Activation of \( D_4 \) Dopamine Receptor Decreases Angiotensin II Type 1 Receptor Expression in Rat Renal Proximal Tubule Cells

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Abstract—The dopaminergic and renin–angiotensin systems interact to regulate blood pressure. Disruption of the \( D_4 \) dopamine receptor gene in mice produces hypertension that is associated with increased renal angiotensin type 1 (AT\(_1\)) receptor expression. We hypothesize that the \( D_4 \) receptor can inhibit AT\(_1\) receptor expression and function in renal proximal tubule cells from Wistar–Kyoto (WKY) rats, but the \( D_4 \) receptor regulation of AT\(_1\) receptor is aberrant in renal proximal tubule cells from spontaneously hypertensive rats (SHRs). The \( D_4 \) receptor agonist, PD168077, decreased AT\(_1\) receptor protein expression in a time- and concentration-dependent manner in WKY cells. By contrast, in SHR cells, PD168077 increased AT\(_1\) receptor protein expression. The inhibitory effect of \( D_4 \) receptor on AT\(_1\) receptor expression in WKY cells was blocked by a calcium channel blocker, nicardipine, or calcium-free medium, indicating that calcium is involved in the \( D_4 \) receptor–mediated signaling pathway. Angiotensin II increased Na\(^+\)-K\(^+\) ATPase activity in WKY cells. Pretreatment with PD168077 decreased the stimulatory effect of angiotensin II on Na\(^+\)-K\(^+\) ATPase activity in WKY cells. In SHR cells, the inhibitory effect of \( D_4 \) receptor on angiotensin II–mediated stimulation of Na\(^+\)-K\(^+\) ATPase activity was aberrant; pretreatment with PD168077 augmented the stimulatory effect of AT\(_1\) receptor on Na\(^+\)-K\(^+\) ATPase activity in SHR cells. This was confirmed in vivo; pretreatment with PD128077 for 1 week augmented the antihypertensive and natriuretic effect of losartan in SHRs but not in WKY rats. We suggest that an aberrant interaction between \( D_4 \) and AT\(_1\) receptors may play a role in the abnormal regulation of sodium excretion in hypertension. (Hypertension. 2015;65:153-160. DOI: 10.1161/HYPERTENSIONAHA.114.04038.)

Key Words: angiotensin type 1 receptor ■ dopamine \( D_4 \) receptor ■ hypertension

Essential hypertension is a major risk factor for stroke, myocardial infarction, heart failure, and kidney failure.\(^1\) The kidney plays a major role in the long-term regulation of blood pressure, and abnormal sodium chloride metabolism is frequently encountered in hypertension.\(^2\) Therefore, many studies have focused on the abnormal renal handling of sodium chloride in the pathogenesis of essential hypertension. Hypertensive subjects have increased sodium transport in several segments of the nephron, including the renal proximal tubule (RPT) and medullary thick ascending limb. The sodium retention in hypertension is caused by enhanced sodium transport per se and a failure to respond appropriately to signals that decrease sodium transport.

Ion transport in the RPT and thick ascending limb of Henle, which is increased in essential hypertension, is regulated by numerous hormones and humoral factors, including angiotensin II and dopamine.\(^2,3\) Paracrine regulation of sodium reabsorption in the proximal tubule by the renin–angiotensin system occurs via several angiotensin receptor subtypes (angiotensin type 1 and 2 [AT\(_1\) and AT\(_2\)]).\(^2,3\) The major effect of angiotensin II on sodium transport is stimulatory, via AT\(_1\) receptors. In the adult spontaneously hypertensive rats (SHR), renal AT\(_1\) receptor expression is similar to that found in normotensive rats but the AT\(_1\) receptor–mediated sodium reabsorption is increased in the RPT of SHRs.\(^4,5\) Proximal tubule fluid reabsorption or transport (NHE3 activity) is higher in SHRs than Wistar–Kyoto (WKY) rats at 5 weeks of age but may not be always increased at 12 weeks of age.\(^6,9\) The ability of an angiotensin converting enzyme inhibitor to decrease proximal tubule fluid reabsorption has been reported to be greater in

Received June 9, 2014; first decision June 20, 2014; revision accepted October 9, 2014.

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The online-only Data Supplement is available with this article at http://hyper.ahajournals.orglookup/supp/doi:10.1161/HYPERTENSIONAHA.114.04038/-/DC1.

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Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.114.04038

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younger SHRs than in older ones, indicating increased sensitivity to endogenous angiotensin II in the younger SHRs,4 that may be related to increased renal AT1 receptors in the younger ones.5 However, the increased sensitivity of RPT transport to angiotensin II in the adult SHRs is not because of increased renal expression of AT1 receptor.6

The dopaminergic system also exerts a paracrine regulatory role on renal sodium transport in the RPT.2,3 Dopamine receptors, like AT1 receptor, are expressed in the brush border and basolateral membranes of the RPT.1 In contrast to the stimulatory effect of the AT1 receptor on sodium transport in the RPT, the major consequence of the activation of dopamine receptors is inhibition of sodium transport.2,3 According to their structure and pharmacology, dopamine receptors are classified into D1-like (D1 and D5 receptors) and D2-like (D2, D3, and D4 receptors) subtypes. D1-like receptors stimulate, whereas D2-like receptors inhibit cAMP production.3

