

Dopamine Decreases Expression of Type-1 Angiotensin II Receptors in Renal Proximal Tubule

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Abstract

Systemic and/or locally produced angiotensin II stimulates salt and water reabsorption in the renal proximal tubule. In vivo, dopamine (DA) may serve as a counterregulatory hormone to angiotensin II's acute actions on the proximal tubule. We examined whether dopamine modulates AT₁ receptor expression in cultured proximal tubule cells (RPTC) expressing DA₁ receptors. Dopamine decreased basal RPTC AT₁ receptor mRNA levels by $67 \pm 7\%$ ($n = 10$; $P < 0.005$) and decreased ¹²⁵I-angiotensin II binding by $41 \pm 7\%$ ($n = 4$; $P < 0.05$). The DA₁-specific agonist, SKF38393 decreased basal AT₁ receptor mRNA levels ($65 \pm 5\%$ inhibition; $n = 5$; $P < 0.025$), and the DA₁-specific antagonist, SCH23390 reversed dopamine's inhibition of AT₁ receptor mRNA expression ($24 \pm 10\%$ inhibition; $n = 8$; NS) and angiotensin II binding ($5 \pm 15\%$; $n = 4$; NS). DA₂-specific antagonists were ineffective. In rats given L-DOPA in the drinking water for 5 d, there were decreases in both proximal tubule AT₁ receptor mRNA expression ($80 \pm 5\%$; $n = 6$; $P < 0.005$) and specific [¹²⁵I] Ang II binding (control: 0.74 ± 0.13 fmol/mg pro vs. 0.40 ± 0.63 fmol/mg pro; $n = 5$; $P < 0.05$).

In summary, dopamine, acting through DA₁ receptors, decreased AT₁ receptor expression in proximal tubule, an effect likely mediated by increased intracellular cAMP levels. Local dopamine production also led to decreased AT₁ receptor expression, suggesting dopamine may reset sensitivity of the proximal tubule to angiotensin II. (*J. Clin. Invest.* 1996. 97:2745–2752.) Key words: angiotensin II • receptor • dopamine • kidney • proximal tubule • L-DOPA

Introduction

The proximal tubule is a major site of salt and water reabsorption in the mammalian nephron, with up to 60% of the filtrate reabsorbed in this segment. Proximal tubule function is under hormonal control, with the vasoconstrictor angiotensin II stimulating reabsorption and the vasodilator dopamine inhibiting reabsorption (1, 2).

Proximal tubule angiotensin II (Ang II)¹ binding sites are

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1. Abbreviations used in this paper: ANG II, angiotensin II; RPTC, rabbit proximal tubule cells.

present on both apical and basolateral membranes (3, 4). Ang II exerts direct effects on proximal tubule transport, independent of alterations in renal or systemic hemodynamics (5–7). We have previously demonstrated that ¹²⁵I-Ang II binding to both basolateral and brush border membranes from either rat or rabbit is inhibited by losartan, indicating the presence of type 1 angiotensin II receptors (AT₁ receptors) on both membranes (8). Other studies have indicated that AT₁ receptors mediate Ang II-mediated increases in proximal tubule volume reabsorption (9).

Previous studies have indicated that dopamine and Ang II serve counteracting functions in the proximal tubule (2, 10, 11). Ang II stimulates net reabsorption via activation of apical Na⁺/H⁺ exchange and basolateral [Na⁺]/[K⁺]ATPase, while dopamine inhibits the activity of these two transporters (12, 13). Furthermore, dopamine inhibits the Ang II-mediated activation of these transporters and Ang II-mediated proximal tubule reabsorption (2, 12). Since alterations in the balance of responsiveness of proximal tubule to angiotensin II and dopamine have important implications for net sodium and water reabsorption and have been implicated in genetic models of hypertension (14), the present studies examined the possibility dopamine may chronically regulate proximal tubule responsiveness to angiotensin II by decreasing AT₁ receptor expression.

Methods

Materials. ¹²⁵I-Ang II, ¹²⁵I-SCH23390 and ¹²⁵I-cAMP radioassay kit were from New England Nuclear (Boston, MA). ³²P-CTP (3,000 Ci/mmol) was from Amersham (Arlington Heights, IL). SCH23390, SKF38393, L-DOPA, dopamine, carbidopa, spiperone, and octoclotheptin were from Research Biochemicals International (Natick, MA). Dideoxyadenosine and dibutyrl cAMP were from Calbiochem (La Jolla, CA).

Animals. Male Sprague-Dawley rats, weighing 150–250 grams were given L-3,4 Dihydroxyphenylalanine (L-DOPA) (2 mg/kg per day) in the drinking water for 5 d (15). Age and weight matched rats were used as controls. The tail-cuff microphonic manometer method was used to measure blood pressures (16).

Isolation of proximal tubules. Modification of the methods of Vinay et al. were used (17, 18). Briefly, renal cortices were gently minced and suspended in a solution containing 115 mM NaCl, 24 mM NaHCO₃, 5 mM KCl, 1.5 mM CaCl₂, 10 mM MgSO₄, 2.0 mM NaH₂PO₄, 10 mM Hepes, pH 7.4 (Buffer A), with the following additions: 5 mM glucose, 1 mM alanine, 0.03% collagenase (Sigma type I), and 0.01% soy bean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO), gassed with 95% O₂/5% CO₂ and maintained at 37°C. The cortical suspension was incubated for 45 min and gently agitated on a rocking platform. The suspension was strained through a large sieve, centrifuged, and resuspended in oxygenated Buffer A, then washed and recentrifuged three times. The resulting pellet was mixed with 100 ml of a 40% Percoll solution with the identical ionic composition as Buffer A and which had been previously gassed with 95% O₂/5% CO₂ and chilled to 4°C. The Percoll solution was centrifuged at 15,000 RPM for 30 min at 4°C in a Beckman J 2-21 high-speed preparative centrifuge using a JA 17 rotor. After centrifugation, the tissue was

separated into four distinct bands, as described by Vinay et al. (17). The lowermost band, which was enriched in proximal tubule segments, was collected.

Cell culture. A previously described line of SV-40 immortalized rabbit proximal tubule cells (RPTC) was used for the present studies (19). Cells were cultured and maintained in DME/F12 supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) at 37°C in 95% air/5% CO₂ atmosphere. Cells were made quiescent by incubation for 48 h in media without fetal bovine serum before administration of agonists. Experiments were performed on confluent cell monolayers of passages 20–30. Primary cultures of rabbit proximal tubule cells were prepared as previously described (20).

