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Enalapril and losartan attenuate mitochondrial dysfunction in aged rats

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ABSTRACT

Renin-angiotensin system (RAS) inhibition can attenuate the effects of aging on renal function and structure; however, its effect on mitochondrial aging is unknown. To investigate whether an angiotensin-converting enzyme inhibitor (enalapril) or an angiotensin II receptor blocker (losartan) could mitigate age-associated changes in kidney mitochondria, male Wistar rats (14 mo old) received during 8 mo water containing either enalapril (10 mg/kg/day) (Enal), or losartan (30 mg/kg/day) (Los), or no additions (Old). Four-month-old untreated rats (Young) were also studied. In Old rats mitochondrial respiratory control, ADP/O, nitric oxide synthase activity, and uncoupling protein 2 levels were lower (46, 42, 27, and 76%, respectively), and Mn-SOD activity was higher (70%) than in Young, Enal, and Los rats. In Old rats mitochondrial hydrogen peroxide production was higher than in both Young (197%) and Enal or Los (40%) rats. In Old rats, kidney GSH/GSSG was lower than in both Young (80%) and Enal (57%) or Los (68%) rats. In Old rats electron microscopy showed effacement of microvilli in tubular epithelial cells, ill-defined mitochondrial cristae, lower mitochondrial numbers, and enhanced number of osmiophilic bodies relative to Young, Enal, or Los rats. In conclusion, enalapril and losartan can protect against both age-related mitochondrial dysfunction and ultrastructural alterations, underscoring the role of RAS in the aging process. An association with oxidative stress modulation is suggested.

Key words: renin-angiotensin system • aging • nitric oxide • glutathione • oxidant damage

Normal kidney aging is associated with the slow development of functional and structural changes. However, function remains suitable for the support of life albeit the kidney becomes more vulnerable to disease. In the presence of hypertension, age-related changes proceed at an accelerated rate eventually leading to kidney failure (1). Even in the absence of hypertension, systolic blood pressure tends to increase with age as a result of the age-related stiffening of the arteries and/or a variety of age-related diseases, including atherosclerosis and diabetes (2), compromising kidney function.

Mitochondria, organelles responsible for most of the cell energy production, are one of the main intracellular sources of oxidants under physiological conditions (3). In addition, since

mitochondria are particularly sensitive to oxidant damage, they seem to play a key role in the aging process. Tissues isolated from aging animals usually have a low number of mitochondria and several changes in mitochondrial structure, such as swelling, shortening of the cristae, and matrix vacuolization. These changes have been related to an increased generation of superoxide anion and hydrogen peroxide and to a decline in the capacity for energy production (3).

Kidney function is highly dependent on mitochondrial ATP supply; hence, kidneys are specially susceptible to the decay of mitochondrial energy production. The renin-angiotensin system (RAS) seems to have a major share in the development of age-related kidney damage (4). Inhibition of the RAS, either with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers, can attenuate the effects of aging on renal structure and function (5, 6). To explain these protective effects, we hypothesized that the RAS might participate in the age-related deterioration of mitochondrial function and/or structure. In this context, the purpose of this study was to investigate whether an ACE inhibitor (enalapril) and an angiotensin II receptor blocker (losartan) could attenuate the functional and structural changes that occur in kidney mitochondria upon aging.

METHODS

Animals and treatments

Fourteen-month-old male Wistar rats were randomly divided into three groups of eight animals each. During the following 8 mo, the rats had free access to drinking water containing either enalapril (10 mg/kg/day) (Enal), or losartan (30 mg/kg/day) (Los), or no additions (Old). A fourth group, consisting of eight 4-mo-old rats (Young) that received drinking water with no additions since birth, was also studied. The rats had free access to standard rat chow (Cargill, Buenos Aires, Argentina). The day before killing systolic blood pressure was evaluated by tail plethysmography (PE-300, Narco Bio-Systems, Austin, TX), and 24 h urine was collected for the determination of urinary albumin excretion (Bind a Rid, Nanorid Products, The Binding Site Ltd, Birmingham, England). Rats were anesthetized with pentobarbital (40 mg/kg body wt ip), blood was drawn from the thoracic aorta, and a systemic perfusion was performed with NaCl 0.9% (w/v) immediately before excision of the kidneys. Creatinine was determined in blood plasma (Randox Laboratories Ltd, Crumlin, N. Ireland). Unless otherwise stated, all reagents were from Sigma Chemical (St. Louis, MO).

