Antioxidant Supplementation Normalizes Elevated Protein Kinase C Activity in the Proximal Tubules of Old Rats

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Aging is associated with increase in oxidative stress. Earlier, we have shown that higher basal protein kinase C (PKC) activity in the proximal tubules (PTs) of old rats contributes to the hyperphosphorylation of Na,K-ATPase and subsequent decrease in basal Na,K-ATPase activity, resulting in diminished natriuretic response to dopamine in these animals. We hypothesized that the increase in PKC activity in PTs of old rats is caused by increased oxidative stress and that antioxidants administration should reduce/normalize the elevated PKC activity in the renal PTs of old rats. We studied the effect of two antioxidants, namely, α-lipoic acid (LA) and tempol, on oxidants level and PKC activity in the PTs of adult (6-month) and old (24-month) Fischer 344 rats. We found that the accumulation of fluorescent dichlorofluorescein (DCF), an indicator of oxidant production, was higher in the PTs of old compared to adult rats. Dietary supplementation with LA for 2 weeks normalized the increased DCF level in old rats. Carboxymethylysine and malondialdehyde, markers of oxidative damage, were elevated in the PTs of old rats, which were normalized to the level of adult rats when tempol was provided in drinking water for 3 weeks. Both LA and tempol treatment also normalized the higher basal PKC activity in the PTs of old rats to the level seen in adult rats. These results suggest that increase in oxidative stress causes an increase in PKC activity, and that antioxidants, while reducing oxidative stress, also normalize PKC activity in the PTs of old rats. Exp Biol Med 229:270–275, 2004

Key words: aging; dopamine; natriuresis; oxidative stress; PKC

Cellular oxidants are produced continuously at a high rate as a by-product of aerobic metabolism. These oxidants include superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^\cdot$) and are known to damage cellular macromolecules such as DNA (1), protein (2), and lipid (3). At the same time, cells are known to possess defense systems to battle these reactive oxidants (4). However, under several conditions, such as aging and hypertension, these defenses do not function properly, and consequently cellular macromolecules become oxidatively damaged.

Mitochondria consume approximately 85% of the cellular oxygen for ATP production and appear to contribute the greatest source of oxidants (5). The increase in cellular oxidant production has been implicated in the decline of mitochondrial function, which can impair normal cellular activities and compromise the cell's ability to adapt to various physiological stresses in many diseases (6, 7) as well as in aging (5, 8).

Aging is associated with increase in oxidative stress and characterized by a general decline in physiological function that leads to morbidity and mortality. Several studies suggest that oxidation is a major contributor to cellular aging and degenerative diseases that accompany aging, such as cancer, immune-system decline, brain dysfunction, cataracts, and cardiovascular disease (9). Dietary antioxidants, namely, ascorbate, tocopherol, and carotenoids, are known to protect against these degenerative diseases (9).

Previously, we have shown that the natriuretic response to dopamine is diminished in old compared to adult rats. This diminished response in old rats is due to the inability of dopamine to inhibit sodium transporters Na,K-ATPase (10) and Na,H-exchanger (11) in renal proximal tubules (PTs). The reasons for reduced inhibition of Na,K-ATPase in the PTs of old rats are due in part to D1 receptor G-protein uncoupling and higher basal protein kinase C (PKC) activity, causing hyperphosphorylation of Na,K-ATPase and hence resulting in a decrease in basal Na,K-ATPase activity in these animals (10–12). In many situations, oxidants are known to stimulate PKC activity (13, 15). In this study we provide evidence that higher basal PKC activity in the PTs of old rats is due to increased oxidant

This paper was funded by NIH grant AG-15031 from National Institute on Aging.

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Received October 30, 2003.
Accepted November 11, 2003.

1535-3702/04/2293-0001$15.00

levels, which is normalized to the level of adult rats when oxidative stress is reduced with antioxidants.

Methods

Animals. Male Fischer 344 rats aged 6-months (adult) and 24-months (old) were bought from the National Institute on Aging (Bethesda, MD) and kept in the University of Houston animal care facility. The institution's Animal Care and Use Committee approved the use of animals.

Diets. Two antioxidants, namely, α-lipoic acid (LA) and 4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl (tempol), were used in two different studies. In one study, adult and old rats were placed on AIN93M standard rodent diet supplemented with 0.4% LA (Research Diets, Brunswick, NJ). The control groups of rats received AIN93M diet without LA. The diets and tap water were freely accessible to rats for 2 weeks. In another study, adult and old rats were given tempol (1 mmol/L) in drinking water for 3 weeks. The control groups of rats received water without tempol.

