Mechanisms of Dopamine D₁ and Angiotensin Type 2 Receptor Interaction in Natriuresis

Shetal H. Padia, Brandon A. Kemp, Nancy L. Howell, Susanna R. Keller, John J. Gildea, Robert M. Carey

Abstract—Renal dopamine D₁-like receptors (D₁Rs) and angiotensin type 2 receptors (AT₂Rs) are important natriuretic receptors counterbalancing angiotensin type 1 receptor–mediated tubular sodium reabsorption. Here we explore the mechanisms of D₁R and AT₂R interactions in natriuresis. In uninephrectomized, sodium-loaded Sprague-Dawley rats, direct renal interstitial infusion of the highly selective D₁R agonist fenoldopam induced a natriuretic response that was abolished by the AT₂R-specific antagonist PD-123319 or by microtubule polymerization inhibitor nocodazole but not by actin polymerization inhibitor cytochalasin D. By confocal microscopy and immunoelectron microscopy, fenoldopam translocated AT₂Rs from intracellular sites to the apical plasma membranes of renal proximal tubule cells, and this translocation was abolished by nocodazole. Because D₁R activation induces natriuresis via an adenylyl cyclase/cAMP signaling pathway, we explored whether this pathway is responsible for AT₂R recruitment and AT₂R-mediated natriuresis. Renal interstitial coinfusion of the adenylyl cyclase activator forskolin and 3-isobutyl-1-methylxanthine induced natriuresis that was abolished either by PD-123319 or nocodazole but was unaffected by specific the D₁R antagonist SCH-23390. Coadministration of forskolin and 3-isobutyl-1-methylxanthine also translocated AT₂Rs to the apical plasma membranes of renal proximal tubule cells; this translocation was abolished by nocodazole but was unaffected by SCH-23390. The results demonstrate that D₁R-induced natriuresis requires AT₂R recruitment to the apical plasma membranes of renal proximal tubule cells in a microtubule-dependent manner involving an adenylyl cyclase/cAMP signaling pathway. These studies provide novel insights regarding the mechanisms whereby renal D₁Rs and AT₂Rs act in concert to promote sodium excretion in vivo. (Hypertension. 2012;59[part 2]:437-445.) • Online Data Supplement

Key Words: dopamine ▪ D₁ receptors ▪ angiotensin ▪ AT₂ receptors ▪ sodium excretion ▪ natriuresis

The recycling of membrane proteins is a dynamic process whereby the distribution among different intracellular compartments and the plasma membrane is determined by the rates of exocytosis and endocytosis of the respective membrane proteins. Various stimuli (including agonists and osmotic stress) enhance exocytosis and/or slow endocytosis, leading to a redistribution of membrane proteins to the cell surface, a process termed “translocation.” Translocation often involves an intact cytoskeleton, and the major building blocks of the cytoskeleton are microtubules and actin microfilaments.¹⁻³

Dopamine (DA) receptors belong to 2 receptor subfamilies, D₁-like (D₁ and D₅) and D₂-like (D₂, D₃, and D₄). D₁-like receptors (D₁Rs) are expressed on both apical and basolateral membranes of renal proximal tubule cells (RPTCs). Activation of D₁Rs by DA accounts for ≈50% of basal sodium (Na⁺) excretion in vivo,⁴⁻⁵ and intrarenal DA deficiency leads to hypertension and reduced longevity.⁶ D₁Rs couple to adenylyl cyclase and cAMP generation, as well as phospholipase C and protein kinase C signaling. On agonist stimulation, D₁Rs are recruited along microtubules⁷ from the interior of RPTCs toward the plasma membrane via cAMP-dependent⁸⁻¹⁰ and not protein kinase C–dependent pathways.¹⁰

