Dopamine D$_1$ Receptor and Protein Kinase C Isoforms in Spontaneously Hypertensive Rats

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Abstract—Dopamine, via D$_1$-like receptors, stimulates the activity of both protein kinase A (PKA) and protein kinase C (PKC), which results in inhibition of renal sodium transport. Since D$_1$-like receptors differentially regulate sodium transport in normotensive and hypertensive rats, they may also differentially regulate PKC expression in these rat strains. Thus, 2 different D$_1$-like agonists (fenoldopam or SKF 38393) were infused into the renal artery of anesthetized normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) (n=5 to 6/drug/strain). Ten or 60 minutes after starting the D$_1$-like agonist infusion, both the infused kidney and the noninfused kidney that served as control were prepared for analysis. The D$_1$-like agonists produced a greater diuresis and natriuresis and inhibited Na$^+$,K$^+$-ATPase activity in proximal tubule (PT) and medullary thick ascending limb (mTAL) to a greater extent in WKY ($\Delta$20±1%) than in SHR ($\Delta$7±1%, P<0.001). D$_1$-like agonists had no effect on PKC-\(\alpha\) or PKC-\(\lambda\) expression in either membrane or cytosol but increased PKC-\(\theta\) expression in PT in both WKY and SHR at 10 minutes but not at 60 minutes. However, membranous PKC-\(\delta\) expression in PT and mTAL decreased in WKY but increased in SHR with either 10 or 60 minutes of D$_1$-like agonist infusion. D$_1$-like agonists also decreased membranous PKC-\(\zeta\) expression in PT and mTAL in WKY but increased it in PT but not in mTAL in SHR. We conclude that there is differential regulation of PKC isoform expression by D$_1$-like agonists that inhibits membranous PKC-\(\delta\) and PKC-\(\zeta\) in WKY but stimulates them in SHR; this effect in SHR is similar to the stimulatory effect of norepinephrine and angiotensin II and may be a mechanism for their differential effects on sodium transport. (Hypertension. 1998;32:1049-1053.)

Key Words: receptors, dopamine \(\bullet\) kidney tubules, proximal \(\bullet\) renal medulla \(\bullet\) protein kinase C

PROTEIN KINASE C (PKC) isoforms have been classified into 3 groups: (1) the conventional PKCs (cPKC-\(\alpha\), -\(\beta\)-, -\(\betaII\), and -\(\gamma\); (2) the novel PKCs (nPKC-\(\delta\), -\(\epsilon\), -\(\theta\), -\(\eta\)/G, and -\(\mu\)); and (3) the atypical PKCs (aPKC-\(\lambda\)(i) and -\(\zeta\)).\(^1\) Several of these isoforms have been identified in various nephron segments.\(^2\) Thus, proximal tubule (PT) expresses PKC-\(\alpha\), -\(\delta\), -\(\epsilon\), and -\(\zeta\) isoforms,\(^3\) and the medullary thick ascending limb of Henle (mTAL) expresses cPKC-\(\alpha\) and -\(\betaII\), nPKC-\(\delta\) and -\(\epsilon\), and aPKC-\(\zeta\) isoforms.\(^4\) PKC-\(\alpha\), -\(\beta\), -\(\delta\), -\(\epsilon\), and -\(\zeta\) were identified in sieved glomeruli, whereas cultured glomerular epithelial and mesangial cells express PKC-\(\alpha\), -\(\delta\), -\(\epsilon\), and -\(\zeta\).\(^5\) PKC-\(\alpha\), -\(\epsilon\), -\(\eta\), -\(\theta\), and -\(\zeta\) have been identified in rabbit cortical collecting duct.\(^6\)