Surgery. At the conclusion of the antioxidant supplementation period, the rats were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg), a midline abdominal incision was made, and kidneys were isolated and used to prepare and enrich proximal tubular suspension according to a routinely used procedure in our laboratory and described elsewhere (10).

Dichlorofluorescein (DCF) Measurement. The level of oxidants in PTs was measured using 2′,7′-dichlorofluorescin diacetate (DCF-DA) as a probe according to Bejma *et al.* (16). Briefly, PTs were suspended in assay buffer (mM): 118 NaCl, 4 KCl, 1 KH_2PO_4, 27.2 $NaHCO_3$, 1.25 $CaCl_2$, 1.2 $MgCl_2$, 10 HEPES, 5 D-glucose, pH 7.4, and loaded with 20 μM DCF-DA for 30 mins at 30°C. The tubules were washed to remove the extracelluar DCF-DA and further incubated at 37°C for 15 mins. This allowed DCF-DA to be cleaved by intracellular esterase to derive DCFH (reduced form), which was oxidized to DCF by cellular oxidants. The reaction was stopped by placing PTs on ice. The oxidized DCF emits fluorescence, which was read on a spectrofluorometer using excitation (503 nm) and emission (525 nm) wavelengths in a final volume of 3 ml containing 40–50 μg PTs. At the end of the measurement, 1 μmol of H_2O_2 (final 330 μM) was added to ascertain that the DCF was at saturating concentration.

Carboxymethylysine (CML) Measurement. Oxidant-induced accumulation of CML, a biomarker of oxidative stress (17), in PTs was measured by noncompetitive ELISA using a previously described method (18) with some modifications. Briefly, PTs were homogenized in a buffer containing (mM) HEPES 10, sucrose 320, EDTA 1, DTT 1, and protease inhibitor cocktail and centrifuged at 12,000 *g* for 10 mins. The supernatant was diluted to a final protein concentration of 0.8–3 μg/ml (linear range of detection) with PBS containing 0.05% NaN_3. The wells of ELISA plate (Nunc-Immuno Plate, MaxiSorp Surface) were coated with 100 μl of the sample and incubated overnight at 4°C. After washing with PBS containing 0.1% tween-20 (T-20; buffer A), the wells were blocked with PBS containing 2% normal goat serum, 1% nonfat dry milk, and 0.05% T-20 (blocking buffer) for 2 hrs at room temperature (RT). The wells were washed with buffer A and incubated with 100 μl mouse monoclonal CML antibody (1:1600) (ICN Biomed, Aurora, OH) in blocking buffer for 2 hrs at RT. After washing, the primary antibody was probed with 100 μl HRP-conjugated goat anti-mouse antibody in blocking buffer (Santa Cruz Biotech Inc., Santa Cruz, CA). Finally, 100 μl of tetramethyl benzidine (Substrate System for ELISA; Sigma Chemical Co., St. Louis, MO) were added and incubated for 20 mins at RT. The reaction was terminated with 100 μl of 1-N sulfuric acid, and the color developed was read at 410 nm in an ELISA plate reader (BIO-TEK Instruments, Inc., Winooski, VT).