Isolation of mitochondria

Kidneys were homogenized at 0–4°C in a solution containing 0.23 M mannitol, 0.07 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4. After centrifugation of the homogenates at 600 *g* for 10 min, the pellet was discarded and the supernatant was centrifuged at 8,000 *g* for 10 min. The pelleted mitochondria were washed once and then resuspended in the same buffered solution (7). For the determination of nitric oxide synthase (NOS) activity, mitochondria were further purified in a self-forming Percoll gradient. Pelleted mitochondria were suspended in 30% (v/v) Percoll in 0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, 0.1% (w/v) BSA, pH 7.4, and centrifuged at 95,000 *g* for 30 min. Purified mitochondria were washed twice with 150 mM KCl, followed by two washes with homogenization solution.

Purity of the preparation was assesed by determining the activities of marker enzymes for other subcellular fractions (glucose-6-phosphatase, 5′-nucleotidase, acid phosphatase, and catalase) recovered in the purified mitochondrial fraction. Contaminations were in the low range (0.13, 0.10, 2.9, and 0.30%, respectively). Electron microscopy examination of the purified mitochondrial fraction confirmed the absence of contaminating organelles or organelle fragments. Protein content was assayed according to Bradford, using BSA as standard (8).

Mitochondrial respiratory rates

Mitochondrial oxygen uptake was evaluated with a two-channel respirometer (Oroboros Oxygraph, Par KG, Graz, Austria). Mitochondrial state 4 rates of O_2 uptake (resting or controlled respiration) were determined in a solution containing 0.23 M mannitol, 0.07 M sucrose, 1 mM EDTA, 4 mM $MgCl_2$, 5 mM potassium phosphate, 0.1% (w/v) BSA, 6 mM malate, 6 mM glutamate, and 20 mM Tris-HCl, pH 7.4, at 37°C. Subsequently, 1 mM ADP was added for the determination of state 3 rates of O_2 uptake (active respiration). Respiratory control ratio was calculated as rate of O_2 uptake in state 3 to the rate of O_2 uptake in state 4. The ADP/O ratio was calculated as nanomoles of ADP added to the reaction medium/nanogram atoms of oxygen used during state 3 respiration (9).

Hydrogen peroxide production

Mitochondrial H_2O_2 production was determined by fluorometry at 350 and 460 nm excitation and emmision wavelengths, respectively. The reaction medium consisted of a solution containing 0.23 M mannitol, 0.07 M sucrose, 20 mM Tris-HCl (pH 7.4), 0.8 μM horseradish peroxidase, 1 μM scopoletin, 0.3 μM SOD, 6 mM malate, 6 mM glutamate, 3 μM antimycin, and 0.1–0.3 mg mitochondrial protein/ml. H_2O_2 (0.05–0.35 μM) was used as standard (10).

Mitochondrial NOS activity

Mitochondrial NOS activity was determined by conversion of [^{14}C]arginine to [^{14}C]citrulline in a solution containing 50 mM potassium phosphate, pH 5.8, 1 μM flavin adenine dinucleotide, 1 μM flavin adenine mononucleotide, 10 μM tetrahydrobiopterine, 0.1 μM calmodulin, 300 μM $CaCl_2$, 100 μM NADPH, 60 mM valine, 50 μM arginine, 0.025 μCi [^{14}C]arginine, and 0.15 mg mitochondrial protein. The assay mixtures were incubated at 37°C for 5 min. The reaction was stopped by addition of 3 vol of a solution containing 2 mM EDTA and 20 mM HEPES, pH 5.5, followed by 6 vol of a Dowex exchange resin (Bio-Rad, Hercules, CA). An aliquot of the supernatant was used for scintillation counting (11).

Western blot analysis of uncoupling protein 2

Mitochondrial proteins were separated on SDS-12.5% polyacrylamide gels and then transferred to PVDF membranes by liquid electroblotting (MiniProtean 3, Bio-Rad) in a transfer solution containing 25 mM Tris, 190 mM glycine, 20% methanol, pH 8.3, at 110 V for 90 min. After transfer, membranes were blocked by incubating with phosphate buffered saline (PBS) containing 5% dry low-fat milk for 1 h at room temperature. Uncoupling protein 2 (UCP2) antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) was diluted in 2% dry

low-fat milk and incubated overnight at 4°C. Membranes were washed with PBS-0.1% Tween 20 and incubated with peroxidase-conjugated donkey anti-goat secondary antibody for 90 min at room temperature. After being washed with PBS-0.1% Tween 20, bound peroxidase-conjugated antibody was revealed with diaminobenzidine tablets (Sigma). Western blots were photographed using a digital camera and analyzed with Scion Image (Scion Corporation, Frederick, MD) software. The amount of protein loaded into gels was normalized by probing the membranes with cytochrome *c* antibody.