Dopamine decreases renal sodium transport by adenylyl cyclase/protein kinase A (PKA) and phospholipase C (PLC)/PKC–dependent and –independent mechanisms.\(^7\)-\(^11\) However, other receptors (eg, angiotensin II [Ang II] and \(\alpha\)-adrenergic receptors) also increase PKC activity but decrease sodium excretion.\(^12\)-\(^15\) We have reported that D$_1$-like receptor agonists increase the expression and activity of PLC-\(\beta\) but decrease the expression and activity of PLC-\(\gamma\) in renal cortical membranes.\(^11\) Norepinephrine was also shown to increase expression and activity of PLC-\(\beta\) but had no effect on PLC-\(\gamma\) expression or activity.\(^15\) PKC mediates some of the effects of dopamine, Ang II, and norepinephrine on sodium transport. Thus, dopamine increases PKC activity and decreases Na$^+$,K$^+$-ATPase in renal PT.\(^8\)-\(^12\),\(^13\) In contrast, norepinephrine and Ang II increase PKC, Na$^+$,K$^+$-ATPase,\(^14\),\(^15\) and Na$^+$/H$^+$ exchanger activity.\(^15\) The mechanism through which these receptors produce opposing effects in the kidney has not been studied but may relate to their differing regulation of PKC isoform expression and activity.

Norepinephrine has been reported to induce the translocation of PKC-\(\alpha\), -\(\beta\)-, -\(\betaII\), -\(\gamma\), -\(\delta\), and -\(\epsilon\) from cytosol to membrane in a thyroid cell line.\(^16\) In airway epithelial cells, \(\alpha\)-adrenergic stimulation produced a sustained translocation of cytosolic PKC-\(\zeta\) and transient translocation of PKC-\(\delta\) to the membrane.\(^17\) Ang II has been reported to increase the expression of PKC-\(\alpha\) and -\(\epsilon\) (and possibly PKC-\(\delta\)) in rat PT.\(^3\),\(^15\) The effect of dopamine receptor stimulation on PKC isoforms expressed in the kidney has not been studied. Moreover, a relationship between dopamine receptor–related PKC isoform expression, sodium excretion, and Na$^+$,K$^+$-ATPase activity has not been reported.

The natriuretic effect of dopamine is impaired in genetic hypertension.\(^7\),\(^19\) This is caused in part by a decreased ability of dopamine via D$_1$-like receptors to stimulate adenylyl
cyclase/PKA and PLC/PKC activity. The decreased ability of D₁-like receptors to stimulate PKC activity in genetic hypertension could be caused by a differential regulation of PKC isoform by dopamine. Therefore, the present study was designed to determine whether D₁-like receptors differentially regulate PKC isoform expression in normotensive and hypertensive rats in vivo. The differential effect of D₁-like receptors on signal transduction and sodium transport has been noted mainly in PT of these rat strains, however, dopamine also decreases sodium pump activity in the mTAL. Therefore, studies were performed in the renal cortex, PT, and mTAL. To ensure that any D₁-agonist effect is caused by D₁-like receptor stimulation, 2 different D₁-like receptor agonists (SKF 38393 and fenoldopam) were used. The drugs were infused into the renal artery to obviate any confounding systemic effects.

Methods

Ten- to 16-week-old spontaneously hypertensive (SHR; n = 12) and Wistar-Kyoto (WKY; n = 13) rats were maintained on standard rat chow until 1 day before the experiment; water was given ad libitum. The rats were anesthetized with pentobarbital (50 mg/kg body wt IP); the temperature was maintained between 36°C and 37°C, and the rats were tracheotomized. Anesthesia was maintained by infusion of pentobarbital at 0.8 mg/100 g body wt per hour. Catheterization of jugular and femoral vessels and left and right ureters and fluid administration were performed as previously described. The right suprarenal artery was catheterized, and normal saline was infused at 40 μL/h. After a 60-minute equilibration period, a 60-minute urine collection period was begun; then the infusate was changed to SKF 38393 (120 ng·kg⁻¹·min⁻¹) or fenoldopam (1 μg·kg⁻¹·min⁻¹) at 40 μL/h. The noninfused left kidney served as a concurrent control for the right infused kidney. After 10 or 60 minutes of infusion, the kidneys were removed and the cortex was separated from the medulla. Renal PT and mTAL were obtained by enzymatic digestion, sieving, and differential centrifugation. The protein concentrations were determined by the Bradford method.