Increasing pieces of evidence show interaction between dopamine and angiotensin II receptors.2 Our previous study also showed a negative interaction between the D1 and AT1 receptors, wherein activation of the D1 receptor inhibits AT1 receptor expression and function in RPT cells.10 Disruption of the D1 dopamine receptor gene in mice produces hypertension that is associated with increased renal AT1 receptor expression.11 The hypotensive effect of a bolus intravenous injection of the AT1 receptor antagonist losartan lasted longer in D1 receptor gene-deficient mice than their wild-type littermates.11 In the kidney, the D1 receptor is expressed in the proximal and distal convoluted tubules, collecting duct, and thick ascending limb of Henle in some species.12 Because the RPT is responsible for approximately 70% of renal sodium reabsorption, we hypothesize that activation of the D1 receptor can inhibit AT1 receptor expression and function in the RPT from WKY rats, and their interaction may be aberrant in cells from SHRs. To test the above hypothesis, we studied D1 receptor and AT1 receptor interaction in immortalized RPT cells from WKY and SHRs. Meanwhile, the antihypertensive and natriuretic effects of AT1 receptor blocker with or without D1 receptor agonist in SHRs and WKY rats were also measured in vivo. These RPT cells behave similarly to freshly obtained RPT cells, at least with regard to dopamine receptors, the AT1 receptor, and responses to G protein stimulation.12

Methods

Cell Culture

Immortalized RPT cells from WKY and SHRs were cultured at 37°C in 95% air and 5% CO2 atmosphere in DMEM/F-1210,13. The cells (80% confluency) were extracted in ice-cold lysis buffer, sonicated, kept on ice for 1 hour, and centrifuged at 16,000 g for 30 minutes. All supernatant samples were stored at −70°C until use.

Preparation of Kidney and RPT Cells

The WKY and SHRs (Taconic, Germantown, NY) were anesthetized with pentobarbital (50 mg/kg, IP), after which the kidneys were harvested and the rats euthanized (pentobarbital, 100 mg/kg, IV).

The renal cortices or cultured RPT cells were homogenized in ice-cold lysis buffer (PBS with 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L PMSF, 10 μg/mL aprotinin, and 10 μg/mL leupeptin), sonicated, kept on ice for 1 hour, and centrifuged at 16,000 g for 30 minutes. The supernatants were stored at −70°C until use for immunoblotting.10,13,14-17 All experiments were approved by the Third Military Medical University Animal Use and Care Committee.

Immunoblotting

Polyclonal rabbit anti-AT1 receptor antibodies4 (1:500) and polyclonal goat anti-D4 receptor antibodies (1:300; Santa Cruz Biotechnology, Inc, Santa Cruz, CA) were used.

Rat RPT cells were treated with vehicle (saline), D4 receptor agonist (PD168077)14,15 (Tocris Cookson Ltd, Bristol, United Kingdom), or D4 receptor antagonist (L745870)16 (Tocris Cookson Ltd, Bristol, United Kingdom), at the indicated concentrations and times. We designed our experiments so that a time control was not needed for each treatment period. Thirty-two hours before cell lysis for immunoblotting, the cells were serum-starved. The cells were treated with PD168077 for 30, 24, 16, 8, 2 hours, or vehicle, as indicated. At 0 hours, the drug-treated and vehicle-treated cells were prepared for immunoblotting. All cells were incubated for 32 hours, including the control cells incubated with vehicle.10

Immunoblotting was performed as previously reported10,11,14,16,17 except that the transblots were probed with the AT1 receptor antibody (1:400). The amount of protein transferred onto the membranes was determined by immunoblotting for α-actin (Santa Cruz Biotechnology Inc, Santa Cruz, CA) and used for the normalization of the receptor densities.10

Determination of the Second Messengers Involved in the D4 Receptor-Mediated Regulation of AT1 Receptor Expression in WKY Cells

To determine the second messenger(s) involved in the D4 receptor-mediated regulation of AT1 receptor expression in WKY cells, several inhibitors or agonists were used as follows: protein kinase C inhibitor (19–31, 10–6 mol/L), protein kinase A inhibitor (14–22, 10–6 mol/L), and calcium channel blocker (nicardipine, 10–6 mol/L). These reagents were added into the incubation medium 15 minutes before the addition of the D4 receptor agonist PD168077. The protein kinase C inhibitor 19 to 31 and nicardipine were purchased from Sigma Co; protein kinase A inhibitor 14 to 22 was purchased from Calbiochem Company21–23 (Darmstadt, Germany).

Confocal Microscopy of the Double-Stained Kidney Sections and RPT Cells

Kidneys from WKY rats were fixed with 4% paraformaldehyde (30 minutes), embedded in paraffin, sectioned (4 μm), and mounted on slides. RPT cells, grown on coverslips, were fixed with 4% paraformaldehyde (30 minutes). The slides were incubated with rabbit anti-AT1 receptor and goat anti-D4 receptor antibodies (1:100 dilution, Santa Cruz) overnight at 4°C, followed by fluorescein isothiocyanate-conjugated mouse anti-goat IgG antibody (1:1000 dilution, green) and rhodamine-conjugated mouse antirabbit IgG antibody (1:1000 dilution, red; Jackson ImmunoResearch Laboratory, West Grove, PA). Immunofluorescence images were acquired (Olympus AX70 laser confocal microscopy) at excitation wavelengths of 350 nm and 507 nm; emission was detected at 450 and 529 nm. Cells or sections that were treated with only fluorescent-conjugated secondary antibodies revealed no immunofluorescence (data not shown).

