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

Ken Chen, Kun Deng,* Xiaoyan Wang,* Zhen Wang, Shuo Zheng, Hongmei Ren, Duofen He, Yu Han, Laureano D. Asico, Pedro A. Jose, Chunyu Zeng

Abstract—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. We hypothesize that the D₄ receptor can inhibit AT₁ receptor expression and function in renal proximal tubule cells from Wistar–Kyoto (WKY) rats, but the D₄ receptor regulation of AT₁ receptor is aberrant in renal proximal tubule cells from spontaneously hypertensive rats (SHRs). The D₄ receptor agonist, PD168077, decreased AT₁ receptor protein expression in a time- and concentration-dependent manner in WKY cells. By contrast, in SHR cells, PD168077 increased AT₁ receptor protein expression. The inhibitory effect of D₄ receptor on AT₁ receptor expression in WKY cells was blocked by a calcium channel blocker, nicardipine, or calcium-free medium, indicating that calcium is involved in the D₄ 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 D₄ receptor on angiotensin II–mediated stimulation of Na⁺-K⁺ ATPase activity was aberrant; pretreatment with PD168077 augmented the stimulatory effect of AT₁ 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 SHR but not in WKY 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. (Hypertension. 2015;65:153-160. DOI: 10.1161/HYPERTENSIONAHA.114.04038.) • Online Data Supplement

Key Words: angiotensin type 1 receptor ▪ dopamine D₄ 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.²³ 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 [AT₁ and AT₂]).²³ The major effect of angiotensin II on sodium transport is stimulatory, via AT₁ receptors. In the adult spontaneously hypertensive rats (SHR), renal AT₁ receptor expression is similar to that found in normotensive rats but the AT₁ receptor–mediated sodium reabsorption is increased in the RPT of SHRs.⁴⁵ 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.⁶⁷ The ability of an angiotensin converting enzyme inhibitor to decrease proximal tubule fluid reabsorption has been reported to be greater in...
younger SHRs than in older ones, indicating increased sensitivity to endogenous angiotensin II in the younger SHRs,6 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.5

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 D2 receptors) and D2-like (D2a, D2b, and D2c 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 ≈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-12.10,13 The cells (80% confluence) were extracted in ice-cold lysis buffer, sonicated, kept on ice for 1 hour, and centrifuged at 16000 g for 30 minutes. The 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 removed and 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 16000 g for 30 minutes. The supernatants were stored at −70°C until use for immunoblotting.10,11,14–16 All experiments were approved by the Third Military Medical University Animal Use and Care Committee.

Immunoblotting

Polyclonal rabbit anti-AT1 receptor antibodies (1:500) and polyclonal goat anti-D1 receptor antibodies (1:300; Santa Cruz Biotechnology, Inc, Santa Cruz, CA) were used.

Rat RPT cells were treated with vehicle (saline), D1 receptor agonist (PD168077)15,17 (Tocris Cookson Ltd, Bristol, United Kingdom), or D1 receptor antagonist (L745870)18 (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 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 D1 Receptor-Mediated Regulation of AT1 Receptor Expression in WKY Cells

To determine the second messenger(s) involved in the D1 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 D1 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 Company (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-D1 receptor antibodies (1:100 dilution, Santa Cruz) overnight at 4°C, followed by fluorescein isothiocyanate–conjugated mouse antigoat 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.21 Rat RPT cells were treated with vehicle (dH2O), and D1 receptor agonist (PD168077), at the indicated concentrations and durations of incubation. To prepare membranes for Na+-K+-ATPase activity assay, RPT cells 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

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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 Na4EGTA, 37.5 mmmol/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 quantitated 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 SHR**

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 determined by adding 100 μL of urine to a final volume of 800 μL reaction mixture. The mixture was then mixed thoroughly 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 quantitated 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.

**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 t test when 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 knock-out (AT1R−/−) mouse by immunostaining (Figure 1A). The specificity of PD168077 as a D4 receptor agonist was also determined by studying the effect of the D4 receptor antagonist, L745870. Consistent with the study shown 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 PD168077 as a D4 receptor agonist was also determined by studying the effect of the D4 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 mol/L), by itself, had no effect on AT1 receptor expression but reversed the inhibitory effect of PD168077 on AT1 receptor expression (Figure 2C). 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).

