Transcriptional Regulation of Renal Dopamine D1 Receptor Function During Oxidative Stress

Anees A. Banday; Mustafa F. Lokhandwala

Abstract—There exists a strong link between oxidative stress, renal dopaminergic system, and hypertension. It is reported that reactive oxygen species attenuate renal proximal tubular dopamine receptor (D1R) function, which disrupts sodium regulation and leads to hypertension. However, the mechanisms for renal D1R dysfunction are not clear. We investigated the role of redox-sensitive transcription factors AP1 and SP3 in transcriptional suppression of D1R gene and subsequent D1R signaling. Human kidney proximal tubular cells were treated with a pro-oxidant l-buthionine sulfoximine (BSO) with and without an antioxidant tempol. In human kidney cells, BSO caused oxidative stress and reduced D1R mRNA and membrane receptor expression. Incubation of human kidney cells with SKF38393, a D1R agonist, caused a concentration-dependent inhibition of Na/K-ATPase. However, SKF38393 failed to inhibit Na/K-ATPase in BSO-treated cells. BSO increased AP1 and SP3 nuclear expression. Transfection with AP1- or SP3-specific siRNA abolished BSO-induced D1R downregulation. Treatment of rats with BSO for 4 weeks increased oxidative stress and SP3–AP1 expression and reduced D1R numbers in renal proximal tubules. These rats exhibited high blood pressure, and SKF38393 failed to inhibit proximal tubular Na/K-ATPase activity. Control rats were kept on tap water. Tempol per se had no effect on D1R expression or other signaling molecules but prevented BSO-induced oxidative stress, SP3–AP1 upregulation, and D1R dysfunction in both human kidney cells and rats. These data show that oxidative stress via AP1–SP3 activation suppresses D1R transcription and function. Tempol mitigates oxidative stress, blocks AP1–SP3 activation, and prevents D1R dysfunction and hypertension. (Hypertension. 2015;65:00-00. DOI: 10.1161/HYPERTENSIONAHA.115.05255.) ● Online Data Supplement

Key Words: antioxidants ■ GTP-binding proteins ■ hypertension ■ kidney ■ kidney tubules, proximal

Kidneys play an important role in the regulation of blood pressure primarily by maintaining sodium homeostasis.1-3 The sodium metabolism is chiefly controlled by a complex interaction among natriuretic and antinatriuretic factors.1-5 Under sodium replete conditions, natriuretic factor dopamine plays a major role in maintaining sodium balance as >50% of excreted sodium is attributed to renal dopamine receptor (D1R) activation and subsequent inhibition of proximal tubular Na/K-ATPase.6,7

Like other G-protein–coupled receptors, D1R is susceptible to oxidative stress.8 It has been shown that in animal models that exhibit oxidative stress, renal D1R function is significantly reduced.9,10 The detrimental link between oxidative stress and D1R function has been reproduced in proximal tubular cell cultures.11 Interestingly, animals with oxidative stress and renal D1R defect exhibit hypertension, thus highlighting the importance of renal D1R in blood pressure regulation and a potential link between oxidative stress and hypertension.9,10 Despite a significant amount of work on the mechanisms of oxidative stress–mediated D1R dysfunction, the role and mechanism of D1R downregulation are not clearly understood. Various studies in animal models of hypertension show that a defect in D1R signaling is independent of receptor expression levels.12,13 In these animals, the failure of D1R to activate downstream pathways is largely because of receptor G-protein uncoupling caused by increased membrane translocation of G-protein receptor kinases (GRK), especially GRK 2 and 4.12-15 The overstimulation of GRKs leads to receptor hyperphosphorylation and subsequent uncoupling from G proteins.12 However, there are reports that show that receptor dysfunction because of G-protein uncoupling is caused by a decrease in D1R expression.16-18 Therefore, this study was undertaken to investigate the mechanism of D1R downregulation during oxidative stress.