Na+-K+-ATPase Activity Assay

Na+-K+-ATPase activity was determined as the rate of inorganic phosphate release in the presence or absence of ouabain.24 Rat RPT cells were cultured in 21 cm2 plastic culture dishes were collected and centrifuged at 3000 g for 10 minutes. The cells were then placed on ice and lysed in 2 mL of lysis buffer (1 mmol/L NaHCO3, 2 mmol/L CaCl2, and 5 mmol/L MgCl2). The cellular lysates were centrifuged at 3000 g for 2 minutes to remove intact cells, debris, and nuclei. The resulting supernatant was suspended in an equal volume of 1 mol/L sodium iodide, and the mixture was centrifuged at 48,000 g for 25 minutes. The pellet (membrane fraction) was washed 2 times and suspended in 10 mmol/L Tris containing...
1 mmol/L EDTA (pH 7.4). Protein concentrations were determined by the Bradford assay (Bio-Rad Laboratories, Hercules, CA) and adjusted to 1 mg/mL. The membranes were stored at −70°C until further use.

Na⁺-K⁺-ATPase activity was measured by adding 100 μL of membrane fraction to an 800 μL reaction mixture consisting of 75 mmol/L NaCl, 5 mmol/L KCl, 5 mmol/L MgCl₂, 6 mmol/L sodium azide, 1 mmol/L Na₂EGTA, 37.5 mmol/L imidazole, 75 mmol/L Tris HCl, and 30 mmol/L histidine (pH 7.4), in the presence or absence of 1 mmol/L ouabain in a final volume of 1 mL and preincubated for 5 minutes in a water bath at 37°C. The reaction was initiated by adding 4 mmol/L Tris ATP; after 15 minutes of incubation at 37°C, the reaction was terminated by adding 50 μL of 50% trichloroacetic acid. Ouabain-insensitive ATPase activity was determined by omitting NaCl and KCl from the reaction mixtures with ouabain. The amount of phosphate produced was quantified by the addition of 1 mL of coloring reagent (10% ammonium molybdate in 10N sulfuric acid and ferrous sulfate mix buffer) to the reaction mixture. The mixture was then mixed thoroughly and centrifuged at 3000g for 10 minutes. The resulting phosphomolybdate was quantified spectrophotometrically at 740 nm, using a standard curve prepared from K₂HPO₄. The difference between total and ouabain-insensitive ATPase activity was taken as Na⁺-K⁺-ATPase activity and expressed as nmol phosphate released per mg protein per minute.

To eliminate the effect of proteases and phosphatases, protease inhibitors (1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL each leupeptin and aprotinin) and a phosphatase inhibitor (50 μmol/L sodium orthovanadate) were added in all solutions after drug or vehicle incubations.

### Determination of the Effect of PD128077 and Losartan on Blood Pressure, Urine Volume, and Urine Sodium of WKY and SHRs

The rats were pretreated with the D₄ receptor agonist PD168077 (10 ng/kg per day, for 1 week), or vehicle (saline). After anesthesia with pentobarbital (50 mg/kg, IP) and cannulation of the left carotid artery, a 30-minute recovery was allowed before systolic blood pressure from the carotid artery was measured, after which time losartan (0.3 mg/kg) was given as a bolus intravenous injection via the carotid vein; systolic blood pressure was then remeasured. Urine and sodium excretions were measured in rats kept in metabolic cages for 24 hours before and after the injection of losartan via tail vein. Urine sodium was measured by an electrolyte analyzer.

### Statistical Analysis

The data are expressed as mean±SEM. Comparison within groups was made by repeated measures ANOVA (or paired t test when only 2 groups were compared), and comparison among groups (or if only 2 groups were compared) was made by factorial ANOVA using the Holm–Sidak test. A value of P<0.05 was considered significant.

### Results

#### D₄ Receptor Localization in RPT Cells and Kidney RPTs

D₄ receptor protein (49 kDa) in the RPT cells was detected by immunoblotting, using renal homogenates as positive control (Figure 1A). The 49 kDa band was D₄ receptor protein-specific because the 49 kDa-band was no longer visible when the antibody was preadsorbed with the D₄ receptor immunizing peptide. Immunofluorescence staining in the Sprague Dawley rat kidney also showed that the D₄ and AT₁ receptors colocalized in the RPT but neither D₄ nor AT₁ receptor was observed in the glomerulus (Figure 1B). The specificity of AT₁ receptor antibody was verified in immortalized proximal tubule cells from AT₁ receptor wild-type (AT₁R+/+) and knockout (AT₁R−/−) mouse by immunostaining (Figure S1A in the online-only Data Supplement) and immunoblotting (Figure S1B).