Similar to DA, the angiotensin peptides of the kidney renin-angiotensin system also contribute to the regulation of Na⁺ homeostasis through actions at different receptors, including angiotensin type 1 (AT₁Rs) and angiotensin type 2 receptors (AT₂Rs). In an effort to define the relationship between the renal dopaminergic system and the renin-angiotensin system in Na⁺ excretion in normal rodents, previous studies from our laboratory have demonstrated that renal interstitial (RI) D₁R activation with fenoldopam (FEN), a highly selective D₁R agonist, induces natriuresis that is abolished by intrarenal coinfusion of the specific AT₂R antagonist PD-123319 (PD).¹¹ Furthermore, FEN-induced natriuresis was accompanied by an increase in apical plasma membrane (AM) but not total RPTC membrane AT₂R expression, as quantified by Western blot analysis.¹¹ Because the mechanisms that underlie the trafficking of proteins to and from the cell surface are important determinants of the
hormonal responsiveness of tissues, the present study examines the roles of the cytoskeleton and cAMP in the redistribution of the natriuretic receptors. We report here that AT$_2$R-mediated natriuresis, either in response to renal D$_1$R stimulation with FEN or direct downstream activation of adenyl cyclase with forskolin (FSK), results in microtubule-dependent AT$_2$R translocation from the cytosol to the AMs of RPTCs in Na$^+$ loaded Sprague-Dawley rats. Taken together, these findings indicate that cAMP plays an important role in microtubule-dependent trafficking of RPTC AT$_2$Rs, which is necessary for the natriuretic response to D$_1$R activation.

**Methods**

**Animal Preparation**

All of the protocols were approved by the animal care and use committee at the University of Virginia and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experiments were conducted on 12-week-old female Sprague-Dawley rats (Harlan) that were housed in a vivarium under controlled conditions (temperature: 21°C; humidity: 60±10%; and light: 8:00 AM to 8:00 PM). For 1 week before and during the experiments, the rats were maintained on a standard high Na$^+$ rat chow containing 4% Na$^+$. On day 6, the rats were placed in metabolic cages, and 24-hour urine samples were collected to measure the urine Na$^+$ excretion rate (U$_{NaV}$). Representative U$_{NaV}$ was 6.34 μmol/min (normal: 0.69 μmol/min). On day 7, the rats were anesthetized for uninephrectomy, carotid artery cannulation for mean arterial pressure measurements (MAPs), and remaining kidney ureter cannulation for quantification of UNaV, as published previously.5,11-13 Please see the online Data Supplement at http://hyper.ahajournals.org for details.

**Renal Cortical Interstitial Infusion**

The RI route of administration was used in these studies to eliminate systemic hemodynamic factors that may play a role in the natriuretic response. The RI catheters were placed as published previously.11-13 for 1-hour RI infusion of vehicle (V), followed by 3 hours of RI infusion of one of the following at a rate of 2.5 μL/min: FEN (1, 3, and 5 μg/kg per minute [each dose for 1 hour]; Sigma)± nodocazol (NOC, a microtubule polymerization inhibitor; 3 μg/kg per minute; Sigma), NOC alone, FEN+PD (10 μg/kg per minute; Sigma), FEN+cytochalasin D (an inhibitor of actin filament polymerization; 0.333 μg/kg per minute; Sigma), cytochalasin D alone, FSK (a direct activator of adenyl cyclase; 1.4 μg/kg per minute; Sigma)±3-isobutyl-1-methylxanthine (IBMX, a selective phosphodiesterase inhibitor that permits accumulation of cAMP; 1.4 μg/kg per minute; Sigma)±NOC, IBMX alone, FSK+IBMX+PD, SCH2390 (SCH, a potent, highly selective D$_2$R antagonist; 10 μg/kg per minute; Sigma)+FSK+IBMX, or SCH alone. Control rats received RI V infusion for the entire 4-hour study. When >1 substance was infused, each was infused via a separate microinfusion catheter. Vetbond tissue adhesive (3M Animal Care Products) was added to the catheter(s) and prevented interstitial pressure loss in the kidney.

**Urine Collection and Blood Pressure Measurements**

Urine was collected from each rat hourly for 4 hours after a 1-hour equilibrium period. Urinary Na$^+$ concentrations were measured using a flame photometer (IL-943, Instrumentation Laboratory) and presented as micromoles per minute. MAP was monitored by a carotid artery catheter via a digital blood pressure analyzer (MicroMed Inc). MAP values were recorded every 5 minutes and averaged for each period.

**In Vivo Kidney Perfusion and Fixation Procedure**

Uninephrectomized Sprague-Dawley rats on a 4% Na$^+$ intake for 1 week received either RI infusion of V, FEN (1 μg/kg per minute), FEN+NOC, or NOC alone for 3 cumulative 1-hour experimental periods. At the end of the in vivo protocol, the rat heart left ventricular cavity was cannulated, and the animal was perfused for fixation before kidney cortex specimen isolation and staining for analysis by quantitative confocal microscopy, as published previously.13,14 Please see the online Data Supplement for details.