Reactive oxygen species modulate a plethora of signaling molecules that could ultimately affect gene expression or protein synthesis.19,20 This occurs, at least in part, by activation or suppression of transcription factors, such as AP1 and SP3.19-24 These transcription factors can regulate genes that control protein synthesis, apoptosis, inflammation, and
cell division individually or by interacting with other transcription factors.22–25 The SP family of transcription factors is characterized by the presence of 3 zinc fingers that form their DNA-binding domain and allow them to bind the GC and GT boxes for the regulation of basal/constitutive expression of a diverse range of viral and cellular genes both under physiological and pathophysiological conditions.22,23 It is reported that the SP3 promoter has a consensus sequence for AP1-binding site.26 AP1 family proteins are basic leucine zipper transcription factors, which represent sequence-specific DNA-binding transcription factors and consist of homo- or heterodimers, which are formed by jun (c-jun, junB, and junD), fos (c-fos, fra-1, and fra-2), or af proteins, in mammalian cells.27 There are reports that oxidative stress activates AP1 directly or via microtubule-associated protein kinase pathways and the D1R promoter possesses binding sequences for AP1.28,29 Therefore, we aimed to investigate the role of AP1 and SP3 in renal D1R gene repression and function during oxidative stress.

Methods

Human kidney (HK2) cells, a human kidney proximal tubular cell line, were obtained from American Tissue Culture Collection (ATCC CRL-2190), maintained, and subcultured in American Tissue Culture Collection-recommended complete media (Invitrogen, 17005-042). Cells were grown to 85% confluence, starved overnight in DMEM-F12, and incubated with 50 μmol/L of l-buthionine sulf-oximine (BSO), tempol (1 mmol/L), and BSO plus tempol for 72 hours. Cells maintained in DMEM-F12 alone served as controls. Malondialdehyde levels were determined by the method of Mihara and Uchiyama30 and using a commercially assay kit (cat number, STA-330; Cell Biolabs Inc).

D1R mRNA and Ligand Binding

Total RNA was isolated from HK2 cells using a RNasea mini kit (QIAGEN) and used for cDNA synthesis and amplification of D1R and glyceraldehyde-3-phosphate dehydrogenase (used as an internal control) using an Advantage cDNA PCR Kit (BD Biosciences, Clontech) as described previously.31 The polymerase chain reaction for D1R and glyceraldehyde-3-phosphate dehydrogenase was performed with commercially available TaqMana primers (Life Technologies).

For ligand-binding assay, membranes were prepared by harvesting the cells in ice-cold PBS followed by differential centrifugation as detailed previously.12 Membranes (50 μg of protein) were suspended in 50 mmol/L of Tris–HCl (pH 7.4, 120 mmol/L of MgCl2), and saturation assay was performed at different concentrations of [3H] SCH23390 using unlabeled SCH23390 (10 μmol/L) to obtain specific binding. Binding was stopped by rapid filtration through Whatman GF/B filters.

Na/K-ATPase Assay

HK2 cells were grown in 24-well plates to measure Na/K-ATPase activity as 86Rb+ uptake.32 After the treatment of cells with BSO or tempol, the plates were washed with PBS and incubated with varying concentrations of SKF38393 for 10 minutes at 37°C. 86Rb+ uptake was initiated by addition of 0.5 mL of DMEM-F12 containing 3 μCi/mL. 86Rb+ in the presence and absence of ouabain (1 mmol/L). Cells were lysed with 0.5 N of NaOH (0.5 mL/well), and radioactivity was measured directly in cell lysate with a gamma counter. Protein was measured in each well to normalize 86Rb+ uptake.

Enzyme-Linked Immunosorbent Assay

As detailed before,33 nuclear fractions were isolated using commercially available NE-PER nuclear and cytosolic protein extraction kit (Pierce). Nuclear translocation of SP3 and AP1 (c-fos) were quantitated by commercially available species-specific ELISA kits (Antibodies-online Inc and Mybiosource). SP3- and AP1-specific optical densities were measured at 450 nm. To correct for optical imperfections, the plate was also read at 540 or 570 nm, and these optical densities were subtracted from the reading recorded at 450 mmol/L.

