Role of oxidative stress in defective renal dopamine D1 receptor-G protein coupling and function in old Fischer 344 rats

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Fardoun, Riham Zein, Mohammad Asghar, and Mustafa Lokhandwala. Role of oxidative stress in defective renal dopamine D1 receptor-G protein coupling and function in old Fischer 344 rats. Am J Physiol Renal Physiol 291: F945–F951, 2006.—Aging is associated with an increase in oxidative stress. Previously, we have reported that dopamine failed to inhibit proximal tubular Na-K-ATPase and to promote sodium excretion in old rats (Beheray S, Kansra V, Hussain T, and Lokhandwala MF. Kidney Int 58: 712–720, 2000). This was due to uncoupling of dopamine D1 receptors from G proteins resulting from hyperphosphorylation of D1 receptors. The present study was designed to test the role of oxidative stress in the age-related decline in renal dopamine D1 receptor function. We observed that old animals had increased malondialdehyde (MDA) levels, a biomarker of oxidative stress, and decreased D1 receptor number and protein in the proximal tubules (PT) compared with adult rats. In old rats, there was increased G protein-coupled receptor kinase-2 (GRK-2) abundance, increased basal serine phosphorylation of D1 receptors, and defective D1 receptor-G protein coupling in PT membranes. Interestingly, supplementation with an antioxidant, tempol (1 mmol/l in drinking water for 15 days), lowered MDA levels and normalized D1 receptor number and protein in old rats to the level seen in adult rats. Furthermore, tempol decreased GRK-2 abundance and D1 receptor serine phosphorylation and restored D1 receptor-G protein coupling in PT of old rats. The functional consequence of these changes was the restoration of the natriuretic response to D1 receptor activation in tempol-supplemented old rats. Therefore, in old rats, tempol reduces oxidative stress and prevents GRK-2 membranous abundance and hyperphosphorylation of D1 receptors, resulting in restoration of D1 receptor-G protein coupling and the natriuretic response to SKF-38393. Thus tempol, by lowering oxidative stress, normalizes the age-related decline in dopamine D1 receptor function.

G protein receptor kinases; antioxidant; tempol; SKF-38393

AGING IS ASSOCIATED WITH AN increase in oxidative stress and is characterized by a general decline in physiological function that leads to morbidity and mortality. Several studies suggest oxidative stress to be a major contributor to cellular aging and degenerative diseases that accompany aging, such as brain dysfunction, cancer, immune system decline, diabetes, and renal and cardiovascular diseases (1). Interestingly, dietary antioxidants, including ascorbate, tocopherol, and carotenoids, have been reported to protect against these diseases (1).

Numerous studies have shown that the aging process causes several structural and functional changes in the kidney (12, 25). Previously, we have reported that the malondialdehyde (MDA) level, an index of oxidative stress, was increased in proximal tubules of old rats compared with adult rats (6). In addition, we found that dopamine failed to inhibit proximal tubular Na-K-ATPase activity and to promote sodium excretion in old rats (8). This was due to reduced D1 receptor expression and binding sites and defective D1 receptor-G protein coupling resulting from hyperphosphorylation of D1 receptors in the renal proximal tubules of these animals (23).

Despite the coexistence of oxidative stress and defective D1 receptor function in old Fischer 344 rats, it is still unclear whether oxidative stress causes the age-related decline in dopamine D1 receptor function. Therefore, in the present study, we wanted to determine whether oxidative stress plays a role in the age-related decline in renal dopamine D1 receptor function by examining the effect of the antioxidant tempol on oxidative stress and D1 receptor function in old Fischer 344 rats. We measured the levels of MDA in control and tempol-supplemented adult (6-mo) and old (24-mo) Fischer 344 rats. Subsequently, we determined D1 receptor protein expression and number and measured G protein-coupled receptor kinase-2 (GRK-2) protein expression in the renal proximal tubular membranes of control and tempol-supplemented adult and old rats. In addition, we determined D1 receptor serine phosphorylation and the coupling of D1 receptors to G proteins in the renal proximal tubular membranes of control and tempol-supplemented adult and old Fischer 344 rats. Finally, we determined the effect of an intravenously administered dopamine D1 receptor agonist, SKF-38393, on urinary sodium and water excretion in these animals.

