-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. For β-actin, the forward primer was 5'-GTGGGTATGGGTAGGTAAGG-3' and the reverse primer was 5'-AGCCGGTAACCCCTCATAG-3' (GenBank accession no. BC 065166). 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'-CCAAGTCACTCTCATCATC-3' and the reverse primer was 5'-CAACATGCGCATATACTTCTA-3' (GenBank accession no. NM-039985). 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. The AT₁ receptor mRNA expression was normalized by β-actin mRNA.

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 (EC₅₀) in AT₁ receptor expression at 7.3 × 10⁻⁷ 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 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 = 0.9 ± 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 U99194.²⁷ Consistent with the study shown in Figure 1A and 1B, PD128907 (10⁻⁷ mol/L per 24 hours) decreased AT₁ receptor expression (control = 1.2 ± 0.1 DU, PD128907 = 0.6 ± 0.1 DU, n = 5; P < 0.05). The D₃ receptor antagonist, U99194 (10⁻⁷ mol/L), by itself, had no effect on AT₁ receptor expression (U99194 = 1.2 ± 0.1 DU) but reversed the inhibitory effect of PD128907 on AT₁ receptor expression (PD128907 + U99194 = 1.1 ± 0.1 DU, n = 5) (Figure 1D).

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...
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). Immuneactive 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 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, 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 because PD128907 decreased AT1 receptor protein in WKY but not in SHR cells.

Figure 5. Immuneactive 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 mice, n = 5, t test).

Figure 6. Concentration response of AT1 receptor expression in A10 cells treated with the D3 agonist PD128907. Immuneactive AT1 receptors were determined at the indicated concentrations for 24 hours (P > 0.05, n = 5, ANOVA, Duncan’s test).
even inhibition have been reported. However, under certain circumstances, such as during moderate sodium loading, \( \text{D}_1 \) and \( \text{D}_2 \)-like receptors can synergistically inhibit sodium transport. The inhibitory effect of dopamine on sodium transport can be antagonized by both \( \text{D}_1 \)-like and \( \text{D}_2 \)-like receptor blockers. A \( \text{D}_2 \)-like agonist, bromocriptine, has also been reported to antagonize the stimulatory effect of angiotensin II on \( \text{Na}^+ / \text{K}^+ \)-ATPase activity in RPTs.

The mechanism for the antagonistic effect of \( \text{D}_2 \)-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 \( \text{D}_2 \)-like agonist, has also been reported to decrease AT1 receptor expression in rat RPTs. However, because bromocriptine has equal affinity to \( \text{D}_2 \) and \( \text{D}_3 \) receptors, it is not known which \( \text{D}_2 \)-like receptor mediates the inhibitory effect of bromocriptine on AT1 receptor expression in rat RPTs.

The current studies show that the \( \text{D}_3 \) receptor participates in the regulation of AT1 receptor expression in RPT cells. A role for \( \text{D}_3 \) receptors cannot be excluded because \( \text{D}_2 \) receptors are also expressed in rat renal cortex. However, the \( \text{D}_2 \) receptors expressed in rat RPTs are probably prejunctional because \( \text{D}_2 \) receptor mRNA is not detected in the immortalized rat RPT cells (unpublished observations). It is also not clear whether the inhibitory effect of \( \text{D}_3 \) receptors on AT1 receptor expression is direct or indirect. The \( \text{D}_3 \) receptor, but probably not the \( \text{D}_2 \) receptor, is the \( \text{D}_2 \)-like receptor that inhibits renin release. Thus, plasma renin levels are elevated in \( \text{D}_3 \) receptor–null mice but not in \( \text{D}_2 \) receptor–null mice. Because RPT cells can also generate angiotensin II, it is possible that a \( \text{D}_3 \) 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 RPT cells. 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 \( \text{D}_3 \) receptors, independent of angiotensin II, can regulate AT1 receptor expression.

The mechanism for the decrease in AT1 receptors caused by \( \text{D}_3 \) receptors was also investigated in this study. Stimulation of the \( \text{D}_3 \) 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 \( \text{D}_3 \) 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 \( \text{D}_3 \) 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 \( \text{D}_3 \) dopamine receptor increases the degradation of the AT1 receptor. Therefore, the possibility that the \( \text{D}_3 \) receptor directly or indirectly increases the degradation of the AT1 receptor cannot be ruled out. In SHRs, the decreased expression of \( \text{D}_3 \) 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 \( \text{D}_3 \) 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 \( \text{D}_3 \) receptor stimulation in the latter rat strain. In addition, in the SHRs, stimulation of an altered \( \text{D}_3 \) 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 \( \text{D}_3 \) and AT1 receptors can directly interact with each other. When \( \text{D}_3 / \text{AT1} \) receptor coimmunoprecipitation is normalized by \( \text{D}_3 \) receptor or AT1 receptor, the basal levels of \( \text{D}_3 / \text{AT1} \) receptor coimmunoprecipitation are higher in WKY cells as compared with SHR cells. However, when data of \( \text{D}_3 / \text{AT1} \) receptor coimmunoprecipitation are normalized by \( \text{D}_3 \) or AT1 receptor, the ratios are different. Normalization by \( \text{D}_3 \) receptor shows that stimulation with PD128907 decreases the percent amount of \( \text{D}_3 \) receptors that coimmunoprecipitate with AT1 receptors to a greater extent in WKY than in SHR cells. This occurs because PD128907 increases \( \text{D}_3 \) receptor expression in WKY but not in SHR cells. Normalization of the data with AT1 receptor shows that PD128907 does not change the \( \text{D}_3 / \text{AT1} \) receptor coimmunoprecipitation in WKY cells but markedly decreases it in SHR cells.

Taking into account that PD128907 increases \( \text{D}_3 \) receptor expression but decreases AT1 receptor expression in WKY cells, we suggest that in WKY cells, PD128907 decreases the amount of \( \text{D}_3 \) receptors bound to AT1 receptors while keeping the same amount of AT1 receptors bound to the \( \text{D}_3 \) receptor. Therefore, more \( \text{D}_3 \) receptors can exert their effects while keeping AT1 receptors in check; \( \text{D}_3 \) 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 \( \text{D}_3 \) receptor expression. The data in Figure 4A and 4B suggest that \( \text{D}_3 \) receptor stimulation with PD128907 makes more AT1 receptors than \( \text{D}_3 \) receptors available to exert their effects, more sodium reabsorption is 1 consequence. \( \text{D}_3 \) receptor–null mice in a C57BL/6 background have higher blood pressures than wild type C57BL/6 mice, confirming our previous study of \( \text{D}_3 \) receptor–deficient mice with mixed B129 and C57BL/6 background. The \( \text{D}_3 \) 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 \( \text{D}_3 \) knockout mice is caused by an increase in angiotensin II levels and/or an increase in AT1 receptors. Consistent with the ability of \( \text{D}_3 \) receptors to inhibit AT1 receptor expression in immortalized RPT cells, AT1 receptor expression is increased in the kidneys of \( \text{D}_3 \) receptor–null mice. It is, therefore, likely that the hypertension in \( \text{D}_3 \) 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.

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None.

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