Animal Studies

Animal studies were performed essentially as detailed in our previous publication.9,12 The protocol was approved by Institutional Animal Care and Use Committee. Briefly, adult male

Figure 1. Effect of l-buthionine-sulfoximine (BSO) and tempol (T) on blood pressure in rats. The inset magnifies the increase in blood pressure in BSO-treated rats. Data represent means±SE from 8 to 10 rats in each group. *P<0.05 vs baseline (at the beginning of the treatment) and #P<0.05 vs control at corresponding time points.

Figure 2. Dopamine D1 receptor (D1R) mRNA level in human kidney (HK2) cells and rat renal proximal tubules. A, D1R mRNA levels in control (C, DMEM-F12), l-buthionine sulfoximine (BSO), tempol (T), and BSO+tempol-treated HK2 cells. B, Renal proximal tubular D1R mRNA levels in C (tape water), BSO, T, and BSO+tempol-treated Sprague-Dawley rats. Experiments were performed in triplicate, and data represent means±SE from 4 to 6 cell passages (cells from passages 2–8) or 5 to 7 rats in each group. *P<0.05 vs C and #P<0.05 vs BSO.
Sprague-Dawley (SD) rats were divided into 4 groups and provided with 30 mmol/L of BSO, 1 mmol/L of tempol, and BSO plus tempol for 4 to 5 weeks in drinking water. Control rats were kept in drinking water. Conscious blood pressure was measured by radio-telemetry (DSI, Minneapolis, MN). Because the change in blood pressure was maximum after 4 weeks of treatment, this time point was selected for further biochemical studies that were performed as detailed previously.9,12

Statistical Analysis

Differences between the means were evaluated using unpaired Student t test or 1-way ANOVA with post hoc Newman–Keuls multiple test, as appropriate. P<0.05 was considered statistically significant.

Results

Treatment of HK2 cells with BSO or tempol for 72 hours had no significant effect on cell viability as measured by trypan blue uptake (% uptake, control: 4.01±0.30; BSO: 4.91±0.41; tempol: 3.81±0.37; BSO+tempol: 4.46±0.43) or lactate dehydrogenase release, (μmol/mg protein per h, control: 0.15±0.01; BSO: 0.19±0.01; tempol: 0.12±0.013; BSO+tempol: 0.14±0.012) and did not change cell morphology (data not shown). However, BSO treatment caused oxidative stress as evidenced by a significant increase in the malondialdehyde level, a lipid peroxidation marker (nmol/mg protein, control: 53.5±2.05, BSO: 86.39±4.22; P<0.05 versus controls). Tempol had no effect on basal malondialdehyde level (tempol: 49.05±5.10) but rescued cells from BSO-induced lipid peroxidation (BSO+tempol: 59.3±4.04, P<0.05 versus BSO). Treatment of SD rats with BSO also increased renal malondialdehyde (pmol/mg protein, control: 75.1±6.5; BSO: 129.2±8.3; P<0.05 versus controls) and urinary 8-isoprostane excretion (pg/mg creatinine, control: 0.95±0.07; BSO: 1.43±0.1; P<0.05 versus controls). An increase in both malondialdehyde and 8-isoprostane was prevented by concurrent tempol supplementation (malondialdehyde: 85.2±7.6; 8-isoprostane 1.07±0.6; P<0.05 versus BSO). Rats treated with BSO exhibited time-depend increase in blood pressure, with maximum increase at 4 weeks of treatment (Figure 1). The rise in blood pressure was mitigated by tempol treatment (Figure 1). In the absence of BSO, tempol had no effect on oxidative stress (malondialdehyde: 70.1±3.2; 8-isoprostane: 0.89±0.06) or blood pressure (Figure 1). BSO and tempol did not affect the food or water intake (data not shown). Tubule viability, as measured by trypan blue uptake or lactate dehydrogenase release, was similar in all experimental groups (data not shown).