Quantitation of AT₁ receptor by polymerase chain reaction. Total RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform method (21). Quantitative PCR was performed as previously described for SV40 rabbit proximal tubule cells (19) and isolated rat proximal tubules (22). For PCR, total RNA (10 µg) and cRNA of an AT₁ receptor Msc1-Msc-1 deletion mutant (100 pg) were mixed and reverse transcribed using murine reverse transcriptase (Pharmacia, First Strand cDNA Synthesis kit, Piscataway, NJ) and a primer specific for the AT₁ receptor in a final reaction volume of 33 µl. The resultant single strand cDNA mixture was then amplified in a Perkin Elmer GeneAmp 9600 PCR System using Taq polymerase (Perkin Elmer/Cetus). The used primers were upstream sense primer (5'-TACATATTGTGTCATGATTCCT-3') and downstream anti-sense primer (3'-GTGAATATTTGGTGGGGAAC-5'). PCR was performed for 35 cycles at 95°C for 20 s, 55°C for 30 s and 72°C for 90 s, followed by a 10-min extension at 72°C. Amplification of intact and mutant AT₁ receptor mRNA by these primers gave 703- and 415-bp fragments, respectively. No amplification occurred in the absence of reverse transcription, indicating that genomic DNA was not being amplified. Preliminary studies indicated linearity of response for at least 40 cycles. Samples were routinely amplified in the presence of α-[³²P] CTP (NEN, Boston, MA; 3,000 Ci/mmol; 2 µCi/sample). For normalization, parallel samples measured amplification of β-actin, using the primers (5':AACCGCGAGAAGATGACCCAGATCATGTTT; and 3':AGCAGCCGTGGCCATCTCTTGCTCGAAGTC) (23). After gel chromatography on 4% agarose gels, the bands corresponding to the AT₁ receptor, deletion fragment and β-actin were excised and counted by scintillation spectrometry. Results are represented as the ratio of intact and deletion fragment AT₁ receptor mRNA amplified, normalized to the amount of amplified β-actin mRNA. This method provides a relative comparison of the amount of AT₁ receptor mRNA present among the different experimental groups (19, 24).

¹²⁵I-Angiotensin II binding. Binding studies were performed using ¹²⁵I-labeled angiotensin II and unlabeled angiotensin II. Cells were made serum-free 12–16 h before study and exposed to the indicated agent every 8 h in the presence of ascorbic acid (1 µM). Cells

were washed in a solution of PBS supplemented with 0.1% bovine serum albumin three times, then exposed to ¹²⁵I-Ang II in PBS-0.1% albumin at 4°C for 4 h, with or without the indicated concentration of unlabeled angiotensin II. After the appropriate incubation, cells were washed three times with PBS-0.1% albumin and cell-associated radioactivity was determined after digestion with 0.05 M NaOH. Binding studies on rat proximal tubule suspensions prepared by Percoll gradient centrifugation as described above were performed using previously described methods for ¹²⁵I-Ang II to membrane preparations (19). Radioactivity was measured in a gamma counter (Beckman 5500B). Specific binding was determined by subtracting the non-specific binding component measured in the presence of 1 µM unlabeled Ang II from total ¹²⁵I-Ang II binding.

DA₁ receptor expression in SV40 RPTC. ¹²⁵I-SCH23390 binding studies were performed upon RPTC incubated in serum-free media for 24–48 h. The cells were washed three times with PBS-0.1% albumin and incubated with ¹²⁵I-SCH23390 (0.1 nM) and increasing concentrations of unlabeled SCH23390 for 40 min at 23°C, then washed three times with ice-cold PBS-0.1% albumin and lysed with 0.05 M NaOH. For determination of DA₁ mRNA expression, PCR primers were used that represented the third and seventh transmembrane regions of the rat DA₁ receptor: upstream primer- 5'ATGTGCTCTACGGCGTCCATT3'; downstream primer- 5'CCACACAAACACATCGAAGGT3'. Samples of total RNA from rat renal cortex, rabbit renal cortex and RPTC were subjected to reverse transcription and then cDNA was amplified for 30 cycles in the presence of the above primers at 94°C for 45 s, 58°C for 45 s, and 72°C for 60 s.

cAMP production. cAMP production was measured by radioimmunoassay as previously described (25).

Statistics. Results are presented as the means±SEM. Results of PCR experiments and ¹²⁵I-Ang II binding to cultured proximal tubule cells were normalized as percentage of control. Statistical comparisons utilized ANOVA and the Bonferroni modification of Student's *t* test, with *P* < 0.05 indicating significance. For analysis of ¹²⁵I-Ang II binding and ¹²⁵I-SCH23390 binding, results were fit with the ligand binding program, Graph Pad Prism (San Diego, CA).

Results

To determine whether SV40 immortalized RPTC expressed a type 1 dopamine receptor (DA₁), specific ¹²⁵I-SCH23390 binding was determined. SCH23390 is a competitive antagonist that binds specifically to DA₁ receptors (26). As Fig. 1A indicates, SV40 RPTC exhibited specific ¹²⁵I-SCH23390 binding, with an apparent *K_d* of $7.52 \pm 0.06 \times 10^{-7}$ M and an apparent *B_{max}* of 228.4 ± 43.2 fmol/mg protein. Spiperone, the DA₂-specific antagonist, did not displace bound ¹²⁵I-SCH23390. RT-

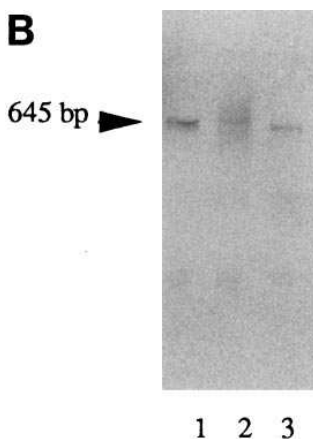
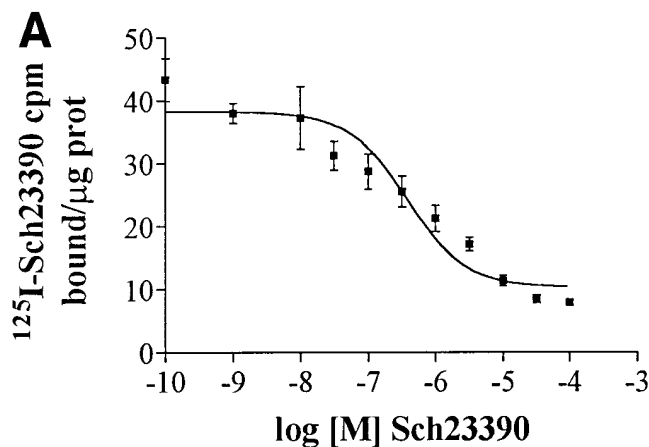


Figure 1. (A) Specific ¹²⁵I-SCH23390 binding. Binding of 0.1 nM ¹²⁵I-SCH23390 in the presence of varying concentrations of unlabeled SCH23390 was determined as described in Methods. (*K_d*: $7.52 \pm 0.06 \times 10^{-7}$ M; *B_{max}*: 228.4 ± 43.2 fmol/mg protein; *n* = 6) (B) RT-PCR amplification of DA₁R mRNA. Primers and PCR protocol were as described in Methods. Lane 1, rat renal cortex; lane 2, rabbit renal cortex; lane 3, SV40 immortalized rabbit proximal tubule cells.

PCR further indicated the presence of DA₁ receptor expression in RPTC using primers corresponding to third and seventh transmembrane regions of the rat DA₁ receptor. As indicated, PCR amplification from total rat renal cortex RNA produced the expected 645-bp fragment and sequencing indicated its identity (Fig. 1 B). Amplification of RNA from either rabbit kidney or RPTC produced DNA fragments of identical size, strongly suggesting the presence of a closely homologous DA₁ receptor subtype.