MATERIALS AND METHODS

Materials

4-Hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (tempol) and [(±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride], SKF-38393, a D1 receptor agonist, were purchased from Sigma (St. Louis, MO). [R(+)-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hydrochloride] ([3H]SCH-23939 hydrochloride) and [35S]guanosine 5’-(γ-thio)triphosphate ([35S]GTPγS) were purchased from NEM Life Sciences. D1 receptor antibody was purchased from either Chemicon (Temecula, CA) for immunoprecipitation studies or Alpha Diagnostics (San Antonio, TX) for Western blotting studies. A phosphorysine antibody was bought from Calbiochem NovaChem (San Diego, CA). Horseradish peroxidase-conju-

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gated anti-mouse antibodies, a GRK-2 polyclonal antibody, and protein A/G-agarose beads were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); a protease inhibitor cocktail was obtained from Boehringer Mannheim (Indianapolis, IN). All other chemicals of the highest purity available were purchased from Sigma.

**Animal Treatment**

Male Fischer 344 rats aged 6 (adult) and 24 mo (old) were bought from the National Institute on Aging (Bethesda, MD) and housed in plastic cages with free access to a normal rodent diet in the University of Houston animal care facility. The study protocol involving the use and care of animals was approved by the Institutional Animal Care and Use Committee. Animals (both adult and old) were divided into four groups: adult-treated and old-treated groups (n = 4 each), which received 1 mmol/l tempol in the drinking water, and adult control and old control groups (n = 4 each), which were kept on tap water, which served as controls. Tempol-supplemented water was changed daily for 15 days. This set of animals (the above 4 groups) was used to carry out biochemical studies. Another set of old control and old-treated rats (n = 4 each) was used to perform renal functional studies, namely, the effect of an intravenously administered dopamine D1 receptor agonist, SKF-38393, on urinary sodium and water excretion.

**Preparation of Renal Proximal Tubular Suspension**

An in situ enzyme digestion procedure as previously described was used to prepare renal proximal tubules (11). Briefly, rats were anesthetized with pentobarbital sodium (50 mg/kg body wt ip), the abdomen was opened, the aorta was catheterized with polyethylene tubing, and the kidneys were perfused with collagenase and hyaluronidase. The kidneys were removed and kept in ice-cold oxygenated Krebs buffer containing (in mM) 1.5 CaCl₂, 110 NaCl, 5.4 KCl, 1 KH₂PO₄, 1 MgSO₄, 25 NaHCO₃, 25 d-glucose, and 2 HEPES (pH 7.6). Transverse sections of the kidneys were obtained, and superficial cortical tissue slices (rich in proximal tubules) were dissected out with a razor blade. The cortical slices were kept in fresh Krebs buffer. Enrichment of proximal tubules was carried out using 20% Ficoll in Krebs buffer. The band at the Ficoll interface was collected and washed in Krebs buffer by centrifugation at 250 g for 5 min. Cell viability was checked in this fraction using the trypan blue exclusion test. This fraction of proximal tubule was used to prepare the proximal tubular membranes. The proximal tubular fraction was suspended and homogenized in homogenization buffer (10 mM Tris·HCl, 250 mM sucrose, 2 mM PMSF, protease inhibitor cocktail; pH 7.4). The suspension was centrifuged at 20,000 g for 25 min at 4°C. The upper fluffy layer of the pellet was resuspended in the homogenization buffer and used for Western blotting and radioligand binding studies (28). Protein was determined by the bichinonic acid method (Pierce Chemical, Rockford, IL) using bovine serum albumin as a standard.

**Index of Oxidative Stress: MDA Measurement**

Oxidant-induced lipid peroxidation in proximal tubules was determined by measuring the MDA level by utilizing the method of Buege and Aust (10). Proximal tubules were homogenized, centrifuged at 2,000 g, and the supernatant was collected. The supernatant was then diluted to 0.4 mg/ml with 1.15% KCl and boiled with 2 ml of 15% trichloroacetic acid, 0.375% thiobisuric acid, and 0.25 N HCl for 15 min. The samples were cooled and centrifuged at 1,000 g for 10 min, and the color of the supernatant was read at 535 on a spectrophotometer. MDA was quantified using the molar extinction coefficient 1.56 × 10⁵ M/cm (10).