Effect of BSO on D1R mRNA and Ligand Binding

Incubation of HK2 with BSO reduced D1R mRNA level when compared with controls (Figure 2A). Treatment of SD rats with BSO also decreased D1R mRNA level in renal proximal tubules (Figure 2B). In both BSO-treated HK2 cells and renal proximal tubules from BSO-treated rats, the decrease in the message level was paralleled by a marked reduction in membrane D1R numbers, whereas the affinity of D1R remained unchanged (Figures 3A–3C and 4A–4C). Antioxidant tempol prevented the decrease in the D1R mRNA level and receptor number (Figures 2–4).
Hypertension May 2015

Effect of BSO on Na/K-ATPase Regulation

In control HK2 cells, SKF38393, a D1R agonist, caused dose-dependent inhibition of Na/K-ATPase activity that peaked at 1 μmol/L concentration (Figure 5A). However, in cells treated with BSO, SKF38393 failed to significantly inhibit Na/K-ATPase activity (Figure 5A and 5B). SKF38393 also failed to inhibit Na/K-ATPase activity in renal proximal tubules from BSO-treated rats when compared with controls (Figure 5C). Tempol treatment mitigated the BSO effect as SKF38393-induced Na/K-ATPase inhibition was comparable in controls versus BSO plus tempol–treated HK2 cells (Figure 5A and 5B) and in proximal tubules from controls versus BSO plus tempol–treated rats (Figure 5C). Tempol or BSO did not affect basal Na/K-ATPase activity (Figure 5B and 5C).

Effect of BSO on SP3–D1R Interaction

BSO treatment in HK2 cells increased nuclear SP3 expression that was blocked by tempol (Figure 6A). Renal proximal tubules from BSO-treated rats also exhibited increased nuclear SP3 expression when compared with controls (Figure 5C). Tempol treatment mitigated the BSO effect as SKF38393-induced Na/K-ATPase inhibition was comparable in controls versus BSO plus tempol–treated HK2 cells (Figure 5A and 5B) and in proximal tubules from controls versus BSO plus tempol–treated rats (Figure 5C). Tempol or BSO did not affect basal Na/K-ATPase activity (Figure 5B and 5C).

Figure 4. Membrane dopamine D1 receptor (D1R) expression in rat renal proximal tubules. A, A representative dose response curve of D1R antagonist [3H]SCH23390 in proximal tubule membranes. B and C, Bmax and Kd obtained from Scatchard plot. Experiments were performed in triplicate, and data represent means±SE from 5 to 7 rats in each group. *P<0.05 vs control (C) and #P<0.05 l-buthionine sulfoximine (BSO). T indicates tempol.

Figure 5. SKF38393-induced inhibition of Na/K-ATPase activity in human kidney (HK2) cells and rat renal proximal tubules. A, A representative dose response curve of SKF38393-induced inhibition of Na/K-ATPase activity in control (C), l-buthionine sulfoximine (BSO), tempol (T), and BSO+tempol treated HK2 cells. SKF38393-induced (1 μmol/L) inhibition of Na/K-ATPase activity in HK2 cells (B) and renal proximal tubules (C). Experiments were performed in triplicate, and data represent means±SE from 4 to 6 cell passages (cells from passages 2–8) or 5 to 7 rats in each group. *P<0.05 vs basal.
tempol blocked BSO-induced SP3 upregulation (Figure 6B). Tempol had no effect on SP3 expression in the absence of BSO in HK2 cells or rat renal tubules (Figure 6A and 6B).

As shown in Figure 7A, siRNA downregulated SP3 by ≈80% when compared with cells incubated with vehicle (transfection reagent alone) or scrambled DNA. As expected, BSO failed to upregulate SP3 in siRNA-transfected cells (Figure 7A). We found that BSO failed to downregulate D1R in SP3 siRNA-transfected cells (Figure 7B). Basal membrane D1R expression was not affected by transfection reagent, scrambled DNA, or SP3 siRNA (Figure 7B). As shown in Figure S1 in the online-only Data Supplement, scrambled sequence had no effect on basal or BSO-induced decrease in membrane D1R protein expression.