Fig. 2 A provides a representative experiment indicating alterations in steady state AT₁ receptor mRNA levels in response to agonist pretreatment. When RPTC were incubated with 10⁻⁵ M dopamine for 4 h, steady state AT₁ receptor mRNA levels significantly decreased to 33±7 of control levels (69±9 vs. 20±4 cpm/cpm of mutant AT₁R/cpm β-actin × 10⁶;

$n = 10$; $P < 0.005$). AT₁ receptor mRNA expression was decreased in a concentration dependent manner, with significant decreases seen at ≥ 10⁻⁶ M and maximum inhibition noted at 10⁻⁵ M (Fig. 2 B). Similar decreases in AT₁ receptor mRNA were noted in primary cultures of rabbit proximal tubule cells treated with dopamine (49±9 vs. 15±3 cpm/cpm of mutant AT₁R/cpm β-actin × 10⁶; $n = 4$; $P < 0.05$).

In RPTC ¹²⁵I-Ang II binding was similarly decreased in a concentration dependent manner (Fig. 2 C). Simultaneous incubation of angiotensin II (10⁻⁹ M) prevented the decreases in AT₁ receptor mRNA seen with incubation with 10⁻⁵ M dopamine (145±43% of control; $n = 8$; NS) (Fig. 2 D).

When proximal tubule cells were incubated for varying periods with dopamine (10⁻⁵ M), significant decreases in AT₁ receptor mRNA expression were noted by 4 h, and receptor ex-

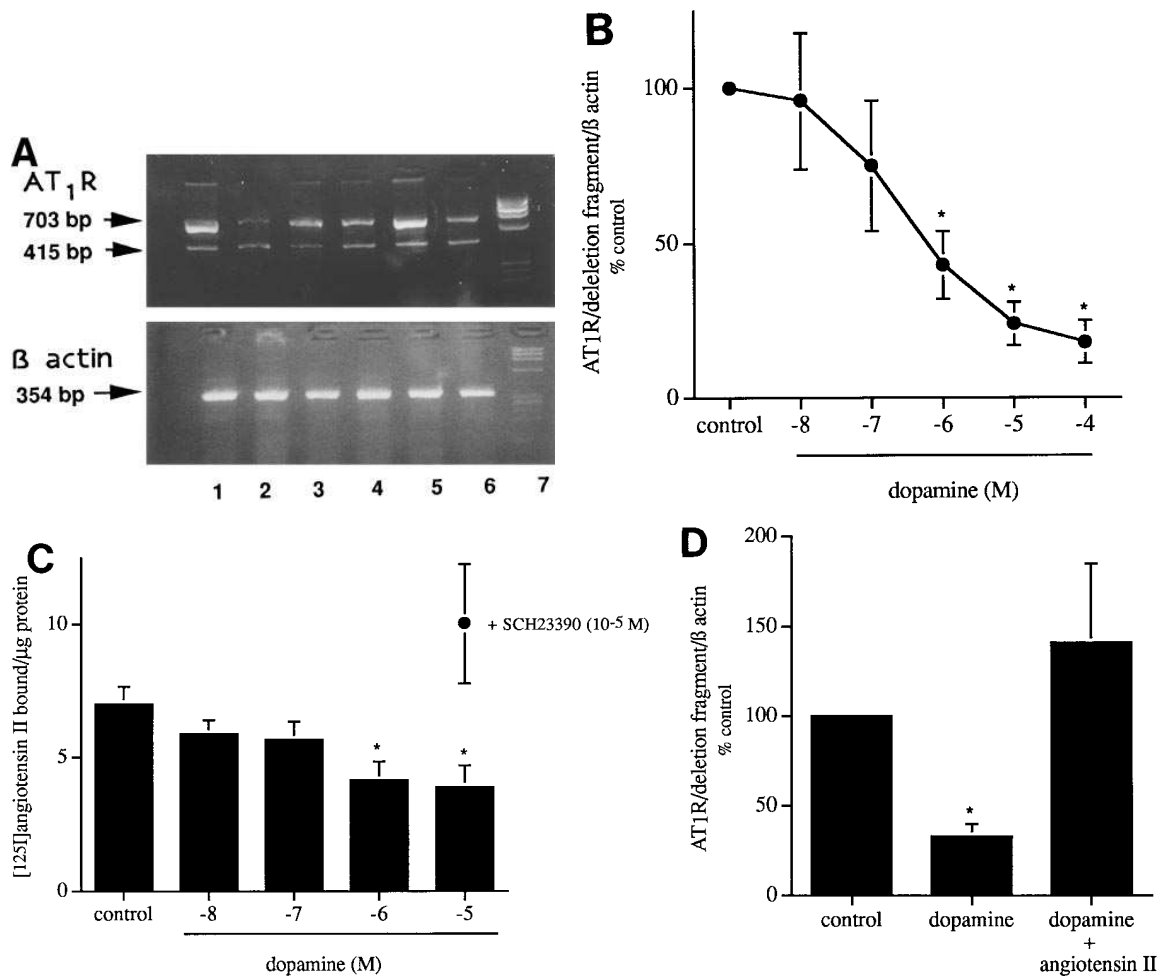


Figure 2. (A) Effect of dopamine on RT-PCR amplification of AT₁R mRNA in SV40 immortalized rabbit proximal tubule cells. Agents were administered to quiescent cells for 4 h and then RNA was harvested. 100 pg of cRNA of an MscI/MscI deletion fragment of rabbit AT₁R was added to 10 μg of total RNA and reverse transcribed. After reverse transcription, PCR was carried out for 35 cycles at 95°C for 20 s, 55°C for 30 s, and 72°C for 90 s using primers as described in Methods. The amplified fragment of intact AT₁R is 703 bp and the MscI/MscI deletion is 415 bp. β-actin was amplified in parallel tubes and its amplified fragment is 354 bp. Lane 1, control; lane 2, dopamine (10⁻⁵ M); lane 3, dopamine (10⁻⁵ M) + SCH23390 (10⁻⁵ M); lane 4, SKF38393 (10⁻⁵ M); lane 5, dopamine (10⁻⁵ M) + Ang II (10⁻⁹ M); lane 6, L-DOPA (10⁻⁴ M); lane 7, size markers. (B) Quantitative PCR of AT₁R mRNA in RPTC after 4 h incubation with the indicated concentration of dopamine. Results are expressed as percent of control values of cpm AT₁R/cpm deletion fragment/cpm β-actin × 10⁶. ($n = 5$; * $P < 0.05$) (C) Specific ¹²⁵I-Ang II binding in RPTC after dopamine incubation. Cells were incubated for 16 h with the indicated concentration of dopamine and specific binding of 0.1 nM ¹²⁵I-Ang II was determined as described in Methods. Also indicated is specific ¹²⁵I-Ang II after preincubation with dopamine (10⁻⁵) + SCH23390 (10⁻⁵). ($n = 5$ –8; * $P < 0.01$ compared to control) (D) Effect of angiotensin II upon dopamine-mediated decrease in AT₁R mRNA in RPTC. Cells were incubated for 4 h with 10⁻⁵ M dopamine ($n = 10$; $P < 0.005$ compared with control) or with 10⁻⁵ M dopamine + 10⁻⁹ M angiotensin II ($n = 8$; NS compared with control).

pression was inhibited for at least 48 h in response to continuous incubation with dopamine (Fig. 3).