Malondialdehyde (MDA) Measurement. Oxidant-induced lipid peroxidation in PTs was determined by measuring MDA level by the method of Buege and Aust (19). The PTs were homogenized, and supernatant was collected as described previously. The supernatant was diluted to 0.4 mg/ml with 1.15% KCl and boiled with 2 ml of 15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25-N HCl for 15 mins. The sample was cooled and centrifuged at 1000 *g* for 10 mins, and the color was read at 535 on a spectrophotometer. MDA was quantitated using molar extinction coefficient $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Protein Kinase C (PKC) Activity Measurement. The PKC activity in PTs was measured using the manufacturer's protocol (catalog no. V5330; Promega Corporation, Madison, WI) and published elsewhere (12). Briefly, the reaction was carried out in a final volume of 25 μl containing (mM) 20 HEPES, pH 7.4, 1.3 $CaCl_2$, 1 DTT, 10 $MgCl_2$, 1 ATP, 0.05% Triton X-100, 0.2 mg/ml phosphatidylserine, and 2 μg fluorescent PepTag C1 peptide substrate (P-L-S-R-T-L-S-V-A-A-K, amino acid sequence). A dye molecule attached to the peptide substrate imparts the hot pink fluorescence. The reaction was started by the addition of 25–50-μg proteins and carried out for 30 mins at 30°C. The samples were heated at 95°C for 10 mins to stop the reaction. The PKC-mediated phosphorylation on PepTag C1 peptide was separated from the nonphosphorylated peptide by electrophoresis on 0.8% agarose. The phosphorylated peptide was cut under UV light, agarose was melted, and the fluorescence intensity was recorded using excitation and emission wavelengths of 568 and 592 nm, respectively, on a spectrofluorometer. The fluorescence intensity was read on a standard curve prepared by using pure PKC enzyme (0–40 ng) as standard supplied with the kit. The PKC enzyme (catalog no. V5261; Promega) supplied was purified from rat brain and is greater than 90% pure as determined by SDS-PAGE, which consists primarily of α, β, and γ isoforms with lesser amount of δ and ζ isoforms.

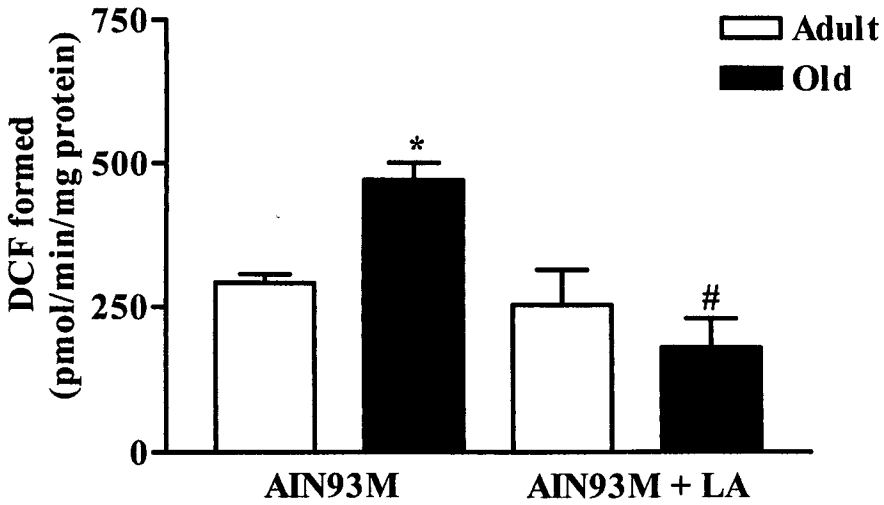

Figure 1. α-Lipoic acid (LA) treatment significantly decreased the level of oxidants in the proximal tubules of old rats. DCFH-DA (20 μM) was loaded with PTs, and the fluorescence from oxidized DCF was read using excitation (503 nm) and emission (525 nm) wavelengths (details in the *Methods* section). Values represent mean ± SEM of five experiments. *Significantly different from adult fed on standard diet (AIN93M) ($P < 0.05$, *t* test). #Significantly different from old rats fed on standard diet ($P < 0.05$, *t* test).

Na,K-ATPase Activity Measurement. The activity of Na,K-ATPase in the PTs was measured colorimetrically by the method of Quigly and Gotterer (20). We have employed this technique routinely in our laboratory and published earlier (10, 12).

Protein Measurement. Proteins were measured using BCA protein assay kit (Pierce, Rockford, IL) and BSA as standards.

Statistics. Results are presented as mean ± SEM. Data were analyzed by ANOVA followed by Bonferroni's multiple comparison test. A value of $P < 0.05$ was considered to be significant.

Materials

The 2′,7′-dichlorofluorescein was bought from Molecular Probes (Eugene, OR). Carboxymethylysine antibody was purchased from ICN Biomed. PKC assay kit and agarose were bought from Promega. All other chemicals used in this study were purchased from Sigma Chemical.