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

To investigate a mechanism of the D4 receptor downregulation of AT1 receptor expression, RPT cells from WKY rats were treated with several agonists or antagonists. The calcium channel blocker nicardipine (10^-6 mol/L), which had no effect on AT1 receptor expression by itself, blocked the inhibitory effect of D4 receptor on AT1 receptor expression in WKY cells (Figure 3A), indicating that calcium was involved as a signaling molecule in the D4 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 mol/L) and protein kinase C inhibitor (19–31, 10^-6 mol/L). None of these reagents was able to block the inhibitory effect of D4 receptor on AT1 receptor expression (data not shown).

To further determine the importance of extracellular calcium entry in the D4 receptor–mediated downregulation of AT1 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 AT1 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 D4 receptor differentially regulates AT1 receptor expression in WKY and SHRs because PD168077 increased AT1 receptor expression in a concentration- and time-dependent manner.
manner in SHR RPT cells (Figure 4A and 4B), whereas the opposite of that was observed in WKY RPT cells (Figures 2 and 3). Additional studies confirmed that PD168077 (10^{-6} mol/L for 24 hours) decreased AT1 receptor expression in WKY cells but increased AT1 receptor expression in SHR cells (Figure 4C). There was colocalization of D4 and AT1 receptors in kidneys (Figure 1B) and RPT cells (Figure 5A). The coimmunoprecipitation of D4 receptor and AT1 receptor was lesser in SHR than WKY RPT cells (Figure 5B).

**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 6A), 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.\(^2,3,10,11,14,16\) Stimulation of the D4-like dopamine receptor inhibits renal renin secretion via inhibition of macula densa cyclooxygenase.\(^27\) By contrast, in rats on low-salt diet, angiotensin II decreases renal dopamine production by increasing renal monoamine oxidase activity.\(^28\) 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.\(^29,30\) D4-like receptors are composed of 3 subtypes: D2, D3, and D4 receptors. It is not known which subtype(s) is involved in this action. Presynaptic D2 receptor is present in nerve cells, whereas postsynaptic D2, D3, and D4 receptors exist in RPT cells.\(^13\) Our previous study found that activation of the D4 receptor decreases AT1 receptor expression in WKY RPT cells.\(^10\) 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.\(^11\) 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 AT₁ receptor expression by the D₄ receptor could be via direct or indirect mechanisms. It is known that angiotensin II can regulate AT₁ receptor expression and the activation of a D₂-like receptor, D₄ receptor, inhibits the renin release. The D₄ receptor is also expressed in the RPT cells, but whether or not the D₄ receptor can affect angiotensin II synthesis in RPT cells is not known. Our previous study showed no difference in plasma renin concentration between D₄−/− and D₄+/+ mice. 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 D₄ receptor agonist (data not shown). We, therefore, suggest that the D₄ receptor, independent of angiotensin II, can regulate AT₁ receptor expression.

The mechanism for the decrease in AT₁ receptor expression caused by the D₄ receptor in WKY rats was also investigated in this study. Calcium plays an important role in the regulation of AT₁ receptor expression; a high calcium diet decreases AT₁ receptor expression in the rat kidney. Elevation of [Ca²⁺]i in RPT cells leads to downregulation of AT₁ receptors in kidney of diabetic rats, and normalization of the [Ca²⁺]i by treatment of the diabetic rats with calcium channel blocker, amlodipine, prevents the elevation of [Ca²⁺]i and downregulation of AT₁ receptor protein and mRNA expressions. 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 D₄ receptor on AT₁ receptor expression in WKY cells. This indicates that calcium is involved as a signaling molecule in the D₄ receptor–mediated downregulation of AT₁ receptor expression in WKY cells.

In summary, we have demonstrated that the D₄ receptor downregulates AT₁ receptor expression in WKY RPT cells via the activation of the calcium channel. The regulation of the AT₁ receptor by the D₄ receptor has physiological significance. Pretreatment of WKY RPT cells with a D₄ receptor agonist for 24 hours reduces the stimulatory effect of AT₁ receptor on Na⁺–K⁺ ATPase activity. However, in SHR RPT 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 SHRs. An aberrant interaction between D₄ and AT₁ receptors may be involved in the pathogenesis of genetic hypertension.