#### D₄ Receptor Decreases AT₁ Receptor Expression in WKY RPT Cells

The D₄ receptor agonist PD168077 decreased AT₁ receptor expression in a concentration- and time-dependent manner in WKY RPT cells. The inhibitory effect was evident at 10⁻⁶ mol/L (Figure 2A). The inhibitory effect of PD168077 (10⁻⁶ mol/L) was noted as early as 2 hours and maintained for ≥30 hours (Figure 2B). The specificity of AT₁ receptor antagonist, L745870. Consistent with the study shown in Figure 2A and 2B, PD168077 (10⁻⁶ mol/L for 24 hours) decreased AT₁ receptor expression. The D₄ receptor antagonist,
L745870 (10^{-6} \text{ mol/L}), by itself, had no effect on AT_1 receptor expression but reversed the inhibitory effect of PD168077 on AT_1 receptor expression (Figure 2C). To demonstrate that PD168077 and L745870 work as expected, we observed the effect of dopamine D_4 receptor agonists and antagonists to cAMP accumulation in WKY RPT cells, and found PD168077 decreased the forskolin-stimulated cAMP accumulation and L-745870 inhibited PD168077-induced reduction of forskolin-stimulated cAMP accumulation, and indicated the agonist and antagonist work as expected (Figure S2).

**Calcium Mediates the Inhibitory Effect of the D4 Receptor on AT1 Receptor Expression in RPT Cells**

To investigate a mechanism of the D_4 receptor downregulation of AT_1 receptor expression, RPT cells from WKY rats were treated with several agonists or antagonists. The calcium channel blocker nicardipine (10^{-6} \text{ mol/L}), which had no effect on AT_1 receptor expression by itself, blocked the inhibitory effect of D_4 receptor on AT_1 receptor expression in WKY cells (Figure 3A), indicating that calcium was involved as a signaling molecule in the D_4 receptor-mediated signal transduction pathway. We also evaluated the involvement of other key cell signaling proteins with the use of a protein kinase A inhibitor (14–22, 10^{-6} \text{ mol/L}) and protein kinase C inhibitor (19–31, 10^{-6} \text{ mol/L}). None of these reagents was able to block the inhibitory effect of D_4 receptor on AT_1 receptor expression (data not shown).

To further determine the importance of extracellular calcium entry in the D_4 receptor–mediated downregulation of AT_1 receptor expression, we studied the effect of PD168077 on RPT cells grown in culture medium with or without calcium. The ability of PD168077 to downregulate the expression of AT_1 receptor protein was lost when the WKY RPT cells were maintained in calcium-free medium, indicating the requirement for cellular calcium entry in this action (Figure 3B).

**D4 Receptor Decreases AT1 Receptor Expression in WKY RPT Cells but Increases It in SHR RPT Cells**

The D_4 receptor differentially regulates AT_1 receptor expression in WKY and SHRs because PD168077 increased AT_1 receptor expression in a concentration- and time-dependent manner (Figure 3B).
Figure 4. Differential effects of D4 receptor on angiotensin type 1 (AT1) receptor expression in renal proximal tubules (RPT) cells from Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHRs). A, Concentration response of AT1 receptor protein expression in SHR RPT cells treated with varying concentrations of a D4 receptor agonist, PD168077, for 24 hours. Results are expressed as the ratio of AT1 receptor and α-actin densities (n=6; *P<0.05 vs control). B, Time course of AT1 receptor protein expression in SHR cells treated with a D4 receptor agonist, PD168077 (10−6 mol/L), for varying durations of incubation. Results are expressed as the ratio of AT1 receptor and α-actin densities (n=6; *P<0.05 vs control). C, Effect of a D4 receptor agonist (PD168077, PD) and a D4 receptor antagonist (L745870) on AT1 receptor expression. WKY and RPT cells were incubated with PD168077 (10−6 mol/L) for 24 hours. Results are expressed as the ratio of AT1 receptor and α-actin densities (n=8; *P<0.05 vs control).

Pretreatment With D4 Receptor Agonist PD168077 Decreases the Stimulatory Effect of AT1 Receptor on Na+-K+ ATPase Activity in WKY RPT but Not in SHR RPT Cells

To investigate the physiological significance of D4/AT1 receptor interaction, the effects of D4 and AT1 receptor stimulation on Na+-K+ ATPase activity were determined in WKY and SHR RPT cells. Stimulation of AT1 receptors by angiotensin II (10−11 mol/L for 15 minutes) increased Na+-K+ ATPase activities in WKY and SHR cells. However, pretreatment with PD168077 (10−6 mol/L) for 24 hours decreased the stimulatory effect of angiotensin II (10−11 mol/L for 15 minutes) on Na+-K+ ATPase activity in WKY cells, but increased it in SHR cells (Figure 4C), which could be accounted for by the disparate regulation of AT1 receptor expression by D4 receptor in WKY and SHR cells, as shown in Figures 2 and 4. Additional studies showed that the intravenous infusion of losartan (0.3 mg/kg) significantly lowered systolic blood pressure (Figure 6B) and increased urine volume and sodium excretion (Figure 6C and 6D) in PD168077-treated (10 ng/kg per day, for 1 week) SHRs, but not similarly treated WKY rats. Because the D4 receptor expression in the kidney and RPT cells is increased in the SHR (Figure S3A and S3B), consistent with a previous report, these data could be taken to indicate that the increase in renal D4 receptor expression in the SHR may be an attempt to compensate for the aberrant D4 receptor function, eg, decreased diuretic and natriuretic effects of the D4 receptor agonist PD168077 in the SHR (Figure S4).