Measurement of Na⁺,K⁺-ATPase Activity

Na⁺,K⁺-ATPase activity was measured in PT and mTAL in the noninfused and the SKF 38393-infused kidney by use of tris-p-nitrophenylphosphate as the substrate. This measurement is relatively insensitive to the endogenous phosphate pool and to metabolic competition for ATP.

Immunoblot Analysis

Immunoblotting was performed as described previously. The immunoblots detected by enhanced chemiluminescence (ECL) were quantified by use of Quantscan (Biosoft). The percent area of each blot was quantified with the total area arbitrarily set at 100%.

Data Analysis

Data are expressed as mean ± SE. Differences between infused and noninfused kidney were evaluated by paired t test. When only 2 variables were being compared between groups, differences were determined by t test. When >2 groups were compared, differences among groups or within groups were determined by ANOVA or ANOVA for repeated measures, respectively, and Scheffe’s test. P < 0.05 was considered significant.

Materials

Male WKY and SHR (250 to 400 g) were from Taconic Farms Inc, (Germantown, NY); D₁-like agonist fenoldopam was from Smith Kline Beecham Pharmaceuticals; SKF 38393, Research Biochemicals International; PKC isozyme antibodies, Gibco-BRL and Upstate Biotechnology Inc; goat anti-mouse or anti-rabbit IgG conjugated to horseradish peroxidase, Jackson ImmunoResearch Labs, Inc; and ECL Western blotting detection reagents RPN2106, Amersham Corp. All other chemicals were from Sigma Chemical Co.

Results

Renal Function Studies

Blood pressure was greater in SHR than in WKY (systolic blood pressure in WKY was 128 ± 1 mm Hg, n = 13, and 217 ± 3 mm Hg in SHR, n = 10; P < 0.05). Urine flow (V) and absolute (UNaV) and fractional sodium excretion (FENa) in the right infused kidney were not different from the left noninfused kidney during the baseline periods (not shown). Sixty-minute infusion of SKF 38393 (SKF) had no effect in the left noninfused kidney but increased V, UNaV, and FENa in the infused right kidney in WKY (V, μL/min: baseline = 4.89 ± 0.48, SKF = 8.59 ± 0.76; UNaV, nmol/min: baseline = 268 ± 64, SKF = 589 ± 172; FENa, %: baseline = 0.17 ± 0.05, SKF = 0.3 ± 0.09. ANOVA for repeated measures, baseline versus SKF, right versus left, Scheffé’s test) but not in SHR (V, μL/min: baseline = 3.73 ± 0.25, SKF = 4.46 ± 0.50; UNaV, nmol/min: baseline = 377 ± 54, SKF = 368 ± 61; FENa, %: baseline = 0.24 ± 0.04, SKF = 0.22 ± 0.04). The changes in V, UNaV, and FENa due to SKF 38393 in WKY were different from control and were greater in WKY (V = 76.3 ± 5.96, UNaV = 113.96 ± 25.94%, FENa = 88.05 ± 31.67%) than in SHR (V = 21.95 ± 5.50%, P < 0.01 versus WKY; UNaV = 1.66 ± 12.74%, P < 0.01 versus WKY; FENa = 0.73 ± 19.80%, P < 0.05 versus WKY). Glomerular filtration rate was not different between WKY and SHR and was not affected by SKF 38393 in either rat strain (data not shown). The effect of the D₁-like agonist fenoldopam on V was similar to that obtained with SKF 38393. In WKY, V increased 22% (4.86 ± 0.93 to 6.20 ± 0.98 μL/min, P < 0.05, n = 5, paired t test) at 10 minutes but was unchanged in SHR (3.36 ± 0.28 to 4.17 ± 0.48 μL/min, P = 0.24, n = 6, paired t test). At 60 minutes in WKY, V increased 57% (3.17 ± 0.54 to 4.94 ± 0.17 μL/min, P < 0.05, n = 5, paired t test), whereas in SHR, V increased by only 36% (3.65 ± 0.20 to 5.76 ± 0.24, P < 0.05, n = 5, paired t test). V at 60 minutes was greater in WKY than in SHR (P < 0.05, t test).