Results

Daily Food Intake and Body Weight of Adult and Old Rats. Supplementation of antioxidants either in diet (for lipoic acid) or in water (for tempol) did not affect the food and water intake of adult (AIN93M vs. AIN93M-LA: 16.0 ± 0.9 vs. 14.0 ± 0.8 g/day/rat; vehicle vs. tempol: 27 ± 1 vs. 28 ± 1 ml/day/rat) and old (AIN93M vs. AIN93M-LA: 17.0 ± 0.8 vs. 15.0 ± 0.6 g/day/rat; vehicle vs. tempol: 24 ± 2 vs. 26 ± 3 ml/day/rat) rats. We did not find any significant changes in the body weight of either adult (AIN93M vs. AIN93M-LA: 378 ± 10 vs. 350 ± 7 g/rat; vehicle vs. tempol: 438 ± 7 vs. 426 ± 3 g/rat) or old (AIN93M vs. AIN93M-LA: 436 ± 4 vs. 425 ± 10 g/rat; vehicle vs. tempol: 444 ± 12 vs. 430 ± 10 g/rat) rats during the period of antioxidant supplementation.

Effect of LA on DCF Accumulation. As shown in Figure 1, DCF accumulation was significantly higher in the PTs of vehicle-treated old compared to adult rats. LA treatment in old rats reduced the DCF level compared to vehicle-treated old rats and normalized to the level of adult rats. LA treatment had no effect on DCF accumulation in adult rats.

Effect of LA and Tempol on CML Accumulation. The CML accumulation in the PTs of vehicle-treated old rats was significantly higher than adult rats (Fig. 2A and B). Both LA (Fig. 2A) and tempol (Fig. 2B) treatment in old rats significantly decreased the CML accumulation compared to vehicle-treated old rats and normalized to the level of adult rats (Fig. 2A and B). Both LA and tempol treatment had no effect on CML accumulation in adult rats (Fig. 2A and B).

Effect of LA and Tempol on MDA Level. MDA level, an indicator of oxidant-induced lipid peroxidation, was higher in the PTs of old compared to adult rats (Fig. 3A and B). Both LA (Fig. 3A) and tempol (Fig. 3B) treatment significantly reduced the MDA level in old rats. Antioxidant supplementation (LA and tempol) normalized the elevated MDA level in old rats to the level of adult rats (Fig. 3A and B). Both LA and tempol had no effect on MDA level in adult rats (Fig. 3A and B).

Effect of LA and Tempol on PKC Activity. In the vehicle-treated groups, the basal PKC activity was 2-fold higher in the PTs of old compared to adult rats (Fig. 4A and B). Both LA (Fig. 4A) and tempol (Fig. 4B) significantly decreased the elevated PKC activity in old rats to the level of adult rats. The antioxidants treatment had no effect on PKC activity in adult rats.

Effect of Antioxidant (LA) on Na,K-ATPase Activity. In the control (AIN93M) groups, the basal Na,K-ATPase activity was lower in the PTs of old compared to adult rats (Fig. 5). LA supplementation normalized the

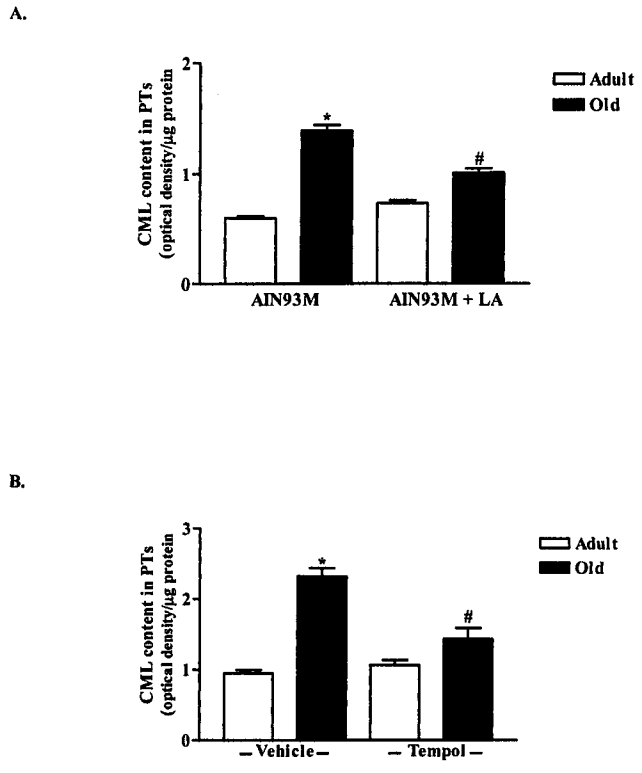

Figure 2. (A) α-Lipoic acid (AIN93M + LA) supplementation in old rats significantly reduced the elevated CML content in the PTs of old rats fed with control (AIN93M) diet. (B) Tempol treatment in old rats normalized the elevated CML content in the PTs of old rats treated with vehicle. Noncompetitive ELISA was used to measure CML content in the PTs (details in the *Methods* section). Bars represent mean ± SEM values of three separate experiments ($N = 3$). *Significantly different ($P < 0.05$) from AIN93M/vehicle-treated adult rats. #Significantly different ($P < 0.05$) from AIN93M/vehicle-treated old rats. Data were analyzed by using ANOVA followed by Bonferroni's multiple comparison tests.