Discussion

Dopamine and angiotensin II are 2 important regulators of sodium and water transport in the kidney serving counteracting functions. Stimulation of the D4-like dopamine receptor inhibits renal renin secretion via inhibition of macula densa cyclooxygenase. By contrast, in rats on low-salt diet, angiotensin II decreases renal dopamine production by increasing renal monoamine oxidase activity. The inhibitory effects of dopamine receptor on the renin–angiotensin system extend to the receptor level. D4-like or D2-like receptors inhibit AT1 receptor–mediated stimulation of sodium transport in the RPT. D2-like receptors are composed of 3 subtypes: D2A, D2B, and D2C receptors. It is not known which subtype(s) is involved in this action. Presynaptic D2 receptor is present in nerve cells, whereas postsynaptic D2A, D2B, and D2C receptors exist in RPT cells. Our previous study found that activation of the D4 receptor decreases AT1 receptor expression in WKY RPT cells. Whether or not the D4 receptor can inhibit AT1 receptor expression is not known. However, there is indirect evidence of a negative D4 and AT1 receptor interaction in D2 receptor null (D2−/−) mice. Renal AT1 receptor expression is higher in D2−/− mice than in the wild-type littermates, and the extent and duration of the hypotensive effect of AT1 receptor blockade is greater and longer in these D2 receptor–deficient mice. We hypothesize that the D4 receptor may have an inhibitory effect on AT1 receptor expression and function in kidney, and an aberrant interaction between D4 and AT1 receptor is involved into the pathogenesis of hypertension. This hypothesis was tested in this study; we found that stimulation of D4 receptor inhibits AT1 receptor expression in WKY RPT cells but increases it in SHR RPT cells. Pretreatment of WKY RPT cells with a D4 receptor agonist for 24 hours inhibits AT1 receptor expression, thereby inhibiting the AT1-stimulated Na+-K+ ATPase activity. By contrast in SHR RPT cells, because of the stimulatory effect of the D4 receptor on AT1 receptor expression, pretreatment for 24 hours with D4 receptor agonist augments the AT1 receptor–mediated stimulation of Na+-K+ ATPase activity,
which would increase renal sodium reabsorption, and lead to increased blood pressure in vivo.

The regulation of \( \text{AT}_1 \) receptor expression by the \( \text{D}_4 \) receptor could be via direct or indirect mechanisms. It is known that angiotensin II can regulate \( \text{AT}_1 \) receptor expression\(^1\) and the activation of a \( \text{D}_4 \)-like receptor, \( \text{D}_4 \) receptor, inhibits the renin release.\(^2\) The \( \text{D}_4 \) receptor is also expressed in the RPT cells,\(^3\) but whether or not the \( \text{D}_4 \) receptor can affect angiotensin II synthesis in RPT cells is not known. Our previous study showed no difference in plasma renin concentration between \( \text{D}_4^{+/+} \) and \( \text{D}_4^{-/-} \) mice.\(^4\) Our present study did not find a difference in renin concentration in the culture medium of WKY RPT cells in the presence or absence of \( \text{D}_4 \) receptor agonist (data not shown). We, therefore, suggest that the \( \text{D}_4 \) receptor, independent of angiotensin II, can regulate \( \text{AT}_1 \) receptor expression.

The mechanism for the decrease in \( \text{AT}_1 \) receptor expression caused by the \( \text{D}_4 \) receptor in WKY rats was also investigated in this study. Calcium plays an important role in the regulation of \( \text{AT}_1 \) receptor expression; a high calcium diet decreases \( \text{AT}_1 \) receptor expression in the rat kidney.\(^5\) Elevation of \([\text{Ca}^2+]_i\) in RPT cells leads to downregulation of \( \text{AT}_1 \) receptors in kidney of diabetic rats, and normalization of the \([\text{Ca}^2+]_i\) by treatment of the diabetic rats with calcium channel blocker, amlodipine, prevents the elevation of \([\text{Ca}^2+]_i\) and downregulation of \( \text{AT}_1 \) receptor protein and mRNA expressions.\(^6\) Our present study found that a decrease in intracellular calcium, caused by a calcium channel blocker or calcium-free medium, blocks the inhibitory effect of the \( \text{D}_4 \) receptor on \( \text{AT}_1 \) receptor expression in WKY cells. This indicates that calcium is involved as a signaling molecule in the \( \text{D}_4 \) receptor–mediated downregulation of \( \text{AT}_1 \) receptor expression in WKY cells.

In summary, we have demonstrated that the \( \text{D}_4 \) receptor downregulates \( \text{AT}_1 \) receptor expression in WKY RPT cells via the activation of the calcium channel. The regulation of the \( \text{AT}_1 \) receptor by the \( \text{D}_4 \) receptor has physiological significance. Pretreatment of WKY RPT cells with a \( \text{D}_4 \) receptor agonist for 24 hours reduces the stimulatory effect of \( \text{AT}_1 \) receptor on Na\(^{+}\)-K\(^{+}\) ATPase activity. However, in SHR RPT cells, the regulation of \( \text{D}_4 \) receptor of \( \text{AT}_1 \) receptor expression and function is aberrant; \( \text{D}_4 \) receptor stimulation (PD128077) increases \( \text{AT}_1 \) receptor expression and augments the stimulatory effect of angiotensin II on Na\(^{+}\)-K\(^{+}\) ATPase activity. Pretreatment with PD128077 for 1 week augments the antihypertensive, diuretic, and natriuretic effects of losartan in SHRs. An aberrant interaction between \( \text{D}_4 \) and \( \text{AT}_1 \) receptors may be involved in the pathogenesis of genetic hypertension.