Na⁺,K⁺-ATPase Activity

SKF 38393 infusion was associated with a greater decrease in Na⁺,K⁺-ATPase activity in PT and mTAL in WKY than in SHR. Thus, in WKY, SKF 38393 decreased Na⁺,K⁺-ATPase activity in the right infused kidney by 21.49 ± 0.81% in PT and 18.66 ± 1.27% in mTAL (compared with the noninfused left kidney, P < 0.05, paired t test). The corresponding values in SHR were PT = −7.54 ± 0.76% and mTAL = −6.95 ± 0.67%.

Subcellular PKC Isoform Expression in Renal Cortex, PT, and mTAL

Five PKC isoforms, cPKC-α (kDa 80), nPKC-δ (kDa 80) and -θ (kDa 79), and aPKC-ζ (kDa 78) and -λ (kDa 74) were found in both cytosolic and membranous fractions of the kidney cortex, PT, and mTAL from both WKY and SHR. Neither PKC-β nor PKC-γ was found in either WKY or SHR (data not shown).
D₁-Like Receptor Agonist Stimulation and PKC Isoform Expression

Because the decreased ability of dopamine, via D₁-like receptors, to produce a natriuresis in SHR is in part due to a decreased ability to stimulate PKC activity,⁴⁻⁶ we measured PKC isoform expression after infusion of 2 different D₁-like agonists, SKF 38393 and fenoldopam. In the control noninfused kidney, PKC-δ expression in membrane but not in cytosol of PT and mTAL was greater in WKY than in SHR (Figure 1A and 1B). In PT and mTAL, SKF 38393 decreased membranous PKC-δ expression in WKY at 60 minutes; in contrast, in SHR, PKC-δ increased at 60 minutes (Figure 1A and 1B). In WKY, another D₁-like agonist, fenoldopam, also decreased PKC-δ expression in renal cortical membranes at 10 and 60 minutes: WKY at 10 minutes (control=27.2±4.0% area, fenoldopam=7.6±0.4% area, P<0.05, n=4, paired t test) and at 60 minutes (control=18.0±1.0% area, fenoldopam=6.0±0.6% area, P<0.05, n=3, paired t test); in contrast, in SHR, PKC-δ increased at 10 minutes (control=22.0±1.0% area, fenoldopam=43.1±4.6% area, P<0.05, paired t test) but tended to return to baseline at 60 minutes. These changes in membranous PKC-δ expression after fenoldopam stimulation resulted in reciprocal changes in cytosolic PKC-δ expression in both WKY and SHR at 10 minutes (data not shown).

In the control noninfused kidney, PKC-ζ expression in membrane but not in cytosol of mTAL was greater in WKY than in SHR. In WKY, 60 minutes of SKF 38393 infusion decreased membranous PKC-ζ expression in PT and mTAL in a way similar to that noted with PKC-δ expression (Figure 2A and 2B). In contrast, in SHR, membranous PKC-ζ increased in PT but was unchanged in mTAL. There were no changes in cytosolic PKC-ζ expression in PT or mTAL in either WKY or SHR. Fenoldopam infusion for 60 minutes also decreased membranous PKC-ζ expression in renal cortex of WKY (control=38.1±6.5% area, fenoldopam=10.6±2.3% area, n=3, P<0.05 paired t test) and tended to increase it in SHR but did not reach statistical significance (data not shown). In cytosol, PKC-ζ expression increased in both WKY (control=29.5±5.0% area, fenoldopam=46.0±6.7% area, P=0.07, n=4, paired t test) and SHR (control=24.0±5.5% area, fenoldopam 46.6±7.6% area, P<0.05, n=4, paired t test) at 10 minutes. As with SKF 38393, cytosolic PKC-ζ expression was unchanged at 60 minutes in either rat strain.