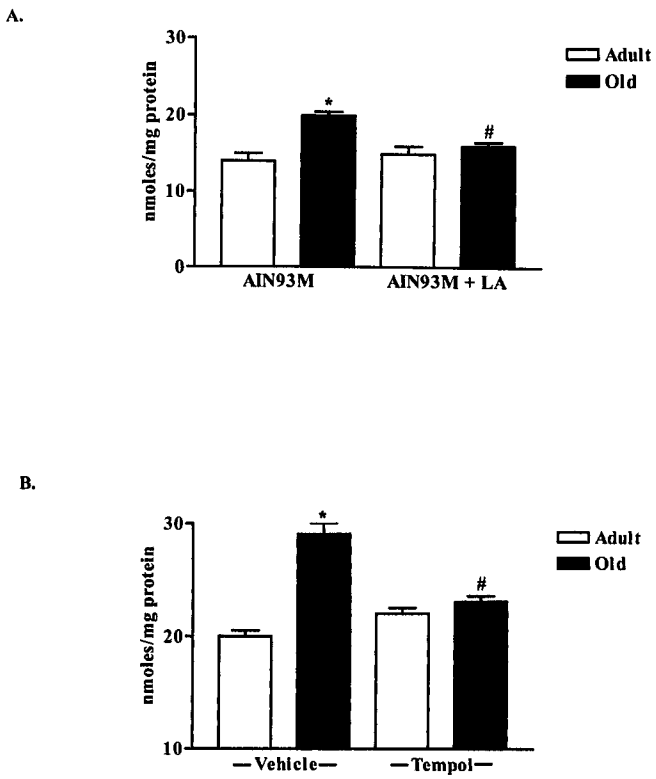

Figure 3. (A) α-Lipoic acid (AIN93M + LA) supplementation in old rats significantly reduced the elevated MDA level in the PTs of old rats fed with control (AIN93M) diet. (B) Tempol treatment in old rats normalized the MDA level in the PTs of old rats treated with vehicle. MDA level in the PTs was measured by absorbing red-colored product of MDA and thiobarbituric acid at 535 nm (details in the *Methods* section). Bars represent mean ± SEM of three separate experiments. *Significantly different ($P < 0.05$) from AIN93M/vehicle-treated adult rats. #Significantly different ($P < 0.05$) from AIN93M/vehicle-treated old rats. Data were analyzed using ANOVA followed by Bonferroni's multiple comparison tests.

Na,K-ATPase activity in old rats to the level seen in adult rats (Fig. 5).

Discussion

Our results show that the levels of oxidants in the proximal tubules were higher in old compared to adult rats, which are in agreement with the previous reports of increased oxidative stress with aging in heart, liver (16, 21), and kidney (22). In our study, we determined different oxidant species in the PTs by measuring different markers of oxidative stress. Dichlorofluorescein (DCF) is the measurement of H_2O_2 (23), while carboxymethylysine (CML) determines the level of both superoxide (24) and hydroxyl radicals (25). Malondialdehyde (MDA) measured in the study is considered the general marker of oxidative stress arising from oxidant-induced lipid peroxidation (19). Two different antioxidants, α-lipoic acid and tempol, were used in the study to see their effect on oxidant levels and PKC activity in renal proximal tubules; α-lipoic acid and tempol have been used previously to reduce the oxidant levels in old Fischer 344 rats (26) and spontaneous hypertensive rats (7), respectively.