**Effect of BSO on AP1 (c-fos)–D1R Interaction**

Treatment of HK2 cells with BSO significantly increased nuclear AP1 (c-fos) expression compared with controls (Figure 8A). In HK2 cells, tempol treatment did not affect AP1 expression; however, tempol abolished BSO-induced AP1 upregulation (Figure 8A). BSO treatment also increased AP1 expression in proximal tubules, and tempol blocked this increase while having no effect on AP1 in the absence of BSO (Figure 8B).

Transfection of HK2 cells with AP1-specific siRNA reduced AP1 expression, and BSO did not upregulate it (Figure 9A). As seen with SP3 downregulation, AP1 downregulation also mitigated BSO-induced reduction of membrane D1R expression (Figure 9B). AP1-specific siRNA had no effect on basal D1R expression (Figure 9B).

**Effect of BSO on SP3–AP1 Interaction**

Transfection of HK2 cells with AP1 siRNA had no effect on basal SP3 expression (Figure 10A). However, AP1 siRNA abolished BSO-induced SP3 upregulation (Figure 10A). However, transfection of cells with SP3 siRNA failed to block BSO-induced AP1 upregulation (Figure 10B). SP3 siRNA had no effect on basal AP1 expression (Figure 10B).

Because SP3- and AP1-specific siRNA were able to mitigate the BSO effect suggesting an increased protein synthesis, we wanted to observe the effect of BSO and tempol on the nuclear to cytosolic ratio of these transcription factors. As shown in Figure S2A and S2B, BSO, tempol, or BSO plus tempol treatment had no effect on the nuclear to cytosolic ratio of SP3 or AP1.

**Discussion**

The present study shows that oxidative stress–mediated activation of transcription factors AP1 and SP3 leads to...
transcriptional D1R downregulation resulting in receptor dysfunction and hypertension. BSO-induced oxidative stress decreased D1R message level and membrane receptor expression, which led to failure of SKF38393, a D1R agonist, to inhibit Na/K-ATPase activity. BSO treatment activated AP1 and SP3, which was blocked by transfecting the HK2 cells with AP1 and SP3 siRNA, respectively. BSO-induced upregulation of SP3 was blocked by AP1 siRNA, whereas SP3 siRNA had no effect on AP1 activation. In addition, transfection of HK2 cells with AP1 and SP3 siRNA prevented BSO-mediated D1R dysfunction. Supplementation of tempol to BSO-treated HK2 cells mitigated oxidative stress, abolished AP1 and SP3 activation, and rescued D1R function. In addition, tempol prevented oxidative and AP3–AP1 upregulation, D1R dysfunction, and development of hypertension in BSO-treated SD rats.

BSO treatment induced oxidative stress as evidenced by increased malondialdehyde and 8-isoprostane levels, and as expected, tempol prevented this phenomenon. Both BSO and tempol did not affect proximal tubular or HK2 cell viability or morphology, suggesting that these compounds are not toxic at the dose used and for the duration of this study. We and others have shown that oxidative stress reduces D1R receptor function in various animal models and cell cultures.

In agreement, we also found that the BSO treatment reduced D1R message level and membrane receptor expression. The decrease in D1R expression was reflected by the loss of receptor function as SKF38393 failed to inhibit Na/K-ATPase. It is well established that the renal dopamine system contributes to >50% of sodium excretion under sodium replete conditions, thus playing a pivotal role in renal sodium metabolism and blood pressure regulation. Therefore, the loss of renal D1R function because of oxidative stress could be a significant risk factor for oxidative stress–associated hypertension. Here in, we also found that oxidative stress was paralleled by a marked increase in blood pressure in BSO-treated rats. The involvement of oxidative stress is further substantiated by the findings that tempol that mitigated oxidative stress was able to prevent D1R downregulation, restore SKF38393-induced Na/K-ATPase inhibition, and maintain normal blood pressure.