Further experiments were designed to determine which dopamine receptor subtype(s) mediated the decrease in AT₁ receptor expression in RPTC. When cells were incubated with the DA₁ subtype agonist SKF38393 (10⁻⁵ M), AT₁ receptor mRNA levels significantly decreased (35±5% of control; *n* = 5; *P* < 0.005) (Figs. 2 A and 4 A). Similarly, simultaneous incubation with the DA₁ specific antagonist, SCH23390 (10⁻⁵ M), in the presence of dopamine, reversed dopamine's decrease in receptor mRNA levels (33±7 vs. 76±10% of control; *P* < 0.025) and [¹²⁵I]-Ang II binding (Figs. 2 C and 4 A). However, no reversal of dopamine inhibition of AT₁ receptor mRNA expression was seen with the DA₂-specific antagonist spiperone (12±7% of control; *n* = 8; NS compared with dopamine) or octoclothepein (25±7% of control; *n* = 8; NS compared with dopamine) (Fig. 4 B).

To determine if endogenously produced dopamine would also inhibit AT₁ receptor expression, RPTC were incubated with the dopamine precursor, L-DOPA. Incubation with L-DOPA (10⁻⁴ M) for 4 h decreased AT₁ receptor mRNA levels by 59±8% (29±6 cpm/cpm of mutant AT₁R/cpm of β-actin ×10⁶; *n* = 8; *P* < 0.01) (Figs. 2 A and 5). This inhibition appeared to be mediated by endogenously produced dopamine since simultaneous incubation with the L aromatic amino acid decarboxylase inhibitor, carbidopa, prevented the L-DOPA inhibition (-16±12% inhibition; *n* = 7; NS compared to control). Similarly, simultaneous incubation with the DA₁ receptor antagonist SCH23390 prevented the inhibition of AT₁ receptor mRNA expression (18±11% inhibition; *n* = 8; NS compared with control), indicating that, as with exogenously administered dopamine, this inhibitory effect was mediated by DA₁ receptors.

In the proximal tubule DA₁ receptors stimulate adenylate cyclase via coupling to G_s. In the cultured proximal tubule cells, addition of dopamine increased cAMP levels from 15±4 pmol/ml to 44±6 pmol/ml (*n* = 4; *P* < 0.025), which was inhib-

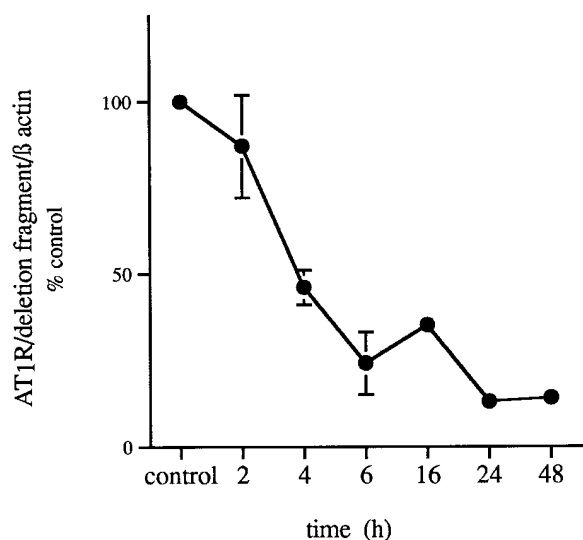


Figure 3. Time course of AT₁R mRNA expression in RPTC in response to dopamine. Cells were incubated for the indicated time with 10⁻⁵ M dopamine and quantitative PCR performed as described in Methods (*n* = 6; *P* < 0.05).

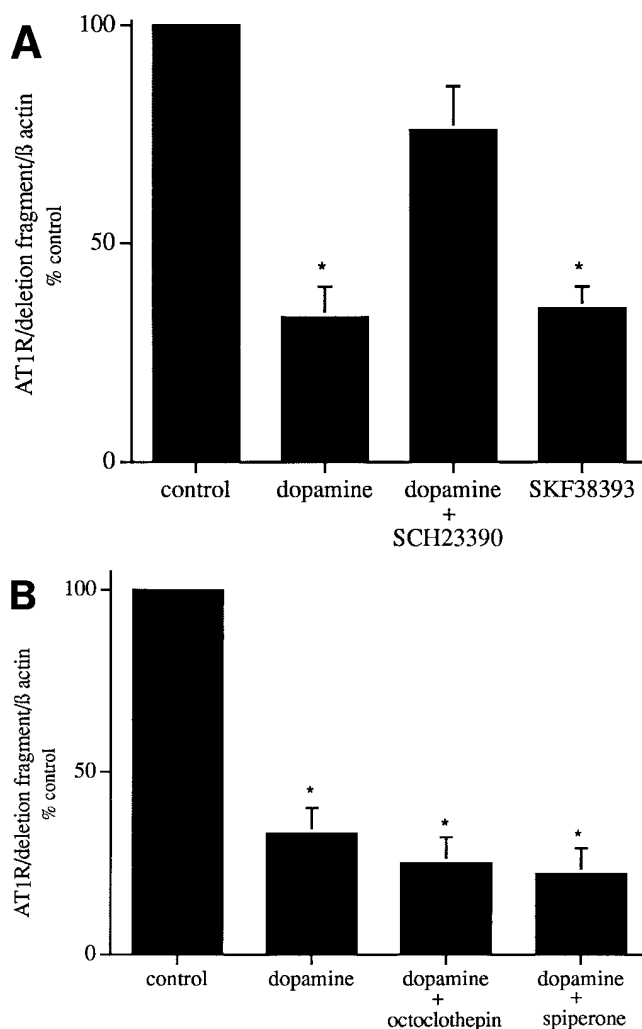


Figure 4. Effect of dopamine agonists and antagonists on AT₁R mRNA expression in RPTC. Cells were incubated with the indicated agents (10⁻⁵ M) for 4 h and quantitative PCR of AT₁R mRNA determined. (A) DA₁ agonist (SKF38393) or antagonist (SCH23390) (*n* = 5; **P* < 0.005 compared to control). (B) DA₂ antagonists (*n* = 8; **P* < 0.01 compared with control).

ited by simultaneous administration of SCH23390 (5±2 pmol/ml) (Fig. 6). Similarly, L-DOPA increased cAMP levels to 28±2 pmol/ml (*P* < 0.05).