Earlier we reported a higher basal PKC activity in the PTs of old rats (12), which seems to be due to overexpression of specific PKC isoforms: PKC-βI and PKC-δ (27). This increased PKC activity causes hyperphosphorylation of Na,K-ATPase, leading to its low activity (12) and consequently low inhibitory response to dopamine in old rats (10). The present study was designed to determine whether increased oxidative stress in renal PTs would cause the increase in basal PKC activity that subsequently leads to a low activity of Na,K-ATPase in old rats. Therefore, antioxidant administration to old rats, while lowering oxidative stress, should normalize the basal elevated PKC and low Na,K-ATPase activities. There seems to be a correlation between oxidants and PKC activity since oxidants are known to increase the PKC activity in many cell types (13–15). The mechanism of oxidant-mediated increase in PKC activity is not known. However, hydrogen peroxide, a known oxidant, increases PKC activity through direct or indirect activation of phospholipase D involving diacyl-

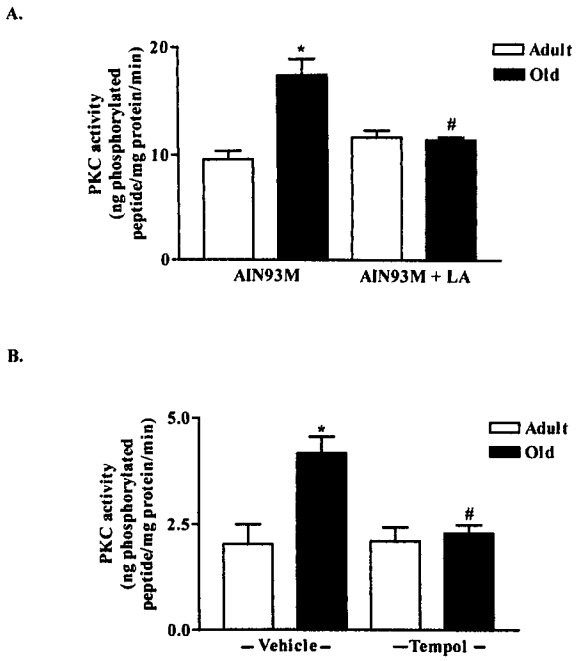

Figure 4. α-Lipoic acid (A) and tempol (B) normalized the higher basal PKC activity in the PTs of old rats to the level of adult rats. PKC activity was measured as the ability of proximal tubular PKC to phosphorylate a specific peptide substrate (details in the *Methods* section). Bars represent mean ± SEM values of five and three experiments for LA and tempol, respectively. *Significantly different from AIN93M/vehicle-treated adult rats ($P < 0.05$). #Significantly different from AIN93M/vehicle-treated old rats ($P < 0.05$). Data were analyzed by ANOVA followed by Bonferroni's multiple comparison tests.

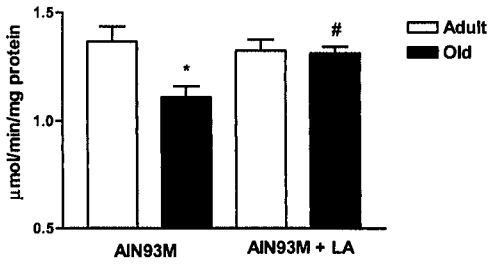

Figure 5. Antioxidant supplementation (α-lipoic acid) normalized the low basal Na,K-ATPase activity in the PTs of old rats. Bars represent mean ± SEM values of four to five experiments. *Significantly different from AIN93M (control)-treated adult rats. #Significantly different from AIN93M (control)-treated old rats. Data were analyzed by ANOVA followed by Bonferroni's multiple comparison tests. A P value <0.05 was considered significant.

glycerol (14), an intracellular messenger molecule required for PKC activation in rat renal PTs (28). In addition, activation of PKC is regulated through oxidant-mediated redox changes in sulfhydryl groups of cysteine-rich regions of PKC (13). In this study, the evidence for oxidant-mediated increase in PKC activity in the PTs of old rats is based on experiments involving antioxidant supplementation to these animals. Both LA and tempol, while reducing the oxidant levels, also normalized the PKC activities in the PTs of old rats. Furthermore, LA also normalized Na,K-ATPase activity in the PTs of old rats. Studies performed in cell lines also support the view of oxidant-induced stimulation of PKC. For instance, tempol reduces the paraquat (generates oxygen-free radicals)-mediated increase in PKC activity in WI-38 human lung cells (29), while α-tocopherol, taurine, and *N*-acetyl-cysteine are known to inhibit PKC activity and TGF β-mediated matrix protein synthesis in response to high glucose, thromboxane, and angiotensin II in mesengial cells (30). These findings, when viewed collectively with results of our study, provide strong evidence that the increase in oxidative stress in the aging kidney is responsible for the higher basal PKC activity, which subsequently leads to a low activity of Na,K-ATPase.