The effect of oxidative stress on D1R receptor expression is not clear. Various reports, including from our own laboratory, show that oxidative stress–mediated D1R dysfunction in obese animals and spontaneously hypertensive rats is confined to receptor G-protein uncoupling without any significant

Figure 8. AP1 (c-fos) nuclear expression in human kidney (HK2) cells and rat renal proximal tubules. Effect of l-buthionine sulfoximine (BSO) on AP1 nuclear expression in BSO, tempol (T), and BSO+tempol–treated HK2 cells (A) and renal proximal tubules from BSO or tempol-treated rats (B). Experiments were performed in triplicate, and data represent mean±SE from 4 to 6 cell passages (cells from passages 2–8) or 5 to 7 rats in each group. *P<0.05 vs control (C) and #P<0.05 vs BSO. OD indicates optical density.

Figure 9. AP1 (c-fos) and D1 receptor interaction. A, AP1 nuclear expression in human kidney (HK2) cells transfected with SP3-specific siRNA or scrambled DNA. B, D1 receptor ligand [3H]SCH23390 binding. Mean±SE from 4 to 6 experiments (cells from passages 2–8) performed in triplicate. *P<0.05 vs vehicle. BSO indicates l-buthionine sulfoximine; and OD, optical density.
change in receptor expression. The failure of D1R receptors to communicate with cognate G proteins is reportedly because of serine hyperphosphorylation. Although the mechanism for receptor hyperphosphorylation could be attributed to a plethora of protein kinases, it is widely perceived that activation of GRKs (serine/threonine kinases) plays a major role in D1R function. GRKs are distributed throughout the cell, and on activation, these kinases are recruited to cell membrane. On translocation to membranes, GRKs have been shown to phosphorylate serine residues of G-protein–coupled receptors, which leads to receptor desensitization. Although this is a well-recognized mechanism for D1R desensitization, there are reports that animal models which exhibit oxidative stress and moderate hypertension and fail to produce natriuresis in response to dopamine, a decrease in receptor number alone could contribute to D1R dysfunction. To identify the role and mechanism of D1R downregulation, we focused on redox-sensitive transcription factors that possess putative binding affinity for D1R promoter. Here in, we found that BSO reduced both the D1R mRNA level and membrane putative binding affinity for D1R promoter. Here in, we found that treatment of HK2 cells and SD rats with BSO activated SP3 and AP1. In HK2 cells, SP3 and AP1 siRNA rescued D1R function, suggesting that activation of these transcriptional factors contributes to D1R receptor dysfunction. Treatment of cells and rats with tempol blocked BSO-mediated activation of these transcription factors. It is worth mentioning that tempol had no effect on basal AP1 and SP3 expression, suggesting that the prevention of BSO-induced activation of AP1 and SP3 could be because of its ability to prevent oxidative stress. However, a direct effect on these transcription factors cannot be ruled out. Nevertheless, the data show that oxidative stress via AP1–SP3 signaling abolished D1R function, which can be rescued by tempol, thus signifying the therapeutic potential of antioxidants in preventing the development of hypertension.

D1R promoter is a cis-acting element located between nucleotides -1154 and -1136 related to the translation start site. Although it is reported that the D1R promoter possesses binding sequences for both SP3 and AP1 and downregulation of AP1 and SP3 mitigated detrimental effects of BSO as it relates to D1R function, it is unclear whether one or both of these factors are involved in D1R transcriptional suppression. To identify an exact link between AP1, SP3, and D1R expressions, we again downregulated SP3 to observe its effect on BSO-induced AP1 activation. Next, we used AP1 siRNA and measured BSO-induced SP3 upregulation. We found that SP3 downregulation failed to block BSO-induced AP1 activation. However, AP1 siRNA abolished BSO-dependent SP3 upregulation. These data show that BSO via AP1 activation upregulates SP3 which in turn contributes to D1R downregulation. It is reported that SP3 promoter has binding sequences for AP1 and an activated SP3 could lead to transcriptional activation or suppression of target genes. Collectively, these findings provide a strong support to our hypothesis that oxidative stress via AP1 activates SP3. Once activated, SP3 transcriptionally downregulates renal D1R causing failure of SKF38393 to inhibit Na/K-ATPase activity. Antioxidant tempol mitigates oxidative stress, abolishes AP1–SP3 activation, and prevents D1R dysfunction.