**Western Blotting of D1 Receptor and GRK-2**

Proximal tubular membrane fractions (5 and 10 μg of protein for D1 and GRK-2, respectively) were resolved by SDS-PAGE. The resolved proteins were electrophoretically transblotted onto a polyvinylidene (PVDF) membrane (Immobilon-P, Millipore, Bedford, MA). The PVDF membrane was blocked with 5% nonfat dry milk overnight at 4°C followed by incubation with either a rabbit polyclonal D1 (1:1,000) or rabbit polyclonal GRK-2 antibody (1:500) for 60 min. A horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:1,000 for D1 and 1:4,000 for GRK-2) was then added for 60 min at room temperature. The PVDF membranes were incubated with enhanced chemiluminescence reagent (Alpha Diagnostics, San Antonio, TX), and the bands were visualized on X-ray film. The bands were quantified by densitometric analysis using Scion Image Software provided by the National Institutes of Health.

**Immunoprecipitation and Detection of Serine Phosphorylation on D1 Receptor**

A previously described method was used for immunoprecipitation of dopamine D1 receptors from the proximal tubular plasma membranes (3). Briefly, proximal tubular plasma membrane fractions (1.5 mg/ml) were suspended in immunoprecipitation buffer containing (in mM) 140 NaCl, 3 KCl, 10 NaHPO₄, 2 KH₂PO₄, 1 orthovannadate, and 1 PMSF, as well as 1% NP-40, 0.5% sodium cholate, 0.1% SDS, and protease inhibitor cocktail, pH 7.4. Next, these samples were subjected to preclearing by adding 100 μl of protein A/G covalently bound to agarose beads (protein A/G-agarose) and incubating them for 1 h at 4°C. The samples were then centrifuged at 13,000 g for 20 s. The supernatants were incubated with 10 μg of D1 receptor antibody overnight to allow the formation of a D1 receptor-antibody complex. Prewashed protein A/agarose was added to the suspension. The samples were vortexed and centrifuged at 14,000 g at room temperature, and the supernatants were used for electrophoresis. To detect serine phosphorylation of D1 receptors, the immunoprecipitated samples (10 μl) were resolved by SDS-PAGE, and the proteins were electrotransferred to a piece of PVDF membrane. The PVDF membrane was blocked with 4% bovine serum albumin in PBS with 0.1% Tween 20. An anti-phosphoserine (1:200 dilution) was incubated with the PVDF membrane for 1 h at room temperature. A horseradish peroxidase-conjugated secondary antibody was used to probe the phosphoserine antibody (1:3,000), and the bands were visualized with an enhanced chemiluminescence reagent kit (Alpha Diagnostics).

**[³H]SCH-23390 Binding**

To determine the number of D1 receptors on the proximal tubular membrane, binding of the D1-receptor antagonist [³H]SCH-23390 to proximal tubular membranes was performed as described previously (20). Briefly, for saturation binding, 50 μg of proximal tubule membrane protein were incubated with 20 nM [³H]SCH-23390 in a final volume of 250 μl binding buffer at 25°C for 90 min. Unlabeled SCH-23390 (10 μM) was used for determining nonspecific binding. Specific binding was calculated as the difference between total and nonspecific bindings.

**Measurement of [³⁵S]GTPγS Binding**

To determine D1 receptor-G protein coupling, a [³⁵S]GTPγS binding assay was performed as described earlier (17). In the presence of [³⁵S]GTPγS, 5 μg of proximal tubular membrane protein were incubated with various concentrations of SKF-38393 (10⁻⁹-10⁻⁶ mol/l) for 1 h at 30°C. Nonspecific binding was determined by adding 100 μM unlabeled GTPγS to the assay media. Specific binding was calculated as the difference between total and nonspecific binding.

**Functional Studies**

The following experiments were performed in old rats not supplemented (control) and supplemented with tempol (treated).
Surgical procedures. As described previously (27, 28), rats were anesthetized with Inactin (100 mg/kg ip). A tracheotomy was performed to facilitate breathing. To measure blood pressure and heart rate and to collect blood samples, the left carotid artery was catheterized with PE-50 tubing. This tubing was connected to a Statham P23AC pressure transducer. Similarly, the left jugular vein was catheterized for infusing saline or drug. For collecting urine samples, a midline abdominal incision was performed and the left ureter was catheterized with PE-10 tubing connected to Tygon tubing. At the completion of surgery, normal saline (1% body wt ml/h) was infused continuously throughout the experimental period to maintain a stable urine output. Blood pressure and heart rate were continuously recorded on a Grass polygraph (model 7D, Grass Instruments, Quincy, MA), and animal body temperature was maintained under normal conditions.