**In Vitro Kidney Section Preparation and Incubation**

Kidneys were harvested from animals under anesthesia and immersed in ice-cold RPMI 1640. Using a McIlwaine Tissue Chopper, 1-mm coronal sections were cut and placed in 6-well plates with 2 mL of prewarmed medium per well. The sections were equilibrated for 30 minutes in a CO$_2$ incubator at 37°C with gentle rocking before treatment with V, FEN, FEN+NOC, or NOC alone for 30 minutes. Sections were fixed for 2 hours in 4% paraformaldehyde made in Tris-buffered saline at room temperature, rinsed 3 times in Tris-buffered saline, immersed in 100 mmol/L of Tris- HCl, and then rinsed 3 times again in Tris-buffered saline before storage in 30% sucrose in Tris-buffered saline overnight at 4°C. The sections were imbedded in OCT compound (Tissue-Tek), and 8-μm frozen sections were prepared, stained, and analyzed by quantitative confocal microscopy, as published previously.13,14 Please see the online Data Supplement for details.

**Immunofluorescence Microscopy**

Kidney sections were incubated with anti-AT$_2$R primary antibody (1:100; H-143 Santa Cruz) for 60 minutes, washed, and then incubated with ALEXA 647 conjugated donkey antirabbit secondary antibody (1:500; Invitrogen) for 60 minutes at room temperature. To identify RPTCs, the preparation was stained further with Texas-Red phalloidin (1:200; Invitrogen), which labels actin-containing structures, including RPTC AMs. Hoechst (10 mg/mL; stock; Invitrogen) was added (1:2500) to identify nuclei. Both phalloidin and Hoechst were added for 60 minutes at room temperature. After several washes, Fluoromount G (Southern Biotech) was applied before being covered with a glass coverslip. Please see the online Data Supplement for details.

**Confocal Microscopy and Quantification of Immunofluorescence Signals**

Confocal microscopy recordings were performed using an Olympus IX81 Spinning Disk Confocal Microscope with excitation at 490 and 467 nm and detection at 510 and 660 nm. Images were captured using identical capture parameters for each section with a ×100 1.2 NA UIS2 water immersion objective and a Hamamatsu 9100-02 EMCCD camera with Slidebook 4.2 software. Images were exported as 16-bit tiff files and analyzed using MacBiophotonics ImageJ v1.38m and the Sync Measure 3D plug-in written by Joachim Walter, as published previously.14 The average AT$_2$R fluorescence intensity 0 to 4 μm from the tip of the apical plasma membrane of RPTCs was designated as AM AT$_2$R fluorescence intensity.

**Immunoelectron Microscopy**

After application of primary and secondary antibodies as described above, the slides were immersed in a solution containing 2.5% glutaraldehyde (EM grade, Electron Microscopy Sciences, Inc [EMS]) in 0.1 mol/L of Dulbecco PBS without calcium and magnesium chloride, held overnight at 4°C, and delivered to the Advanced Microscopy Facility for further processing for transmission electron microscopy. All of the subsequent processing was carried out at 24°C unless otherwise noted. Slides with attached sections were washed in distilled water (4×5 minutes), postfixed for 1 hour in 1% osmium-tetroxide, dehydrated through a graded ethanol series followed by transition into 100% acetone, and infiltrated with epoxy resin (EPON 812, EMS). Although the resin on the slides was used.
still liquid, an embedding capsule (BEEM, EMS) filled with epoxy resin was inverted directly over each section and the entire unit (slide with section and capsule) polymerized for 48 hours at 60°C. To separate capsules with underlying embedded tissue sections from the slides, each slide was immersed in boiling water, followed by immersion in liquid nitrogen, and returned to boiling water a second time. Ultrathin sections (70–80 nm) were prepared with a Diatome diamond knife (Diatome, EMS) on a Leica Ultracut UCT ultramicrotome, collected on 200 mesh copper grids (EMS), and contrast stained using a double-lead procedure (Daddow) as follows: 5 minutes in lead citrate; 15 minutes in uranyl acetate (3.0% in 50.0% acetone); and a final 5 minutes in lead citrate. Thin sections were examined in a JEOL 1230 transmission electron microscope (Japan Electron Optics Limited, Tokyo, Japan), and digital images of proximal kidney tubules were acquired with an SIA 12-C slow-scan 16.8 megapixel camera (Scientific Instruments and Applications). Immunogold labeling of AM AT₂Rs was quantified from 6 different electron micrographs of RPTC brush borders (each micrograph brush border area: 0.17 μm²) for each experimental condition. Immunogold labeling of basolateral membrane AT₂Rs was quantified from 5 electron micrographs of RPTC basolateral membrane foldings (totaling an area of 0.25 μm²) for each experimental condition.