In the control noninfused kidney, PKC-α, -λ, and -θ expression was not different between WKY and SHR. The expression of PKC-α, -θ, or -λ was not altered by the
60-minute infusion of either D1-like agonist in renal cortex, PT, or mTAL in either WKY or SHR (data not shown). However, the 10-minute infusion of fenoldopam increased membranous PKC-θ expression in both WKY (control = 18.0 ± 5.1% area, fenoldopam = 34.0 ± 4.4% area, n = 4, \(P < 0.05\), paired \(t\) test) and SHR (control = 17.4 ± 4.7% area, fenoldopam = 30.5 ± 4.1% area, n = 4, \(P < 0.05\), paired \(t\) test).

**Discussion**

The demonstration of cPKC-α, nPKC-δ and -θ, and aPKC-λ and -ζ in rat renal cortical and PT subcellular fractions is in agreement with earlier studies.4,5 In addition, the present studies demonstrate the presence of nPKC-δ and -θ and aPKC-ζ in mTAL. Our studies also confirm previous reports that failed to show the expression of cPKC-β1 and -γ in rat renal cortex.2,6

Norepinephrine and Ang II receptor stimulation increase sodium transport at least in part via increases in PKC activity.14,15 In normotensive animals, dopamine, via D1-like receptors, decreases sodium transport in renal PT in part by the stimulation of PKC activity.9,12,13 It is not readily apparent how an increase in PKC activity due to norepinephrine, Ang II, and dopamine can lead to an increase in sodium transport in one instance (norepinephrine and Ang II) and a decrease in another instance (dopamine). The differential effect on sodium transport may be due to differential effects on PKC isoforms; D1-like agonists (presumably acting on D1-like receptors) had no effect on PKC-α and PKC-λ but decreased PKC-δ and PKC-ζ expression, the opposite of the effect of norepinephrine and Ang II.14,15 In renal PT, Ang II has been reported to increase the membranous expression of PKC-α and PKC-δ at high concentrations and PKC-δ and PKC-ζ at low concentrations.3,18 Adrenergic stimulation has been reported also to increase membranous PKC-δ and PKC-ζ expression.16,17

The decrease in PKC-δ and PKC-ζ expression after 60 minutes of D1-like agonist infusion in WKY is associated with an increase in sodium excretion and a decrease in Na+,K+-ATPase activity in PT in normotensive rats. The role of the decrease in PKC isoform expression on Na+,K+-ATPase activity in PT after 60 minutes of D1-like agonist infusion in normotensive rats remains to be determined since we did not measure PKC activity. Previous studies have shown that dopamine and D1-like agonists increase PKC activity in renal PT,13,14 and they have been suggested as mediators of inhibition of Na+,K+-ATPase activity.12,14 However, the dopamine and D1-like agonist–mediated increase in PKC activity has been documented only in the first 20 minutes of stimulation.1,10 Moreover, the effects of PKC stimulation on Na+,K+-ATPase activity in PT vary with time, with an early stimulation followed by an inhibition.7 Beyond 15 minutes, inhibition of PKC activity does not affect the ability of dopamine to inhibit Na+,K+-ATPase activity in PT.8 These data taken together suggest that D1-like receptors regulate PKC isoform expression differently from adrenergic and Ang II receptors. The differential regulation of PKC isoform expression by these receptors could account for their differential effect on sodium transport in normotensive animals. Ang II and α1-adrenergic receptors increase sodium transport and stimulate Na+,K+-ATPase activity in PT, effects opposite of those noted with D1 agonists.14,24 A role of PKC dopamine–mediated inhibition of Na+,K+-ATPase activity in mTAL has not been supported by studies that make use of PKC blockers.15 Our novel finding that D1-like agonist stimulation is associated with a decrease in PKC-δ and PKC-ζ expression in WKY suggests involvement of PKC in the D1-like action even in this nephron segment.