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**Figure 5.** Colocalization and coimmunoprecipitation of \( \text{D}_4 \) and angiotensin type 1 (\( \text{AT}_1 \)) receptors in Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) renal proximal tubules (RPT) cells. A, Colocalization of \( \text{D}_4 \) and \( \text{AT}_1 \) receptors in WKY and SHR RPT cells. The cells were washed, fixed, and immunostained for \( \text{D}_4 \) receptor and \( \text{AT}_1 \) receptor, as described in the Methods. Colocalization appears as yellow after merging the images of fluorescein isothiocyanate–tagged \( \text{D}_4 \) receptor (green) and rhodamine-tagged \( \text{AT}_1 \) receptor (red). B, Coimmunoprecipitation of \( \text{D}_4 \) and \( \text{AT}_1 \) receptors in WKY and SHR RPT cells. The cells were immunoprecipitated with \( \text{D}_4 \) receptor antibodies and immunoblotted with \( \text{AT}_1 \) receptor antibodies (*\( P < 0.05 \) vs WKY; \( n=3 \)). One immunoblot (43 kDa) is depicted in the inset. For PC, \( \text{AT}_1 \) receptor antibody was used, and for NC, IgG was used instead of \( \text{D}_4 \) receptor antibody as the immunoprecipitant. PC indicates positive control; and NC, negative control.
Dopamine, produced in neural and non-neural tissues, is now recognized to serve an important role in the regulation of sodium balance and blood pressure. According to their structure and pharmacology, dopamine receptors are classified into D1-like (D1 and D5) and D2-like (D2, D3, and D4) receptors. D1-like receptors stimulate, whereas D2-like receptors inhibit, cAMP production.3,12 Previous studies have found that the activation of D1-like or D2-like receptor inhibits AT1 receptor–mediated stimulation of sodium transport in RPTs and RPT brush border membranes.29,30 Other studies have also shown that stimulation of the D1, D3, and D4 receptors inhibits AT1 receptor expression and function.10,12,14,15 Our present study found that the D4 receptor, similar to D1, D3 and D5 receptors, is also involved in this process. Because D1-like and D2-like receptors synergistically increase sodium excretion,32 it is possible that the D4 receptor, together with the D1, D3, and D4 receptors, synergistically inhibits AT1 receptor expression and function in WKY RPT cells. However, this conjecture needs to be confirmed in future studies.

**Figure 6.** Effect of D4 receptor agonist on the function of the angiotensin type 1 (AT1) receptor. A, Effect of pretreatment with D4 receptor agonist on the stimulatory effect of AT1 receptor on Na+-K+-ATPase activity in Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) renal proximal tubules cells. The cells were pretreated with D4 receptor agonist, PD168077 (10−6 mol/L for 24 hours), or vehicle (dH2O) for 24 hours. After washing for 15 mins, the cells were treated with angiotensin II (10−11 mol/L) for 15 mins. Results are expressed as μmol phosphate released per mg protein per minute (*P<0.05 vs control; #P<0.05 vs angiotensin II alone; n=8/group). B, Effect of AT1 receptor blockade on systolic blood pressure (SBP) in WKY and SHRs with long-term D4 receptor agonist treatment. The rats were pretreated with D4 receptor agonist, PD168077 (10 ng/kg per day), or vehicle (saline) for 1 week. Losartan (0.3 mg/kg) lowered SBP to a greater extent in PD168077-treated than vehicle-treated SHRs. The mice were anesthetized with pentobarbital and SBPs measured from the left carotid artery. SBPs were obtained after a 30 minute-stabilization period (n=3; *P<0.05 vs vehicle-treated SHR or WKY rats at the same time point). C, Effect of a D4 receptor agonist, PD168077, and AT1 receptor antagonist on urine volume in WKY and SHRs. PD168077 and losartan were used as in Figure 6B. Twenty-four–hour urine volume (normalized by body weight) was measured in rats kept in metabolic cages after losartan (0.3 mg/kg) or vehicle injection (n=5; *P<0.05 vs vehicle-treated SHRs; #P<0.05 vs losartan-treated SHRs). D, Effects of PD168077 and AT1 receptor antagonist on urine sodium in WKY and SHRs. Urine sodium excretion was measured in losartan-treated (0.3 mg/kg) or vehicle-treated rats (n=5; *P<0.05 vs vehicle-treated SHRs; #P<0.05 vs losartan-treated SHRs).

**Sources of Funding**

These studies were supported in part by grants from the National Natural Science Foundation of China (31130029, 30925018), the National Basic Research Program of China (2012CB517801), and the US National Institutes of Health (5P01HL074940).

**Disclosures**

None.

**References**


Novelty and Significance

What Is New?