The hallmark of the aging process, which is associated with oxidative stress, is the loss of sensitivity of receptors to agonist stimulation. This appears to be particularly true in striatal (31) and peripheral (11) dopamine systems. Decreased receptor number and defective signal transduction seem to be responsible for a marked decline in natriuresis (11) and cognitive and motor functions (31) in response to D1-receptor stimulation. The diminished natriuretic response to dopamine was due to hyperphosphorylation and down-regulation of D1 receptor in the PTs of old rats (32). One approach to normalize the defective dopamine receptor function in aging would be to increase the number of high-affinity dopamine receptors. A study in this direction has been conducted with acetyl L-carnitine (ALCAR) supplementation to aged mice, which enhanced dopamine release and attenuated the loss of the D1 subclass of striatal dopamine receptors associated with aging (33). However, ALCAR is known to cause a decline in antioxidant status and an increase in oxidant production and oxidative damage in isolated hepatocytes from old but not young rats (34). Therefore, comprehensive experiments utilizing a number of different antioxidant treatment need to be performed to examine the influence of lowering oxidant levels on restoring D1 receptor–G protein coupling and natriuretic response to D1-receptor activation.

In summary, antioxidant administration, while reducing oxidative stress, normalized basal higher PKC and low Na,K-ATPase activities in old rats. The higher PKC activity is one of the contributing factors for the diminished natriuretic response to dopamine in old rats. Therefore, antioxidant supplementation, while normalizing PKC activity, may prove to be beneficial in restoring D1 receptor function and natriuretic response to dopamine in old rats.

1. Fraga CG, Shigenaga MK, Park JW, Degan P, Ames BN. Oxidative damage to DNA during aging: 8-hydroxy-2N-deoxyguanosine in rat organ DNA and urine. Proc Natl Acad Sci U S A 87:4533–4537, 1990.
2. Stadtman ER. Protein oxidation and aging. Science 257:1220–1224, 1992.
3. Marnett LJ, Hurd H, Hollstein MC, Esterbauer DE, Ames BN. Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mutat Res 148:25–34, 1985.