Membrane Preparations and Western Blot Analysis
After in vitro kidney section preparation, RPTC AMs were isolated as published previously with slight modifications.11,14 Please see the online Data Supplement for details. AMs were incubated with rabbit AT₂R polyclonal antibody (1:100 dilution, H-143 Santa Cruz) standardized to villin monoclonal antibody (1:2500 dilution, Immunootech), which is enriched in RPTCs.11 Membranes were subsequently incubated with infrared secondary antibodies (antimouse IRDye 680 nm and antirabbit IRDye 800 nm, each at 1:15 000, Licor Biosciences). Immunoreactivity and quantitative assessment of band densities were performed using the Odyssey Infrared Imaging System (Licor Biosciences). Results are reported as a ratio of AT₂R/villin expression.

Statistical Analysis
Data are presented as mean±1 SE. ANOVA with a repeated-measures term was used to analyze for variation between the groups. A 2-tailed Student t test was used to compare individual means between groups. A P<0.05 was considered statistically significant.

Results
Effects of AT₂R Antagonism and Microtubule Polymerization Inhibition on D₁R-Induced Natriuresis in Rats on High Na⁺ Intake
Figure 1A demonstrates that cumulative RI FEN infusion results in increased UNaV across the duration of the experiment and that coinfusion of PD or NOC abolishes this response to control values. FEN increased UNaV from a baseline of 0.23±0.03 to 0.58±0.06 μmol/min (P<0.001) at 1 μg/kg per minute, 0.70±0.07 μmol/min (P<0.0001) at 3 μg/kg per minute, and 0.69±0.07 μmol/min (P<0.0001) at 5 μg/kg per minute. There were no significant changes in UNaV in the V-infused kidneys. As shown in Figure 1B, compared with time control during which only V was infused, RI FEN, FEN+PD, FEN+NOC, or NOC alone infusions did not alter MAP from baseline values. Identical experiments were performed in which the actin microfilament polymerization inhibitor cytochalasin D failed to affect FEN-induced natriuresis (please see Figure S1, available in the online Data Supplement at http://hyper.ahajournals.org).
Confocal Microscopy Analysis of Renal Proximal Tubule AT2R Redistribution in Response to In Vivo Infusions and In Vitro Incubations

Figure 2A through 2D demonstrate high power (×600) confocal micrographs of the rat renal cortex labeled with phalloidin, a marker for filamentous actin that is enriched in RPTC AM (red), antibodies to the AT2R (green), and Hoechst nuclear stain (blue). As indicated by the colocalization of AT2R and phalloidin (yellow), FEN treatment markedly increased AT2R localization in the apical plasma membrane (AM) of renal proximal tubule cells (RPTCs). The addition of nocodazole (NOC), an inhibitor of microtubule polymerization, to FEN (C and H) abolished AM AT2R recruitment both in vivo and in vitro, whereas NOC treatment alone (D and I) failed to show any difference in AT2R localization compared with vehicle (V) treatment. The bar at the bottom of D and I represents 10 μm. E and J represent the quantification of RPTC AM AT2R fluorescent intensity both in vivo and in vitro, respectively. Compared with V-treated kidneys, FEN-treated kidneys (■) demonstrated greater RPTC AM AT2R fluorescence intensity both in vivo and in vitro (1765±113 versus 1111±19, P<0.00001, and 1013±20 versus 630±19, P<0.00001, respectively). Both in vivo and in vitro, FEN+NOC-treated kidneys (○) abolished the increase in AM AT2R fluorescent intensity, and NOC treatment alone (▲) failed to show any significant difference in AT2R localization compared with control conditions (□). For in vivo quantifications, each data point represents mean±1 SE of 22 independent measurements of RPTCs. For in vitro quantifications, each data point represents mean±1 SE of 16 independent measurements of RPTCs.