To investigate whether dopamine-stimulated cAMP production was involved in decreased AT₁ receptor expression, cultured proximal tubule cells were incubated with dopamine (10⁻⁵ M) with or without the adenylate cyclase inhibitor, dideoxyadenosine (10⁻⁴ M) (27) and AT₁ receptor mRNA levels were assayed (Fig. 7). Dideoxyadenosine alone did not significantly alter receptor expression (64±10% of control; *n* = 6; NS). Dopamine alone decreased receptor expression to 27±6% of control (*P* < 0.0025); however, simultaneous administration of dideoxyadenosine reversed the dopamine-mediated inhibition of AT₁ receptor expression (80±24% of control; NS compared with control). Similar to our previous report (19), administration of the nonhydrolyzable analog of cAMP, dibutyl-cAMP, decreased steady state levels of AT₁ receptor mRNA levels to 32±6% of control (*n* = 6; *P* < 0.005 com-

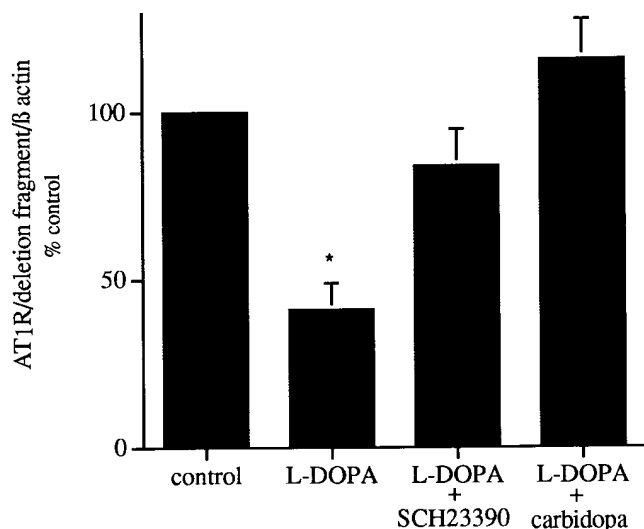


Figure 5. Effect of L-DOPA on AT₁R mRNA expression in RPTC. Cells were incubated for 4 h with L-DOPA (10^{-4} M) with or without simultaneous incubation with the DA₁ antagonist SCH23390 (10^{-5} M) or the aromatic L-amino acid decarboxylase inhibitor, carbidopa (10^{-4} M). ($n = 8$; * $P < 0.01$ compared with control).

pared with control); simultaneous administration of dideoxyadenosine did not affect dibutyryl-cAMP-mediated receptor inhibition ($28 \pm 5\%$ of control; $P < 0.0025$).

To determine whether the observed decrease in proximal tubule AT₁ receptor expression also occurred in vivo, rats were treated with L-DOPA for 5 days. L-DOPA treatment did not affect body weight (control: 227 ± 7 grams; L-DOPA: 226 ± 5 grams; $n = 9$; NS) or blood pressure (MAP: control: 78 ± 3 mmHg; L-DOPA: 80 ± 2 mmHg; $n = 9$; NS). The animals

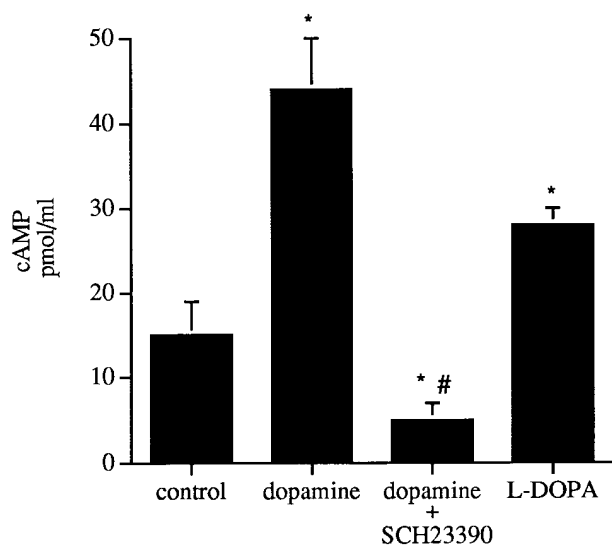


Figure 6. Effect of dopamine on cAMP production in RPTC. Cells were incubated for 10 min with the indicated agent (10^{-5} M dopamine, 10^{-5} M SCH23390, 10^{-4} M L-DOPA) in the presence of the phosphodiesterase inhibitor, isobutyl-methylxanthine (10^{-4} M) and cAMP was measured by RIA as described in Methods. $n = 4$; * $P < 0.05$ compared to control.

were killed and proximal tubule RNA was extracted. As indicated in Fig. 8, compared with age matched controls, administration of L-DOPA decreased proximal tubule AT₁ receptor mRNA levels by $80 \pm 5\%$ (49 ± 8 vs. 9 ± 2 cpm/cpm of mutant AT₁R/cpm of β -actin $\times 10^6$; $n = 6$; $P < 0.005$). In separate animals, proximal tubule suspensions were prepared and 125 I-Ang II binding was determined. L-DOPA treatment significantly decreased specific 125 I-Ang II binding (control: 0.74 ± 0.13 fmol/mg pro. vs. 0.40 ± 0.63 fmol/mg pro; $n = 5$; $P < 0.05$).

Discussion

The major finding of this study is that dopamine decreased AT₁ receptor mRNA expression and 125 I-angiotensin II binding in cultured proximal tubule cells and in rat proximal tubules in vivo. The studies in the SV40 immortalized cultured rabbit proximal tubule cells indicated that the inhibition of AT₁ receptor mRNA expression and 125 I-angiotensin II binding was mediated by DA₁ receptors. Dopamine stimulated increased cAMP production; furthermore, the adenylate cyclase inhibitor, dideoxyadenosine, prevented dopamine-mediated inhibition of AT₁ receptor expression but did not prevent the inhibition induced by a cell permeant, nonhydrolyzable cAMP analog. Dopamine may inhibit AT₁ receptor mRNA expression by increasing cAMP levels, in contradistinction to Ang II, which upregulates AT₁ receptor expression in this nephron segment by decreasing cAMP levels (19).

In addition to the present investigations, studies in mesangial cells, vascular smooth muscle cells and adrenocortical cells have also noted that increasing intracellular cAMP levels decreases steady state AT₁ receptor expression (24, 28, 29). Since the 5' promoter region of rat AT₁ receptor expression is known to contain a cAMP response element, further studies will be required to determine whether cAMP decreases receptor expression by transcriptional and/or posttranscriptional mechanisms. In this regard, studies suggest that angiotensin II, which decreases AT₁ receptor mRNA levels in vascular smooth muscle cells, appears to act through both transcriptional and post-transcriptional mechanisms (28).

Although both DA₁ and DA₂ receptors have been noted in kidney, DA₁ receptors have been localized primarily to proximal tubules (30–32). DA₁ receptor mRNA has been identified in microdissected rat proximal convoluted tubules (33) and in two cell lines with proximal tubule characteristics, OK cells and LLC-PK-1 cells (34, 35). DA₁ receptors in each cell type coupled to G_s, with activation of adenylate cyclase (34–36).

Ang II stimulates Na⁺/H⁺ exchange in the proximal tubule at least in part by activation of G_i, leading to an inhibition of adenylate cyclase and a decrease in cAMP levels (4). Conversely, dopamine inhibits proximal tubule Na⁺/H⁺ exchange at least in part by activation of G_s, leading to an increase in adenylate cyclase activity and cAMP levels (2, 12, 13, 35–38).

Ang II has also been shown to stimulate and dopamine to inhibit [Na⁺]/[K⁺]ATPase directly in proximal tubule. Sodium pump activation may require simultaneous activation of both DA₁ and DA₂ receptors in the proximal tubule (13). However, our studies with specific DA₂ antagonists failed to indicate a role for a DA₂ receptor mediated inhibition of AT₁ receptor expression (12).