4. Sies H. Oxidative stress: from basic research to clinical application. Am J Med 91:31S–38S, 1991.
5. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci U S A 91:10771–10778, 1994.
6. Schnackenberg CG. Oxygen radicals in cardiovascular-renal disease. Curr Opin Pharmacol 2:121–125, 2002.
7. Schnackenberg CG, Wilcox CS. Two-week administration of tempol attenuates both hypertension and renal excretion of 8-iso prostaglandin $F_{2\alpha}$. Hypertension 33:424–428, 1999.
8. Peterson KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Sculman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 300:1140–1142, 2003.
9. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A 90:7915–7922, 1993.
10. Kansra V, Hussain T, Lokhandwala MF. Alterations in dopamine DA1 receptor and G proteins in renal proximal tubules of old rats. Am J Physiol 273:F53–F59, 1997.
11. Beheray S, Kansra V, Hussain T, Lokhandwala MF. Diminished natriuretic response to dopamine in old rats is due to an impaired D1-like receptor-signaling pathway. Kidney Int 58:712–720, 2000.
12. Asghar M, Kansra V, Hussain T, Lokhandwala MF. Hyper-phosphorylation of Na-pump contributes to defective renal dopamine response in old rats. J Am Soc Nephrol 12:226–232, 2001.
13. Gopalkrishna R, Anderson WB. Reversible oxidative activation and inactivation of protein kinase C by the mitogen/tumor promotor periodate. Arch Biochem Biophys 285:382–387, 1991.
14. Taher MM, Garcia JG, Natarajan V. Hydroperoxide-induced diacylglycerol formation and protein kinase C activation in vascular endothelial cells. Arch Biochem Biophys 303:260–266, 1993.
15. Schuppe-Koistinen I, Moldeus P, Bergman T, Cotgreave IA. S-Thiolation of human endothelial cell glyceraldehydes-3-phosphate dehydrogenase after hydrogen peroxide treatment. Eur J Biochem 227: 1033–1037, 1994.
16. Bejma J, Ramires P, Ji LL. Free radical generation and oxidative stress with aging and exercise: differential effects in the myocardium and liver. Acta Physiol Scand 169:343–351, 2000.
17. Requena JR, Fu MX, Ahmed MU, Jenkins AJ, Lyons TJ, Thorpe SR. Lipoxidation products as biomarkers of oxidative damage to protein during lipid peroxidation reactions. Nephrol Dial Transplant 11:48–53, 1996.
18. Koo JR, Vaziri ND. Effects of diabetes, insulin and antioxidants on NO synthase abundance and NO interaction with reactive oxygen species. Kidney Int 63:195–201, 2003.
19. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 52:302–310, 1978.
20. Quigley JP, Gotterer, GS. Distribution of Na,K-stimulated ATPase activity in intestinal mucosa. Biochem Biophys Acta 173:456–468, 1969.
21. Hagen TM, Moreau R, Suh JH, Visili F. Mitochondrial decay in the aging rat heart: evidence for improvement by dietary supplementation with acetyl-l-carnitine and/or lipoic acid. Ann N Y Acad Sci 959:491–507, 2002.
22. Ruiz-Torres MP, Gonzalez-Rubio M, Lucio-Cazana FJ, Ruiz-Villaespesa A, Rodriguez-Puyol M, Rodriguez-Puyol D. Reactive oxygen species and platelet activating factor synthesis in age related glomerulosclerosis. J Lab Clin Med 124:489–495, 1994.
23. Cathcart R, Schwier E, Ames NB. Detection of picomole levels of hydrogen peroxides using a fluorescent dichlorofluorescein assay. Anal Biochem 134:111–116, 1983.
24. Shibata N, Hirano A, Hedley-Whyte ET, Dal Canto MC, Nagai R, Uchida K, Horiuchi S, Kawaguchi M, Yamamoto T, Kobayashi M. Selective formation of certain advanced glycation end products in spinal cord astrocytes of humans and mice with superoxide dismutase-1 mutation. Acta Neuropathol 104:171–178, 2002.
25. Nagai R, Ikeda K, Higashi T, Sano H, Jinnouchi Y, Araki T, Horiuchi S. Hydroxyl radical mediates N epsilon-(carboxymethyl)lysine formation from Amadori product. Biochem Biophys Res Commun 234:167–172, 1997.
26. Jung HS, Eric TS, Jason DM, Brian C, Alma ER, Balz F, Tory MH. Oxidative stress in the aging rat heart is reversed by dietary supplementation with (R)-α-lipoic acid. FASEB J 15:700–706, 2001.
27. Asghar M, Hussain T, Lokhandwala MF. Over-expression of PKC-βI and -δ contributes to higher PKC activity in the proximal tubules of old Fischer 344 rats. Am J Physiol Renal Physiol 285: F1100–F1107, 2003.
28. Chen C, Beach RE, Lokhandwala MF. Dopamine fails to inhibit renal tubular sodium pump in hypertensive rats. Hypertension 21:364–372, 1993.
29. Kuo ML, Lee KC, Lin JK, Huang TS. Pronounced activation of PKC, ornithine decarboxylase and c-jun proto-oncogene by paraquat-generated active oxygen species in WI-38 human lung cells. Biochim Biophys Acta 1268:229–236, 1995.
30. Studer RK, Craven PA, DeRubertis FR. Antioxidant inhibition of protein kinase C signaled increases in transforming growth factor-beta in mesangial cells. Metabolism 46:918–925, 1997.
31. Joseph JA, Roth GS. Cholinergic systems in aging: the role of oxidative stress. Clin Neuropharmacol 15:508A–509A, 1992.
32. Asghar M, Hussain T, Lokhandwala MF. Higher basal serine phosphorylation of D1A receptors in proximal tubules of old Fischer 344 rats. Am J Physiol 283:F350–F355, 2002.
33. Sershen H, Harsing LJ, Banay SM, Hashim A, Ramacci MT, Lajtha A. Effect of acetyl-L-carnitine on the dopaminergic system in aging brain. J Neurosci Res 30:555–559, 1991.
34. Hagen TM, Ingersoll RT, Wehr CM, Lykkesfeldt J, Vinarsky V, Bartholomew JC, Song MH, Ames BN. Acetyl-L-carnitine fed to old rats partially restores mitochondrial function and ambulatory activity. Proc Natl Acad Sci U S A 95:9562–9566, 1998.