AT2R antibody with the immunizing peptide abolished the AT2R signal (green) in the immunofluorescence and confocal micrographs (data not shown). Figure 2E quantifies AT2R fluorescence intensity as a function of its distance from the apical tip of RPTCs. Compared with V-infused kidneys, FEN-infused kidneys demonstrated greater RPTC AM AT2R fluorescence intensity (1765±113 versus 1111±19; P<0.00001). Neither FEN+NOC-infused kidneys nor NOC alone infusion showed significant differences in AM AT2R fluorescence intensity compared with V-infused kidneys. Figure 2F through 2I demonstrate the corresponding conditions after in vitro incubations with V (Figure 2F), FEN (10 μmol/L, Figure 2G), FEN+NOC (Figure 2H), or NOC alone (10 μmol/L, Figure 2I). As shown by the colocalization of AT2R and phalloidin (Figure 2G; yellow color), incubation with FEN alone in vitro also resulted in increased AM AT2R expression compared with V, FEN+NOC, or NOC alone.
conditions. In vitro FEN-treated RPTCs also demonstrated higher AM AT₂R fluorescence intensity compared with control conditions (1013 ± 1006 versus 630 ± 19; \( P < 0.00001 \); Figure 2J).

**Immunoelectron Microscopy Analysis of Renal Proximal Tubule AT₂R Redistribution After 3-Hour In Vivo Infusions**

Figure 3A provides a low-power micrograph of a renal proximal tubule cell (RPTC). Figure 3B, 3C, 3E, and 3F demonstrate immunogold labeling of AT₂R in brush border microvilli of RPTCs after in vivo infusion of V (B), fenoldopam (FEN; C), FEN + nocodazole (NOC; D), or NOC alone (F). Black dots represent immunogold (10-nm particles) labeling of brush border angiotensin type 2 receptors (AT₂Rs) after each experimental infusion. The bar at the bottom of F represents 0.2 μm. D depicts the quantification of immunogold-labeled AT₂Rs of RPTC brush borders from 6 different electron micrographs (each micrograph brush border area: 0.17 μm²) for each experimental condition. The electron micrographs confirm increased brush border AT₂R density after RI infusion of FEN. Data represent the mean ± 1 SE. *\( P < 0.05 \) and **\( P < 0.01 \) from FEN-infused kidneys.

**Role of RI cAMP in AT₂R-Mediated Natriuresis**

After in vivo FSK+IBMX infusion, RI cAMP levels measured via microdialysis increased significantly from a baseline value of 22.1 ± 3.9 to 38.1 ± 5.1 pmol/min (\( P < 0.05 \)) during period 1, 48.6 ± 7.7 pmol/min (\( P < 0.01 \)) during period 2, and 34.8 ± 5.9 pmol/min during period 3 (Figure 4). RI FSK infusion was not sufficient to increase RI cAMP levels significantly. After RI infusion of V (\( N = 19 \)), FSK alone (\( N = 12 \)), or IBMX alone (\( N = 7 \)), \( U_{NaV} \) failed to increase significantly from baseline (Figure 5A). However, after the addition of IBMX to FSK (\( N = 17 \)), \( U_{NaV} \) increased from 0.21 ± 0.02 to 0.41 ± 0.06 μmol/min in period 1 (\( P < 0.01 \)) to 0.46 ± 0.06 μmol/min in period 2 (\( P < 0.001 \)), and 0.35 ± 0.04 μmol/min in period 3 (\( P < 0.05 \)). The addition of PD (\( N = 8 \)) or NOC (\( N = 8 \)) to FSK+IBMX abolished the natriuretic responses (Figure 5A). MAP responses remained unchanged in response to any of the RI infusions (Figure 5B).

**Effects of Renal D₁R Blockade on FSK+IBMX-Induced Natriuresis**

As demonstrated in Figure 6A, coinfusion of SCH, an inhibitor of D₁Rs, failed to affect FSK+IBMX–induced natriuresis in vivo. In the presence of D₁R antagonism, FSK+IBMX (\( N = 12 \)) significantly increased \( U_{NaV} \) from a...
Baseline value of 0.24±0.02 to 0.37±0.05 µmol/min (P<0.05) during period 1, 0.42±0.05 µmol/min (P<0.05) during period 2, and 0.30±0.03 µmol/min during period 3. RI infusion of SCH alone (N=8) had no effect on UNaV or MAP responses (Figure 6B).