Renal functional studies. The effect of SKF-38393 on sodium and water excretion was determined in control and tempol-treated old rats. The protocol (27, 28) consisted of a 45-min stabilization period after the surgery followed by five consecutive 30-min collection periods: C1, C2, SKF, R1, and R2. During C1 and C2, saline alone was infused; during SKF, SKF-38393 (1 μg·kg⁻¹·min⁻¹ in saline) was infused; and during R1 and R2 (recovery), only saline was infused. Urine samples were collected throughout the 30-min periods, and blood samples (500 μl) were collected at the end of each period. Plasma was separated by centrifuging blood samples at 1,500 g for 15 min at 4°C. Urine and plasma samples were stored at −20°C until analyzed for creatinine, sodium, and potassium content. These samples were used to calculate urine flow, urinary sodium excretion, and fractional excretion of sodium.

Urine and plasma analysis. Sodium and potassium concentrations in the urine and plasma were measured using a flame photometer 480 (Ciba Corning Diagnostics, Norwood, MA). Plasma and urine creatinine levels were measured with a creatinine analyzer (model 2, Beckman, Fullerton, CA). Urine volume was measured gravimetrically, and urine flow (ml/min) was calculated. The fractional excretion of sodium (percentage) was calculated based on the clearance of sodium and creatinine (7).

Results

Effect of Tempol on MDA Level

In the proximal tubules of old control rats, MDA levels were significantly higher compared with adult rats. Treatment with the antioxidant tempol significantly reduced MDA levels in old treated rats, similar to the MDA level in adult rats (Fig. 1). Tempol had no effect on MDA levels in adult rats (Fig. 1).

Effect of Tempol on GRK-2 Expression in Proximal Tubular Membranes of Old Rats

In the proximal tubules of old control rats, there was an approximately twofold increase in GRK-2 immunoreactivity compared with that of adult control rats, indicating an increased translocation of GRK-2 to the proximal tubular membranes in old control rats (Fig. 2). Tempol supplementation significantly reduced GRK-2 protein density on the proximal tubular membranes of old treated rats similar to the level seen in adult rats (Fig. 2).

Tempol supplementation did not affect GRK-2 protein density in adult rats (Fig. 2).

Effect of Tempol on Serine Phosphorylation of D1 Receptors in the Proximal Tubules of Old Rats

In the proximal tubules of old rats, basal serine phosphorylation of D1 receptors was about twofold higher compared with adult rats (Fig. 3). Tempol supplementation in old rats caused a significant reduction in the basal serine phosphorylation of D1 receptors and normalized it to the level of adult rats (Fig. 3). Serine phosphorylation of the D1 receptor was unaffected by tempol in adult rats (Fig. 3).

Effect of Tempol on D1 Receptor Protein Expression

The Western blot analysis of D1 receptor revealed reduced D1 receptor protein expression in old rats. Tempol supplementation to

Statistical Analysis

Data are presented as means ± SE. Differences between means were evaluated using the unpaired t-test and ANOVA with post hoc tests (Newman-Keuls) for comparisons between groups and for within-group variations, respectively. A P value <0.05 was considered statistically significant. Statistical analysis was done using GraphPad Prism (version 3.02, GraphPad Software, San Diego, CA).

Fig. 1. Effect of tempol on oxidative stress in the renal proximal tubules of old control (OC), old-treated (OT), adult control (AC), and adult-treated (AT) Fischer 344 rats. MDA, malondialdehyde. Values are means ± SE of 4 different experiments (animals) performed in triplicate. *P < 0.05, significantly different from OC rats by ANOVA followed by a Newman-Keuls multiple comparison test.

Fig. 2. Effect of tempol on G protein-coupled receptor kinase-2 (GRK-2) expression in proximal tubular membranes of OC, OT, AC, and AT Fischer 344 rats. Top: representative blot of GRK-2 and the loading control actin. Bottom: densitometric analysis of GRK-2 protein. Values are means ± SE; n = 4. *P < 0.05 compared with OC rats by 1-way ANOVA followed by Newman-Keuls multiple comparison test.
old rats normalized D1 receptor protein expression similar to the level seen in adult rats (Fig. 4). Tempol did not affect the protein expression of D1 receptor in adult rats (Fig. 4).

Effect of Tempol on G Protein Coupling of D1 Receptors and D1 Receptor Number on the Proximal Tubular Membranes of Old Rats

To determine whether tempol supplementation normalizes D1 receptor number in old rats, a single-point radioligand binding with 20 nM $[^{3}H]SCH-23390$, a D1-receptor antagonist, was performed. In old rats, there was an ~60% reduction in specific $[^{3}H]SCH-23390$ binding. Tempol treatment in old rats significantly increased the specific $[^{3}H]SCH-23390$ binding, without affecting ligand binding in adult rats (Fig. 5A).