- The dopaminergic and renin–angiotensin systems interact to regulate blood pressure. Disruption of the D4 dopamine receptor gene in mice produces hypertension that is associated with increased renal angiotensin type 1 (AT1) receptor expression. In these studies, we found that the D4 receptor downregulates AT1 receptor expression in Wistar–Kyoto renal proximal tubules cells via the activation of the calcium channel. The regulation of the AT1 receptor by the D4 receptor has physiological significance. Pretreatment of Wistar–Kyoto renal proximal tubules cells with a D4 receptor antagonist for 24 hours reduces the stimulatory effect of AT1 receptor on Na+/K+ ATPase activity. However, in spontaneously hypertensive rats' renal proximal tubules cells, the regulation of D4 receptor of AT1 receptor expression and function is aberrant; D4 receptor stimulation (PD128077) increases AT1 receptor expression and augments the stimulatory effect of angiotensin II on Na+/K+ ATPase activity. Pretreatment with PD128077 for 1 week augments the antihypertensive, diuretic, and natriuretic effects of losartan in spontaneously hypertensive rats. We suggest that an aberrant interaction between D4 and AT1 receptors may play a role in the abnormal regulation of sodium excretion in hypertension.

What Is Relevant?

- The present study reinforces the role of dopamine D4 receptor in hypertension and shows the different regulation of D4 receptor on AT1 receptor expression and function in Wistar–Kyoto rats and spontaneously hypertensive rats. The aberrant interaction between D4 and AT1 receptors may be involved in the pathogenesis of genetic hypertension. The results imply that the regulation of the AT1 receptor by the D4 receptor may be an effective therapeutic approach for essential hypertension.

Summary

The present study reinforces the role of renal dopamine receptor in hypertension and shows that the D4 receptor downregulates AT1 receptor expression in Wistar–Kyoto renal proximal tubules cells via the activation of the calcium channel. However, the regulation of D4 receptor of AT1 receptor expression and function is aberrant in hypertension. The aberrant interaction between D4 and AT1 receptors may be involved in the pathogenesis of genetic hypertension.
Activation of D4 Dopamine Receptor Decreases Angiotensin II Type 1 Receptor Expression in Rat Renal Proximal Tubule Cells

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Hypertension. 2015;65:153-160; originally published online November 3, 2014;
doi: 10.1161/HYPERTENSIONAHA.114.04038

An erratum has been published regarding this article. Please see the attached page for:
/content/suppl/2016/04/11/HYPERTENSIONAHA.114.04038.DC2

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/65/1/153

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2014/11/03/HYPERTENSIONAHA.114.04038.DC1
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Figure S2 in the online-only Data Supplement has been corrected to show that the experiments were conducted in the presence of forskolin to stimulate cAMP accumulation, except for the basal study. The figure legend that previously read, “Effect of D₄ receptor agonist, PD168077 (10⁻⁶M/30min) and D₄ receptor antagonist, L745870 (10⁻⁶M/30min), on cAMP accumulation in WKY RPT cells. The cells were incubated with the indicated reagents. Results are expressed as the percent of control (n=8, *P<0.05 vs. others, #P<0.05 v.s. PD168077 alone),” has been changed to read, “Effect of D₄ receptor agonist, PD168077 (10⁻⁶M/30min) and D₄ receptor antagonist, L745870 (10⁻⁶M/30min), on forskolin-stimulated cAMP accumulation in WKY RPT cells. The cells were incubated with the indicated reagents. Results are expressed as the percent of basal cAMP accumulation (n=8, *P<0.05 vs. others, #P<0.05 vs. PD168077 alone).”

On p 156 in the article, left column, starting on line 3, the sentence that previously read, “To demonstrate that PD168077 and L745870 work as expected, we observed the effect of dopamine D₄ receptor agonists and antagonists to cAMP accumulation in WKY RPT cells, and found PD168077 increased the cAMP accumulation and L745870 inhibited the increased cAMP accumulation by PD168077, and indicated the agonist and antagonist work as expected (Figure S2),” has been changed to read, “To demonstrate that PD168077 and L745870 work as expected, we observed the effect of dopamine D₄ receptor agonists and antagonists to cAMP accumulation in WKY RPT cells, and found PD168077 decreased the forskolin-stimulated cAMP accumulation and L-745870 inhibited PD168077-induced reduction of forskolin-stimulated cAMP accumulation, and indicated the agonist and antagonist work as expected (Figure S2).”

The correct Figure S2 is shown below.

The authors apologize for these errors.

These corrections have been made to the current online version of the article, which is available at http://hyper.ahajournals.org/content/65/1/153.full.
Activation of D₄ dopamine receptor decreases AT₁ angiotensin II receptor expression in rat renal proximal tubule cells

Short title: D₄ receptor-mediated regulation of AT₁ receptors in kidney

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Figure S1A. Immunofluorescence staining of AT$_1$ receptors in RPT cells from AT$_1$R+/+ and AT$_1$R -/- mice. AT$_1$ receptors appear as red. These studies were repeated at least three times. (scale bar = 50 mm)
Figure S1B. AT₁ receptors expression in RPT cells from AT₁R+/+ and AT₁R -/- mice. The protein (100mg) from AT₁R +/+ cell lysate (lane 1) and AT₁R -/- cell lysate (lane 2) were subjected to immunoblotting with anti-AT₁R antibody (1:400). The band was not visible in protein from AT₁R -/- cells lysate.
**Figure S2.** Effect of D₄ receptor agonist, PD168077 (10⁻⁶M/30min) and D₄ receptor antagonist, L745870 (10⁻⁶M/30min), on cAMP accumulation in WKY RPT cells. The cells were incubated with the indicated reagents. Results are expressed as the percent of control (n=8, *P<0.05 v.s. others, #P<0.05 v.s. PD168077 alone).
Figure S3A. D₄ receptor expression in RPT cells from WKY and SHRs. Results are expressed as the ratio of D₄ receptor and α–actin densities (n = 10, *P<0.05 vs. control).
**Figure S3B.** $D_4$ receptor expression in kidneys from WKY and SHRs. Results are expressed as the ratio of $D_4$ receptor and $\alpha$–actin densities ($n = 4$, *$P<0.05$ vs. control).
**Fig. S4A**