Western Blot Analysis of Apical Membrane AT2R Expression

Compared to V-incubated kidneys (N=6), FSK+IBMX (N=6) significantly increased AM AT2R expression (0.0065±0.0004 and 0.0039±0.0003 relative fluorescence units, respectively; P<0.001; Figure 7). The addition of SCH (N=6) did not affect the increase in AM AT2R expression induced by FSK+IBMX treatment. However, none of the individual agents (FSK, IBMX, or SCH alone) altered AM AT2R expression compared with V.

Discussion

These studies demonstrate that direct RI D1R activation with FEN, as well as downstream activation of cAMP (in the presence of D1R blockade), induces microtubule-dependent translocation of AT2Rs to the AM of RPTCs and AT2R-mediated natriuresis. Given that renal D1Rs signal through cAMP, and RI accumulation of cAMP is important in the regulation of AT2R-mediated natriuresis, these studies provide novel insight into a mechanism whereby renal D1Rs and AT2Rs act in concert to promote Na+ excretion in vivo.

It is well known that there is a complex interplay between the intrarenal dopaminergic and renin-angiotensin systems in the control of Na+ excretion and blood pressure. The natriuretic effect of D1Rs is enhanced during AT1R blockade,15,16 and DA, through its actions at D1Rs, decreases AT1R expression and angiotensin II binding sites in RPTCs.16 Because renal AT2Rs mediate the natriuretic effects of AT1R blockade12 and specific AT2R antagonism with PD abolishes FEN-induced natriuresis,11 there exists a clear need to investigate the precise mechanisms by which renal AT2Rs mediate D1R-induced natriuresis. The present studies, therefore, examined the key components of D1R-induced natriuresis.
(microtubule-dependent trafficking and cAMP signaling) and their effects on AT₂R-mediated Na⁺ excretion.

First, a dose-response relationship for FEN-induced natriuresis was determined and shown to peak during the 3-μg/kg-per-minute infusion period. Previous studies carried out under identical experimental conditions using a constant dose of FEN for the entire duration of the study (1 μg/kg per minute for 3 hours) did not show a peak effect on natriure-
Thus, intrarenal D₃R activation demonstrates a dose-specific and not time-dependent maximization of Na⁺ excretion. MAP responses were not significantly affected by 3 hours of cumulative FEN infusion, indicating that the observed natriuresis was not a result of systemic hemodynamic factors. Abolition of FEN-induced natriuresis by AT₂R blocker PD occurred as early as the first experimental period with continuation throughout the duration of the protocol, indicating early and sustained AT₂R dependence of D₃R-induced natriuresis. Importantly, the high Na⁺ diet under which these experiments were conducted was demonstrated previously not to reduce RI or tissue levels of angiotensin II/III, which would be available to activate translocated AT₂Rs.¹¹ AT₂R activation has been shown to induce natriuresis, likely via its downstream NO and cGMP signaling pathways.¹⁷ These observations prompted interest in the acute and nongenomic mechanisms of D₁R-induced AT₂R responses.

One of the results of D₁R activation in salt-loaded animals involved an increase in AM RPTC AT₂R localization. Neither total¹¹ nor basolateral RPTC AT₂R expression is changed in response to FEN infusion, suggesting that apically distributed AT₂Rs participated in the natriuretic response. Previous experiments have established that D₁Rs translocate to the cell surface in response to D₁R activation in cultured kidney cells, kidney section preparations, and isolated proximal tubules⁷–⁹ and that this response requires an intact microtubule network.⁷ The present studies extend these findings to the natriuretic mechanism of renal AT₂Rs in vivo. Using NOC, which disrupts the microtubule network but preserves the actin microfilaments of RPTCs,¹³ we found complete abolition of FEN-induced natriuresis and AT₂R translocation. Although NOC may affect the renal transport of other molecules/receptors involved in D₁R-induced natriuresis in vitro,¹⁸ NOC infusion alone failed to alter basal Na⁺ excretion or AT₂R localization compared with V infusion in vivo. Thus, microtubules are not only necessary for D₁R recruitment but also for AT₂R recruitment in response to FEN, suggesting a common pathway for the natriuretic function of these receptors.