SKF-38393, a D1-receptor agonist, elicited a significant increase in $[^{35}S]GTP_{\gamma}S$ binding in the proximal tubular membranes from adult rats but failed to stimulate $[^{35}S]GTP_{\gamma}S$ membrane binding in old rats (Fig. 5B). Treatment with tempol restored the SKF-38393-induced $[^{35}S]GTP_{\gamma}S$ binding in proximal tubular membranes from old rats similar to the level seen in adult rats (Fig. 5B). Tempol did not affect $[^{35}S]GTP_{\gamma}S$ binding in adult rats (Fig. 5B).

Effect of Tempol on SKF-38393-Mediated Natriuresis in Old Rats

In old control rats, SKF-38393 did not produce any changes in urine volume (Fig. 6A), urinary sodium excretion (Fig. 6B), and fractional excretion of sodium (Fig. 6C), a finding similar to the one reported in our previous study (8). Interestingly, in tempol-supplemented old rats, SKF-38393 produced marked natriuresis and diuresis and caused an increase in fractional...

Fig. 3. Serine-phosphorylation of D1 receptors in proximal tubular membranes of OC, OT, AC, and AT Fischer 344 rats. Proximal tubular cell membranes were used for immunoprecipitation of D1 receptors. Immunoprecipitated samples were then used for immunoblotting of serine-phosphorylated D1 receptors and total D1 receptors. Top: representative immunoblots of serine-phosphorylated D1 receptors and total D1 receptors. Bottom: densitometric analysis of serine-phosphorylated D1 receptor protein, normalized to immunoprecipitated D1 receptor protein density. Values are means ± SE; n = 4. *P < 0.05 compared with OC rats by 1-way ANOVA followed by Newman-Keuls multiple comparison test.

Fig. 4. Effect of tempol on D1 receptor protein expression in renal proximal tubular membranes of OC, OT, AC, and AT Fischer 344 rats. A: total no. of D1 receptors on the proximal tubular membrane as determined by binding of 20 nM $[^{3}H]SCH-23390$, a D1-receptor antagonist, to the proximal tubular membrane. Unlabeled SCH-23390 (10 μmol/l) was used for determining nonspecific binding. Specific binding was calculated as the difference between total binding and nonspecific binding. B: proximal tubular membranes from all the 4 groups were incubated with $[^{35}S]GTP_{\gamma}S$, unlabeled GTP$_{\gamma}$S (for nonspecific), and SKF-38393 ($10^{-9}$–$10^{-6}$ mol/l) at 30°C for 60 min. Values are means ± SE; n = 4. *P < 0.05 compared with OC rats by 1-way ANOVA followed by Newman-Keuls multiple comparison test.
excretion of sodium (Fig. 6, A–C) but no change in GFR (Fig. 6D). In addition, there were no significant differences between the body weights of old control and old treated rats (350 ± 16 vs. 355 ± 10 g, respectively).

DISCUSSION

Several studies have indicated an association of increased oxidative stress with aging in the heart, liver, and kidney (9, 14, 31). The antioxidant tempol has been used previously to reduce the oxidative stress in old rats (22) and spontaneously hypertensive rats (33, 34). However, it is unclear whether this phenomenon is responsible for impaired D1 receptor function in old rats. The results presented in our study provide direct evidence for the involvement of oxidative stress in D1 receptor dysfunction observed in old rats. This is supported by the observation that tempol, a membrane-permeable free radical scavenger, ameliorated oxidative stress and normalized the D1 receptor coupling to G protein and functional responsiveness to D1 receptor activation. It should be noted that we have recently observed similar effects with tempol on restoration of renal dopamine D1 receptor function in streptozotocin-treated and obese Zucker rats, and both of these models of type I and type II diabetes exhibit oxidative stress (7, 28).

It is reported that increased phosphorylation of serine residues in D1 receptors is responsible for the attenuation of the natriuretic effects of dopamine in various animal models, including spontaneously hypertensive rats, obese Zucker rats, and old Fischer 344 rats (3, 8, 35, 37). In this regard, it is important to note that higher basal serine phosphorylation of the D1 receptor has been reported in proximal tubular culture from patients with essential hypertension (13, 32). It appears that an increase in the basal serine phosphorylation of D1 receptors in hypertension (13, 37), obesity (7, 35), and aging (3) leads to their uncoupling from G proteins, resulting in the loss of the downstream signal. This is supported by our findings that reducing D1 receptor serine phosphorylation by tempol increases receptor-G protein coupling, leading to the restoration of functional responsiveness of the receptor in tempol-supplemented old rats.