Figure 5. Colocalization and coimmunoprecipitation of D₄ and angiotensin type 1 (AT₁) receptors in Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) renal proximal tubules (RPT) cells. A, Colocalization of D₄ and AT₁ receptors in WKY and SHR RPT cells. The cells were washed, fixed, and immunostained for D₄ receptor and AT₁ receptor, as described in the Methods. Colocalization appears as yellow after merging the images of fluorescein isothiocyanate–tagged D₄ receptor (green) and rhodamine-tagged AT₁ receptor (red). B, Coimmunoprecipitation of D₄ and AT₁ receptors in WKY and SHR RPT cells. The cells were immunoprecipitated with D₄ receptor antibodies and immunoblotted with AT₁ receptor antibodies (*P<0.05 vs WKY; n=3). One immunoblot (43 kDa) is depicted in the inset. For PC, AT₁ receptor antibody was used, and for NC, IgG was used instead of D₄ receptor antibody as the immunoprecipitant. PC indicates positive control; and NC, negative control.
Perspectives

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.

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Disclosures

None.

References


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**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 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 tubule cells with D1 receptor agonist 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 D1 receptor of AT1 receptor expression and function is aberrant; D1 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 AT 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 tubule cells via the activation of the calcium channel. However, the regulation of D1 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.
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http://hyper.ahajournals.org/content/67/6/e22.full.pdf

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2014/11/03/HYPERTENSIONAHA.114.04038.DC1
http://hyper.ahajournals.org/content/suppl/2016/04/11/HYPERTENSIONAHA.114.04038.DC2

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 v.s. 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

Ken Chen¹,², Kun Deng*¹,², Xiaoyan Wang³, Zhen Wang¹,², Shuo Zheng¹,², Hongmei Ren¹,², Duofen He¹,², Yu Han¹,², Laureano D. Asico³, Pedro A. Jose³, Chunyu Zeng¹,²

¹Department of Cardiology, Department of Cardiology, Daping Hospital, The Third Military Medical University, Chongqing Institute of Cardiology, Chongqing, P.R.China; ²Chongqing Institute of Cardiology, Chongqing, P.R.China; ³Division of Nephrology, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA.

**Address correspondence to:**
Chunyu Zeng, MD, PhD
Department of Cardiology
Daping Hospital
The Third Military Medical University
Chongqing City, 400042; P.R.China
Tel/Fax: 011-86-23-68757808
E-mail: cyzeng1@hotmail.com

*co-first author
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.
**Fig. S2**

*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. $D_4$ receptor expression in RPT cells from WKY and SHRs. Results are expressed as the ratio of $D_4$ receptor and $\alpha$–actin densities ($n = 10$, *P*<0.05 vs. control).
Figure S3B. D₄ receptor expression in kidneys from WKY and SHRs. Results are expressed as the ratio of D₄ receptor and α–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D₄受体下调大鼠肾脏近端小管上皮细胞AT₁受体的表达

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

Ken Chen, Kun Deng, Xiaoyan Wang, Zhen Wang, Shuo Zheng, Hongmei Ren, Duofen He, Yu Han, Laureano D. Asico,
Pedro A. Jose, Chunyu Zeng

曾春雨 陈显 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D₄受体可以建立小鼠的高血压模型，而其肾脏AT₁受体的表达则显著增加。本研究设想多巴胺D₄受体可抑制Wistar-Kyoto（WKY）大鼠肾脏近端小管上皮细胞AT₁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。本研究结果数据显示，D₄受体激动剂PD168077呈浓度及时间依从性地抑制WKY细胞AT₁受体蛋白的表达，而在SHR细胞，PD168077则上调了AT₁受体蛋白的表达，D₄受体对AT₁受体的抑制作用可能被钙通道阻断剂或钙拮抗剂所阻断，提示Ca²⁺参与了D₄受体介导的信号通路。Ang II明显增强了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，D₄受体对AT₁受体的抑制作用受损，而PD168077预处理增强了Ang II对Na⁺-K⁺ATP酶活性的刺激作用。同时，体内实验证实，在SHR大鼠，PD128077预处理1周可明显增强氨氯地平的抗高血压及降钠利尿作用，而在WKY大鼠，PD128077则无此作用。本研究提示多巴胺D₄受体对AT₁受体的异常调节在原发性高血压的发生中具有重要作用。


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 裴秋艳 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（ISHI）患者的疗效尚未明确。本研究入选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要小于CH组（P<0.05）。术后3、6、12个月时随访24小时动态血压，ISH组SBP及DBP分别下降10、13、15 mmHg和6、6、9 mmHg，而CH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个月时的降幅明显减小。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。