The presence of components of the renin-angiotensin system in proximal tubule suggests that locally produced angiotensin II may modulate proximal tubule function (39, 40). In

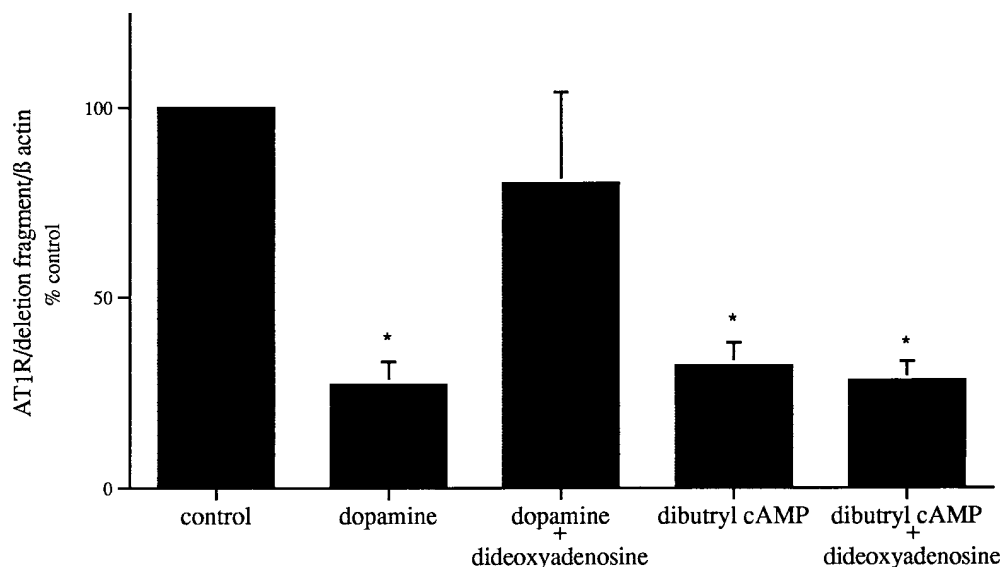


Figure 7. Effect of dideoxyadenosine on dopamine inhibition of AT₁R mRNA expression in RPTC. Cells were incubated for 4 h with dopamine (10^{-5} M) or dibutyl cAMP (10^{-4} M) in the presence or absence of the adenylyl cyclase inhibitor, dideoxyadenosine (10^{-4} M). ($n = 6$; * $P < 0.005$ compared to control).

the kidney, angiotensinogen mRNA has been localized to the proximal tubule (41). In addition, renin and angiotensin converting enzyme have also been identified in the proximal tubule (4). Angiotensin II concentrations in rat proximal tubule lumen have been determined by free flow micropuncture and found to be in the range of 10^{-8} M, compared to concentrations in the range of 10^{-10} M in systemic plasma (39, 42).

The proximal tubule is also the site of local dopamine production. Proximal tubules produce dopamine by uptake of L-DOPA ([L]-3-2[3,4-dihydroxyphenyl]-L-alanine) from tubular filtrate via a sodium dependent cotransport, followed by decarboxylation to dopamine by AADC (L-aromatic amino acid decarboxylase) (43). AADC is found in high concentrations in the proximal tubule (13, 44, 45). Urinary dopamine concentrations exceed circulating levels, and the major source of urinary dopamine is thought to be from proximal tubule synthesis rather than from plasma or renal nerves, since inhibition of AADC decreases urinary excretion of dopamine. LLCPK-1 cells increase cAMP production in response to L-DOPA, which can be inhibited by either the DA₁ antagonist, SCH23390 or the AADC inhibitor, carbidopa, suggesting

that intracellular dopamine synthesis can contribute to DA₁-mediated increases in cAMP (35).

In the present studies, the effect of dopamine or DA₁ agonists upon AT₁ receptor mRNA levels was mimicked by administration of L-DOPA and was blocked by simultaneous administration of either carbidopa or SCH23390. Furthermore, administration of L-DOPA to animals also decreased AT₁ receptor mRNA levels in the proximal tubule, indicating a role for local dopamine production in regulating AT₁ receptor mRNA expression.

Our previous studies indicated that salt depletion increased AT₁ receptor mRNA and receptor density in proximal tubule (19). In rats, salt depletion also increased proximal tubule angiotensinogen mRNA levels (41), and a sodium deficient diet for 7 d increased renal angiotensin I and angiotensin II levels (41). Conversely, sodium loading increases urinary dopamine levels (31). The mechanism for this increased dopamine excretion has been attributed to increased DOPA concentrations in plasma and tubular lumen (46) and increased proximal tubule DOPA uptake via a sodium-dependent cotransport process (44). Therefore, volume expansion may serve to decrease

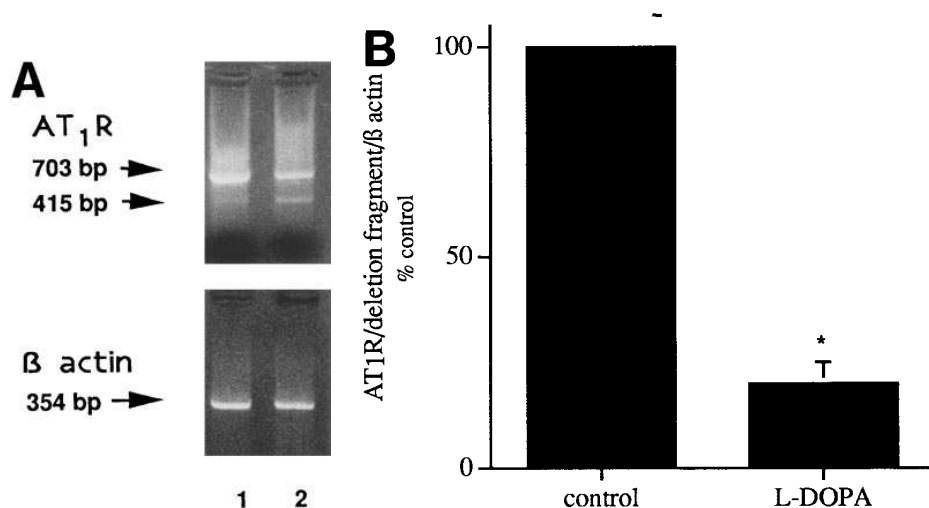


Figure 8. Effect of L-DOPA treatment on AT₁R mRNA expression in rat proximal tubules. Sprague-Dawley rats were administered L-DOPA (2 mg/kg per day) in the drinking water for 5 d, proximal tubule RNA was harvested and RT-PCR was performed as described in Fig. 2. (A) Representative PCR reaction. Lane 1, control; lane 2, L-DOPA. (B) Results of six experiments. * $P < 0.005$ compared with control.

proximal tubule AT₁ receptor expression by decreasing local and/or systemic angiotensin II production and increasing proximal tubule dopamine production.

It is of interest that dopamine also interferes with angiotensin II-mediated aldosterone secretion in the adrenal, since it is known that sodium depletion and angiotensin II also up-regulate adrenal angiotensin II receptors (47). Dopamine's effects have been attributed to hormonal regulation of the sodium-dependent modulation of adrenal angiotensin responsiveness (48). Although the question of whether dopamine alters adrenal angiotensin II receptor density has not yet been studied, these observations suggest that a mechanism by which dopamine may chronically modulate salt and water balance is by altering responsiveness to angiotensin II in the adrenal gland and the proximal tubule.