Therefore, because D₁Rs signal through cAMP/protein kinase A to mediate natriuresis, we examined the role of RI cAMP generation in AT₂R-mediated natriuresis. Although RI FSK infusion alone was insufficient to induce a significant rise in RI cAMP, the addition of IBMX to FSK caused a significant and sustained increase in RI cAMP during the first 2 experimental periods. It is likely that the selective inhibition of cAMP degradation with IBMX was necessary for significant cAMP accumulation because of rapid degradation of the second messenger in vivo. Interestingly, the pattern of increase in UNa⁺V induced by FSK+IBMX paralleled the rise and fall of RI cAMP levels. FSK+IBMX-induced natriuresis was clearly dependent on renal AT₂Rs, because the effect was abolished by PD, but microtubulin-dependent trafficking was also an important component, because NOC also inhibited the effect.

Whether direct agonist stimulation of renal D₁Rs is necessary for AT₂R-mediated natriuresis is a question that was addressed using SCH, a highly specific D₁R antagonist, in conjunction with FSK and IBMX. In the present study, intrarenal D₁R blockade with SCH did not reduce basal Na⁺ excretion acutely, consistent with our previous studies.¹¹ During SCH administration, RI infusion of FSK+IBM induced natriuresis that required AT₂R activation, emphasizing the importance of D₁R-induced cAMP generation over direct agonist-dependent activation of D₁Rs. Thus, one mechanism by which AT₂Rs and D₁Rs interact in high Na⁺ conditions to mediate natriuresis is related to D₁R-cAMP signaling, which, in turn, provides the stimulus necessary for AT₂R translocation and natriuresis. Because the effect was independent of specific D₁R-induced activation of adenylyl cyclase, stimulation of other receptors that signal through cAMP/protein kinase A pathways may be advantageous in promoting AT₂R-mediated natriuresis in vivo. Indeed, administration of parathyroid hormone, through its cAMP/protein kinase A–dependent but not phospholipase C/protein kinase C–dependent signaling, has been shown to redistribute Na⁺ transporters such as Na⁺–hydrogen exchanger-3 and inhibit Na⁺–K⁺–ATPase activity in a direction favoring natriuresis and diuresis.¹⁹ Renal AT₂Rs are known to inhibit Na⁺–K⁺–ATPase activity,²⁰ and increased RI cAMP may regulate this process.

In summary, we have shown that renal AT₂Rs are required for D₁R-mediated natriuresis and that both AT₂R cellular trafficking and natriuretic activities are regulated by downstream cAMP signaling pathways. We also have demonstrated that renal AT₂Rs are translocated to the AMs of RPTCs by a microtubule-dependent pathway that is independent of D₁R activation. These results demonstrate an interaction between renal D₁Rs and AT₂Rs that counterbalances Na⁺ reabsorption mediated by AT₂Rs.

**Perspectives**

These studies demonstrate that AT₂R-mediated natriuresis, either in response to renal D₁R stimulation with FEN or direct downstream activation of adenylyl cyclase with FSK, involves microtubule-dependent AT₂R translocation to the AMs of RPTCs in Na⁺-loaded rats. Taken together, these findings indicate that cAMP is a key mediator of the interaction between renal D₁Rs and AT₂Rs to induce natriuresis during high-salt states. Furthermore, targeting the common trafficking pathways of these receptors would affect the natriuretic capacity of 2 of the most powerful systems governing Na⁺ homeostasis in the body. Such endeavors hold significant clinical implications in cardiovascular medicine and hypertension where the focus has predominantly been on AT₁R blockade and inhibition of the renin-angiotensin system.

**Sources of Funding**

This work was supported by National Institutes of Health grants 08-HL-093353 (to S.H.P.), R01-HL-087998 (to R.M.C.), R01-HL-095796 (to R.M.C.), and T32-DK-07646 (to R.M.C.).

**Disclosures**

None.
References


Mechanisms of Dopamine D₁ and Angiotensin Type 2 Receptor Interaction in Natriuresis

Hypertension. 2012;59:437-445; originally published online December 27, 2011;
doi: 10.1161/HYPERTENSIONAHA.111.184788
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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/59/2/437

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

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

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/