One possible explanation for hyper-serine phosphorylation of dopamine D1 receptors in old rats is an increased abundance of GRK-2 on proximal tubular membranes.

On its activation, GRK-2 translocates to the cellular membrane, and it is known to phosphorylate D1 receptors (13, 32, 35). Therefore, increased abundance of GRK-2 on membranes is considered an index of its activity. Recently, we have reported an increased abundance of membranous GRK-2 leading to D1 receptor serine phosphorylation and receptor-G protein uncoupling in proximal tubular cells treated with hydrogen peroxide (2). This phenomenon requires the involvement of PKC (2). These studies suggest that oxidants indirectly stimulate GRK-2, leading to increased D1 receptor phosphorylation and G protein uncoupling. A similar mechanism of D1 receptor phosphorylation and G protein uncoupling is possible in renal proximal tubules of old rats, which are associated with oxidative stress. However, we did not measure PKC activity in the
proximal tubules in this study, as we have already reported earlier a twofold increase in basal PKC activity in proximal tubules of old rats (4), which decreased on antioxidant supplementation to these animals (6). In addition, PKC has been shown to provide anchors for GRK-2 on the plasma membrane and increase GRK-2 activity (24, 36). Moreover, studies from our laboratory in other animal models associated with oxidative stress such as obese Zucker rats showed that basal PKC activity is elevated in these animals, which, in turn, is responsible for an increase in GRK-2 translocation to the plasma membrane (3, 6, 28, 32).

However, studies by Lombardi et al. (26) showed oxidative stress-mediated downregulation of GRK 2 via calpain-dependent pathways in whole cell lysates of lymphocytes. Therefore, it is possible that oxidative stress may act differently, involving different mechanistic pathway in different cell types and cellular fractions (membranes vs. cytosol vs. whole cell lysate). It has been shown that GRKs phosphorylate agonist-occupied receptors (15, 30). However, several studies have shown a ligand-independent phosphorylation of D1 receptors in renal proximal tubules of spontaneously hypertensive, obese Zucker, and old Fischer rats (3, 13, 35). For example, an increase in GRK-4 activity and expression in proximal tubular cells from humans with essential hypertension has also been reported (32) and suggested to be the cause for increased D1 receptor phosphorylation and desensitization. It seems that in situations associated with oxidative stress, GRKs can be activated in a ligand-independent manner. In this study, we believe that tempol, by reducing oxidative stress, decreased membranous GRK-2 abundance, and therefore, decreased the basal serine phosphorylation of D1 receptors.

Tempol is a stable, metal-independent, low-molecular-weight, cell-permeable superoxide dismutase. It has been shown to have beneficial effects in normalizing blood pressure in various models of hypertension as well as in restoring renal dysfunction in hypertension and improving endothelial dysfunction in streptozotocin-induced diabetes (16, 27, 29, 33, 34). In addition to reducing MDA levels, membranous GRK-2 abundance, and basal serine phosphorylation of D1 receptors in old treated rats, tempol normalized D1 receptor number and protein in old rats to the level seen in adult rats. Moreover, tempol restored the functional responsiveness to D1 receptor activation in old treated rats. This is reflected by marked natriuresis and diuresis and a significant increase in the fractional excretion of sodium in these animals compared with old control rats. Restoration of dopamine receptor function in old treated rats is most likely attributed to tempol-induced normalization of D1 receptor-G protein coupling as a result of decreased D1 receptor serine phosphorylation. We did not measure the natriuretic response to dopamine in adult rats, because we did not observe any differences between adult control and adult treated groups in our biochemical studies.

In summary, we found that oxidative stress leads to an age-related decline in renal dopamine D1 receptor function. This is due to an oxidant-induced increase in PKC activity (2), membranous translocation of GRK-2, D1 receptor phosphorylation, receptor-G protein uncoupling, and loss of functional responsiveness of the receptor in old rats. Antioxidant (tempol) supplementation to old rats, while decreasing oxidative stress, prevented membranous translocation of GRK-2, decreased D1 receptor phosphorylation, and restored dopamine receptor-G protein coupling and the natriuretic effect of D1 receptor activation. Tempol may prove to be beneficial in restoring the age-related decline in renal function.

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REFERENCES