In two experimental models of hypertension, spontaneously hypertensive rats (SHR) and Dahl salt sensitive rats, defective coupling of dopamine to adenylate cyclase activation has been noted (49–53). In hypertensive humans, both defective renal dopamine production in response to salt loading and decreased proximal tubule responsiveness to dopamine have also been reported (54). Prehypertensive SHR express increased angiotensin II receptor density in proximal tubule and have increased proximal tubule reabsorption in response to angiotensin II infusion (55, 56). These findings suggest the possibility that in salt sensitive hypertension, defects in the proximal tubule dopaminergic system may prevent normal modulation of proximal tubule angiotensin II receptor density.

In summary, the present findings suggest that in addition to acute inhibition of proximal tubule volume reabsorption, dopamine may also serve to inhibit tonically AT₁ receptor RNA expression and angiotensin II receptor density in the proximal tubule. Therefore, the balance between locally produced angiotensin II and dopamine may be important not only in the acute regulation of proximal reabsorption but also in the chronic resetting of proximal tubule responsiveness to angiotensin II stimulation.

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References

- Cogan, M.G. 1990. Angiotensin II: a powerful controller of sodium transport in the early proximal tubule. *Hypertension*. 15:451–458.
- Aperia, A. 1994. Dopamine action and metabolism in the kidney. *Curr. Opin. Nephrol. Hypertens.* 3:39–45.
- Brown, G.P., and J.G. Douglas. 1982. Angiotensin II binding sites on isolated rat renal brush border membranes. *Endocrinology*. 111:1830–1836.
- Brown, G.P., and J.G. Douglas. 1983. Angiotensin II-binding sites in rat and primate isolated renal tubular basolateral membranes. *Endocrinology*. 112: 2007–2014.
- Burns, K.D., T. Homma, and R.C. Harris. 1993. The intrarenal renin-angiotensin system. *Semin. Nephrol.* 13:13–30.
- Schuster, V.L., J.P. Kokko, and H.R. Jacobson. 1984. Angiotensin II directly stimulates sodium transport in rabbit proximal convoluted tubules. *J. Clin. Invest.* 73:507–515.
- Liu, F.-Y., and M.G. Cogan. 1988. Angiotensin II stimulation of hydrogen ion secretion in the rat early proximal tubule. Modes of action, mechanism, and kinetics. *J. Clin. Invest.* 82:601–607.
- Burns, K.D., T. Inagami, and R.C. Harris. 1993. Cloning of a rabbit kid-

- ney cortex AT₁ angiotensin II receptor that is present in proximal tubule epithelium. *Am. J. Physiol.* 264:F645–F654.
- Xie, M.-H., F.-Y. Liu, P.C. Wong, P.B.M.W.H. Timmermans, and M.G. Cogan. 1990. Proximal nephron and renal effects of DuP 753, a nonpeptide angiotensin II antagonist. *Kidney Int.* 38:473–479.
- Chen, C.J., S. Apparsundaram, and M.F. Lokhandwala. 1991. Intrarenally produced angiotensin II opposes the natriuretic action of the dopamine-1 receptor agonist fenoldopam in rats. *J. Pharm. Exp. Ther.* 256:486–491.
- Gesek, F.A., and A.C. Schoolwerth. 1991. Hormone responses of proximal Na(+)-H(+) exchanger in spontaneously hypertensive rats. *Am. J. Physiol.* 261:F526–F536.
- Aperia, A., U. Holtback, M.L. Syren, L.B. Svensson, J. Fryckstedt, and P. Greengard. 1994. Activation/deactivation of renal Na(+),K(+)-ATPase: a final common pathway for regulation of natriuresis. *FASEB J.* 8:436–439.
- Bertorello, A., T. Hokfelt, M. Goldstein, and A. Aperia. 1988. Proximal tubule Na+-K+-ATPase activity is inhibited during high salt diet: evidence for DA-mediated effect. *Am. J. Physiol.* 254:F795–F801.
- Felder, R.A., S. Kinoshita, A. Sidhu, K. Ohbu, and F.J. Kaskel. 1990. A renal dopamine-1 defect in two genetic models of hypertension. *Am. J. Hypertens.* 3:96S–99S.
- Stier, C.T., H.D. Itskovitz, and Y.H. Chen. 1993. Urinary dopamine and sodium excretion in spontaneously hypertensive rats. *Clin. Exp. Hypertens.* 15: 105–123.
- Iwai, N., and T. Inagami. 1992. Identification of a candidate gene responsible for the high blood pressure of spontaneously hypertensive rats. *J. Hypertens.* 10:1155–1157.
- Vinay, P., A. Gougoux, and G. Lemieux. 1981. Isolation of a pure suspension of rat proximal tubules. *Am. J. Physiol.* 241:F403–F411.
- Harris, R.C., and T.O. Daniel. 1989. Epidermal growth factor binding, stimulation of phosphorylation and inhibition of gluconeogenesis in rat proximal tubule. *J. Cell Physiol.* 139:383–391.
- Cheng, H.-F., B.N. Becker, K.D. Burns, and R.C. Harris. 1995. Angiotensin II regulates its receptor sites in rabbit proximal tubule. *J. Clin. Invest.* 95: 2012–2019.
- Harris, R.C. 1992. S6 kinase activity in proximal tubule cells. Evidence for activation by epidermal growth factor and angiotensin II in vitro and by contralateral nephrectomy in vivo. *Am. J. Physiol.* 263:F127–F134.
- Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.
- Cheng, H.-F., K.D. Burns, and R.C. Harris. 1994. Reduced proximal tubule angiotensin II receptor expression in experimental diabetes mellitus. *Kidney Int.* 46:1603–1610.
- Briggs, J.P., K. Todd-Turla, J.B. Schnermann, and P.D. Killen. 1993. Approach to the molecular basis of nephron heterogeneity: Application of reverse-transcription-polymerase chain reaction to dissected tubule segments. *Semin. Nephrol.* 13:2–12.
- Makita, N., N. Iwai, T. Inagami, and K.F. Badr. 1992. Two distinct pathways in the down-regulation of type-1 angiotensin II receptor gene in rat glomerular mesangial cells. *Biochem. Biophys. Res. Commun.* 184:142–146.
- Harris, R.C. 1989. Response of rat inner medullary collecting duct to epidermal growth factor. *Am. J. Physiol.* 256 (Renal Fluid and Electrol. 25) F1117–F1124.
- Barnett, A., H.S. Ahn, W. Billard, E.H. Gold, J.D. Kohli, D. Glock, and L.I. Goldberg. 1986. Relative effects of SCH23390 and its analogs in three tests for D₁/DA₁ dopamine receptor antagonism. *Eur. J. Pharmacol.* 128:249–253.
- Zenser, T.V., and R.W. Wannemacher, Jr. 1976. Inhibition of cholera toxin-stimulated intestinal epithelial cell adenylate cyclase by adenosine analogs. *Proc. Soc. Exp. Biol. Med.* 152:126–129.
- Lassegue, B., R.W. Alexander, G. Nickenig, M. Clark, T.J. Murphy, and K.K. Griendling. 1995. Angiotensin II down-regulates the vascular smooth muscle AT₁ receptor by transcriptional and post-transcriptional mechanisms: evidence for homologous and heterologous regulation. *Mol. Pharmacol.* 48: 601–609.
- Bird, I.M., J.I. Mason, and W.E. Rainey. 1994. Regulation of type 1 angiotensin II receptor messenger ribonucleic acid expression in human adrenocortical carcinoma H295 cells. *Endocrinology*. 134:2468–2474.
- Hegde, S.S., A. Ricci, F. Amenta, and M.F. Lokhandwala. 1989. Evidence from functional and autoradiographic studies for the presence of tubular dopamine-1 receptors and their involvement in the renal effects of fenoldopam. *J. Pharmacol. Exp. Ther.* 251:1237–1245.
- Lokhandwala, M.F., and F. Amenta. 1991. Anatomical distribution and function of dopamine receptors in the kidney. *FASEB J.* 5:3023–3030.
- Huo, T., and D.P. Healy. 1989. Autoradiographic localization of dopamine DA₁ receptors in rat kidney with [³H] Sch 23390. *Am. J. Physiol.* 257:F414–F423.
- Yamaguchi, I., P.A. Jose, M.M. Mouradian, L.M. Canessa, F.J. Monsma, Jr., D.R. Sibley, K. Takeyasu, and R.A. Felder. 1993. Expression of dopamine D1A receptor gene in proximal tubule of rat kidneys. *Am. J. Physiol.* 264:F280–F285.
- Nash, S.R., N. Godinot, and M.G. Caron. 1993. Cloning and characterization of the opossum kidney cell D1 dopamine receptor: expression of identi-