**Figure S4A.** Effect of the intrarenal infusion of D₄R agonist PD168077 on urine flow in WKY and SHRs. Varying doses of PD168077 (0.5–5.0 μg/kg per minute) were infused into the right suprarenal artery of anesthetized rats. (*P<0.05 vs. control, repeated-measures ANOVA, Holm–Sidak test; # P<0.05 vs. SHRs, t-test, n = 4)**
Figure S4B. Effect of the intrarenal infusion of D₄R agonist PD168077 on sodium excretion in WKY and SHRs. Varying doses of PD168077 (0.5–5.0 mg/kg per minute) were infused into the right suprarenal artery of anesthetized rats on regular diet. (*P<0.05 vs. SHR, repeated-measures ANOVA, Holm–Sidak test; # P<0.05 vs. control, t-test, n = 4).
肾（摘要）

激活多巴胺D_{1}受体下调大鼠肾脏近端小管上皮细胞AT_{1}受体的表达

Activation of D4 Dopamine Receptor Decreases Angiotensin II Type 1 Receptor Expression in Rat Renal Proximal Tubule Cells

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曾春雨 陈昱 译

多巴胺能系统与肾素-血管紧张素系统共同调节着血压水平。有研究报道，敲除多巴胺D_{1}受体可建立小鼠的高血压模型，而其肾脏AT_{1}受体的表达则显著增加。本研究设想多巴胺D_{4}受体可抑制Wistar-Kyoto（WKY）大鼠肾脏近端小管上皮细胞AT_{1}受体的表达及功能。并且，这种调节作用的失衡可能参与了大鼠自发性高血压（spontaneously hypertensive rats，SHR）的发生。本研究结果显
示，D_{1}受体激动剂PD168077呈浓度及时间依赖性地抑制WKY细胞AT_{1}受体蛋白的表达，而在SHR细胞，PD168077则上调了AT_{1}受体蛋白的表达。D_{4}受体对AT_{1}受体的抑制作用可能通过钙通道阻断剂或钙通道调节或通过Ca^{2+}参与了D_{4}受体介导的信号通路。Ang II 显著增加了WKY细胞Na^{+}-K^{+}ATP酶的活性，而PD168077预处理可显著抑制Ang II的作用。在SHR细胞，D_{4}受体对AT_{1}受体的抑制作用更明显，而PD168077预处理增强了Ang II对Na^{+}-K^{+}ATP酶活性的抑制作用。同时，体内研究证实，在SHR大鼠，PD128077预处理1周可明显增强氯沙坦的抗高血压及排钠利尿作用，而在WKY大鼠，PD128077则无此作用。本研究提示多巴胺D_{4}受体对AT_{1}受体的异常调节在原发性高血压的发生中具有重要作用。

（Hypertension. 2013;65:153-160.）

肾去神经术（摘要）

经皮肾去交感神经术对单纯收缩期高血压患者降压效应减小

Reduced Effect of Percutaneous Renal Denervation on Blood Pressure in Patients With Isolated Systolic Hypertension

Sebastian Ewen, Christian Ukena, Dominik Linz, Ingrid Kindermann, Bodo Cremers, Ulrich Laufs, Stefan Wagenpfeil, Roland E. Schmieder, Michael Böhm, Felix Mahfoud

朱彦琪 译 蔡秋艳 审校

肾去交感神经术能够降低某些难治性高血压患者的血压，但是对单纯收缩期高血压（ISH）患者的疗效尚未明确。本研究入组126例患者，63例为ISH（收缩压≥140 mmHg，舒张压<90 mmHg），63例为混合型高血压（CH，收缩压≥140 mmHg，舒
张压≥90 mmHg），定义为已经使用3种或以上的降压药物之后，基线诊室血压依然≥140 mmHg。肾去交感神经术治疗后3、6、12个月时随访发现，ISH组患者收缩压（SBP）分别下降17、18、17 mmHg，舒张压（DBP）分别下降5，4，4 mmHg；CH组患者SBP分别下降28、27、30 mmHg，DBP分别下降13、16、18 mmHg。在各个时间点，ISH组患者血压的下降值都小于CH组（P<0.05）。术后3、6、12个月时随访24小时动态血压，CH组SBP及DBP分别下降10、13、15 mmHg和6，6，9 mmHg，而ISH组分别下降4，8，7 mmHg（P=0.032，P=0.001，P=0.009）和3，4，2 mmHg（P=0.08，P=0.001，P=0.130）。动态血压检测中，SBP在第3，12个月，DBP在第12个月时的降幅明显更小。结论：肾去交感神经术治疗能够降低ISH患者的诊室血压及动态血压，但是其降压程度弱于CH患者中的效果。

（Hypertension. 2013;65:193-199.）