There is a defect in the regulation of the D1-like receptor in the PT in human essential hypertension and in some models of rodent genetic hypertension.7,10–21 The defect in the regulation of the D1-like receptor function, which results in a decreased ability of dopamine to produce a natriuresis, has been thought to be caused in part by a decreased ability of dopamine to stimulate PKC activity in renal PT.19 Recently, PKC isoform gene expression has been studied in the brain and heart of SHR. These studies showed that in the cerebral cortex, PKC-γ mRNA was present in greater abundance in SHR than in WKY, whereas PKC-e mRNA was present in similar quantities in SHR and WKY. However, in ventricular myocytes, PKC-e mRNA was present in greater abundance in SHR than in WKY.25 In the renal cortex and PT, we did not find any differences in PKC isoform expression in the cytosol. However, membranous PKC-δ in PT and mTAL and PKC-ζ in mTAL were greater in WKY than in SHR. The significance of these differences remains to be determined.

The present studies show that D1-like receptor stimulation differentially affects PKC isoform expression in WKY and SHR. Both fenoldopam and SKF 38393 produced a greater diuresis in WKY than in SHR after 10 and 60 minutes of infusion. SKF 38393 did not affect renal blood flow or glomerular filtration rate but increased absolute and fractional sodium excretion in WKY but not in SHR. As in WKY, D1-like agonist infusion also did not affect PKC-α and PKC-λ expression in SHR at 10 or 60 minutes. However, after 10- or 60-minute infusion of D1-like agonists, membranous PKC-δ expression decreased in renal cortex, PT, and mTAL of WKY but increased in SHR. Membranous PKC-ζ expression was also decreased by SKF 38393 in PT of WKY at 60 minutes, the opposite of that found in SHR. However, fenoldopam increased membranous PKC-θ expression at 10 minutes in both WKY and SHR.

Several studies have reported that a dopamine or D1-like receptor–mediated stimulation of PKC activity is important in the dopamine or D1-like receptor–mediated inhibition of Na+,K+-ATPase activity in renal PT in the short-term period, <20 minutes.9,12,13 Moreover, a failure of dopamine and D1-like agonists to increase PKC activity has been reported to be a cause of the failure of these ligands to inhibit Na+,K+-ATPase activity in renal PT in SHR.13 We have found that D1-like agonists inhibit the expression of PKC-δ and PKC-ζ in WKY but stimulate it in SH and 10 and 60 minutes. The only PKC isoform we found to be stimulated by D1-like agonist is PKC-θ. It is unlikely that this PKC isoform is responsible for the short-term stimulation of PKC activity and inhibition of Na+,K+-ATPase activity in renal PT by dopamine and D1-like agonists because PKC-θ was increased in both SHR and WKY, yet a diuresis caused by fenoldopam was noted in the latter but not in the former. It is possible that
other PKC isoforms not measured in this study are responsible for the PKC activation noted with dopamine and D1-like agonists. This is entirely speculative since we did not measure total PKC or isoform activity in the kidney of these rat strains after D1-like agonist stimulation. Although we did not determine the effect of specific PKC isoforms on sodium transport, overexpression of PKC-δ in NIH 3T3 cells caused an increase in sodium/phosphate cotransport. Thus, the differential effect of D1-like receptors on PKC isoform expression could in part explain the decreased ability of D1-like agonists to inhibit renal sodium handling in SHR. It may be noteworthy that the effect of D1-like receptor stimulation on PKC isoform expression in SHR is similar to the effects of norepinephrine and Ang II stimulation in normotensive rats.3,16–18 Because both Ang II and norepinephrine increase PT sodium transport and stimulate Na+/K+-ATPase activity,14,24 similarities in PKC isoform expression due to these ligands and D1-like agonists in SHR may explain in part the decreased ability of D1-like receptors to induce a natriuresis in SHR.

In conclusion, D1-like receptors regulate PKC isoform expression differently than adrenergic and Ang II receptors. Moreover, D1-like receptor regulation of rat renal PKC isoform expression differs between normotensive and hypertensive rats. The aberrant effect of D1-like agonists in SHR is similar to the stimulatory effect of norepinephrine and Ang II in normotensive rats and may be a mechanism for the decreased natriuretic effect of D1-like receptors in genetically hypertensive rats.

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