- cal D1A and D1B dopamine receptor mRNAs in opossum kidney and brain. *Mol. Pharm.* 44:918–925.
35. Grenader, A., and D.P. Healy. 1991. Locally formed dopamine stimulates cAMP accumulation in LLC-PK1 cells via a DA1 dopamine receptor. *Am. J. Physiol.* 260:F906–F912.
 36. Kinoshita, S., A. Sidhu, and R.A. Felder. 1989. Defective dopamine-1 receptor adenylate cyclase coupling in the proximal convoluted tubule from the spontaneously hypertensive rat. *J. Clin. Invest.* 84:1849–1856.
 37. Felder, C.C., T. Campbell, F. Albrecht, and P.A. Jose. 1990. Dopamine inhibits Na⁺-H⁺ exchanger activity in renal BBMVs by stimulation of adenylate cyclase. *Am. J. Physiol.* 259:F297–F303.
 38. Cheng, L., P. Precht, D. Frank, and C.T. Liang. 1990. Dopamine stimulation of cAMP production in cultured opossum kidney cells. *Am. J. Physiol.* 258:F877–F882.
 39. Seikaly, M.G., B.S. Arant, Jr., and F.D. Seney, Jr. 1990. Endogenous angiotensin concentrations in specific intrarenal fluid compartments in the rat. *J. Clin. Invest.* 86:1352–1357.
 40. Braam, B., K.D. Mitchel, J. Fox, and L.G. Navar. 1993. Proximal tubular secretion of angiotensin II in rats. *Am. J. Physiol.* 264:F891–F898.
 41. Ingelfinger, J.R., W. Min Zuo, E.A. Fon, K.E. Ellison, and V.J. Dzau. 1990. In situ hybridization evidence for angiotensinogen messenger RNA in the rat proximal tubule. *J. Clin. Invest.* 85:417–423.
 42. Fox, J., S. Guan, A.A. Hymel, and L.G. Navar. 1992. Dietary Na and ACE inhibition effects on renal tissue angiotensin I and II and ACE activity in rats. *Am. J. Physiol.* 262:F902–F909.
 43. Lokhandwala, M.F., and S.S. Hegde. 1990. Cardiovascular dopamine receptors: role of renal dopamine and dopamine receptors in sodium excretion. *Pharmacol. Toxicol.* 66:237–243.
 44. Baines, A.D., and W. Chan. 1980. Production of urine free dopamine from DOPA: a micropuncture study. *Life Sci.* 26:253–259.
 45. Chan, Y.L. 1976. Cellular mechanisms of renal tubular transport of L-DOPA and its derivatives in the rat: micropuncture studies. *J. Pharm. Exp. Ther.* 199:17–24.
 46. Carey, R.M., G.R. Van Loon, A.D. Baines, and E.M. Ortt. 1981. Decreased plasma and urinary dopamine during dietary sodium depletion in man. *J. Clin. Endocrinol. Metab.* 52:903–909.
 47. Hauger, R.L., G. Aguilera, and K.J. Catt. 1978. Angiotensin II regulates its receptor sites in the adrenal glomerulosa. *Nature (Lond.)* 27:176–178.
 48. Missale, C., C. Lombardi, R. DeCortis, M. Memo, M.O. Carruba, and P.C. Spano. 1989. Dopaminergic receptor mechanisms modulating the renin-angiotensin system and aldosterone secretion: an overview. *J. Cardiovasc. Pharmacol.* 4 (Suppl 4):S29–S39.
 49. Ohbu, K., F.J. Kaskel, S. Kinoshita, and R.A. Felder. 1995. Dopamine-1 receptors in the proximal convoluted tubule of Dahl rats: defective coupling to adenylate cyclase. *Am. J. Physiol.* 268:R231–R235.
 50. Ohbu, K., and R.A. Felder. 1993. Nephron specificity of dopamine receptor-adenylate cyclase defect in spontaneous hypertension. *Am. J. Physiol.* 264:F274–F279.
 51. Chen, C.J., and M.F. Lokhandwala. 1992. An impairment of renal tubular DA-1 receptor function as the causative factor for diminished natriuresis to volume expansion in spontaneously hypertensive rats. *Clin. Exp. Hypertension.* 14:615–628.
 52. Sidhu, A., P. Vachvanichsanong, P.A. Jose, and R.A. Felder. 1992. Persistent defective coupling of dopamine-1 receptors to G proteins after solubilization from kidney proximal tubules of hypertensive rats. *J. Clin. Invest.* 89:789–793.
 53. Chen, C., R.E. Beach, and M.F. Lokhandwala. 1993. Dopamine fails to inhibit renal tubular sodium pump in hypertensive rats. *Hypertension.* 21:364–372.
 54. Clark, B.A., R.M. Rosa, F.H. Epstein, J.B. Young, and L. Landsberg. 1992. Altered dopaminergic responses in hypertension. *Hypertension.* 19:589–594.
 55. Matsushima, Y., M. Kawamura, and S. Akabane. 1988. Increases in renal angiotensin II content and tubular angiotensin II receptors in prehypertensive spontaneously hypertensive rats. *J. Hypertension.* 6:791–796.
 56. Thomas, D., P.J. Harris, and T.O. Morgan. 1988. Altered responsiveness of proximal tubule fluid reabsorption of peritubular angiotensin II in spontaneously hypertensive rats. *J. Hypertension.* 6:407–410.