Limitations

BSO by inhibiting glutamate–cysteine ligase activity causes a robust decrease in glutathione levels, both GSH and GSSG, which could increase a plethora of reactive oxygen species. We did not measure reactive oxygen species in this study; however, tempol can metabolize both H2O2 and superoxide that are commonly associated with BSO treatment. It has been previously shown that AP1 activation causes transcriptional upregulation of glutamate–cysteine ligase, which could lead to mitigation of oxidative stress. However, BSO is a potent inhibitor of glutamate–cysteine ligase, and we have previously shown that glutathione levels are significantly low in rats treated with BSO. We did not investigate whether tempol-mediated normalization of AP1–SP3 signaling in BSO-treated rats is because of general reduction of oxidative stress or a more direct effect of tempol on these transcription factors. However, it is worth mentioning that tempol, in the absence of BSO, had no effect on AP1–SP3 activation.
indicating that the effect of tempol could be confined to mitigation of oxidative stress. We found that BSO in the absence or presence of tempol did not affect the nuclear to cytosolic ratio of SP3 or AP1. It seems that oxidative stress increased the protein content of these transcription factors that are then translocated to the nucleus. We did not study the mechanisms for these phenomena because it was beyond the scope of our present study.

**Perspectives**

This study identifies a novel molecular mechanism for D1R downregulation during oxidative stress. We show that redox-sensitive transcription factors involving AP1–SP3 cascade can suppress D1R transcription, which leads to receptor downregulation and loss of receptor function. Antioxidant tempol while mitigating oxidative stress maintains normal AP1–SP3 signaling and prevents D1R dysfunction and hypertension. These data underline the role of oxidative stress in renal D1R defect, which could be a contributing factor to the development of hypertension given the importance of renal dopamine system in sodium and blood pressure regulation.

**Sources of Funding**

This work was funded by National Institutes of Health and National Institute of Diabetes and Digestive and Kidney (DK98509).

**Disclosures**

None.

**References**


Novelty and Significance

What Is New?
- Redox-sensitive transcriptional pathway AP1–SP3 suppresses dopamine receptor transcription, leading to receptor downregulation and dysfunction.
- Antioxidant can prevent activation of redox-sensitive transcription factors and thus protect G-protein–coupled receptor function during oxidative stress.

What Is Relevant?
- The data reinforce the role of oxidative stress in abrogating renal dopamine receptor function and the potential implication in hypertension.
- This study signifies the translational potential as a more direct therapeutic approach can be focused toward the specific transcription factors in oxidative stress–associated hypertension.

Summary
Oxidative stress activated AP1, which upregulates SP3 in human kidney cells and rat renal proximal tubules. The enhanced AP1–SP3 signaling suppresses dopamine receptor transcription causing receptor downregulation, which leads to the failure of SKF38393 to inhibit Na/K-ATPase activity. Antioxidant tempol mitigates oxidative stress, normalizes AP–SP3 signaling, and prevents dopamine receptor downregulation and hypertension.
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ONLINE SUPPLEMENT

Transcriptional Regulation of Renal Dopamine D1 Receptor Function during oxidative stress

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Figure S1. Dopamine D1 receptor (D1R) expression in HK2 cells incubated with vehicle or scrambled sequence DNA and exposed to L-buthionine-sulfoximine (BSO) for 72 hr. Upper images are representative immunoblots and bars represent mean ± SE from 4-5 experiments.

Lanes: 1—vehicle, 2—scrambled sequence, 3—vehicle plus BSO and 4—scrambled sequence plus BSO. *P<0.05 vs. vehicle and #P<0.05 vs. scrambled sequence.
Figure S2. Nuclear to cytosolic ratio of SP3 (A) and AP1 (B) in HK2 cells treated with BSO and/or tempol. Bars represent mean ± SE from 4-5 experiments.