Total (GSH + GSSG) and oxidized glutathione (GSSG)

Tissue samples were homogenized with 4 vol of 0.33 M $HClO_4$, and the resulting suspension was centrifuged at 5,000 *g* for 10 min. After neutralization with 1.75 M K_3PO_4, the supernatants were used for *1*) the determination of GSSG + GSH using the 5,5′-dithiobis(2-nitrobenzoic acid) spectrophotometric assay (12); and *2*) GSSG determination by following NADPH oxidation at 340 nm (12). Results are expressed as nanomoles of GSH equivalents per gram of wet tissue and nanomoles of GSSG per gram of wet tissue, respectively.

Antioxidant enzyme activities

Tissue samples were homogenized with 4 vol of 120 M KCl-30 mM potassium phosphate, pH 7.4, and centrifuged at 600 *g* for 10 min. The supernatant was used for the assesment of antioxidant enzyme activities. Total SOD activity (CuZn-SOD+Mn-SOD) was determined after the inhibition of cytochrome *c* reduction by superoxide anion at 550 nm (13). One unit of SOD was defined as the amount of enzyme necessary to cause a 50% inhibition of the reduction of cytochrome *c*. To assess Mn-SOD activity (expressed as units/mg mitochondrial protein), the same procedure was performed in the presence of 2 mM NaCN. Glutathione reductase (GSSG-Rd) activity was determined by following NADPH oxidation at 340 nm (14). One unit of GSSG-Rd was defined as the amount of enzyme that catalyzes the oxidation of one micromole of NADPH per minute. Glutathione peroxidase (GPx) activity was determined in the presence of GSSG-Rd by following the oxidation of NADPH at 340 nm (15). One unit of GPx was defined as the amount of enzyme that catalyzes the oxidation of one micromole of NADPH per minute. Catalase activity was evaluated by following the oxidation of H_2O_2 at 240 nm and calculating the pseudo-first order constant of the reaction (16). Catalase content was expressed as picomoles of catalase per milligram of protein.

Electron microscopy

Immediately after excision of the kidneys, tissue samples were cut into 1 mm^3 blocks, fixed in 3% (w/v) glutaraldehyde in a solution containing 0.1 M sodium phosphate buffer, pH 7.2, and postfixed in 2% (w/v) osmium tetroxide in the same solution. After being embedded in an Epon resin, thick sections (5 μm) were cut and examined by light microscopy to select areas containing proximal tubules. The selected blocks were then cut with an ultramicrotome into thin sections (0.5 μm) and examined with a Zeiss EM 10C electron microscope. Morphometric analysis of tubular epithelial cell ultrastructures was performed on 6 micrographs from different sections of each block, with a magnification of x6,000 (whole cell) and x20,000 (mitochondria) and a x2 enlargement of paper copies. Quantification was performed by determining the number of cells showing alterations, preservation, or complete

absence of microvilli. The results were expressed as the percentage of cells that exhibited either of these characteristics. Osmiophilic bodies, mitochondria, and mitochondria with poorly defined cristae were counted and expressed as the numbers of a particular structure per cell. Mitochondrial cristae were counted and expressed as number of cristae per μm of mitochondrial contour.

Statistical methods

Values are means ± SE. Nonparametric Kruskall-Wallis statistics (Statview 5.0, SAS Institute Inc., Cary, NC) were used to establish the significance of between-group differences. P values <0.05 were considered significant.

RESULTS

Effect of enalapril and losartan treatments on animal outcome

Food intake showed no differences among groups (5.5±0.1 g/100 g body wt/day) throughout the study. Table 1 shows the biological outcome for the study groups. At the end of the treatments, body weight was similar in the rats from the Old, Enal, and Los groups. Kidney weight was similar in the four groups studied. Plasma creatinine and urinary albumin excretion were significantly higher in the Old group relative to the Enal, Los, and Young groups. In the Enal and Los groups, plasma creatinine and urinary albumin excretion were significantly higher than in the Young group. Systolic blood pressure was significantly higher in the Old group compared with the Enal, Los, and Young groups.

Age-related changes in kidney mitochondrial respiratory control, ADP/O ratios, H_2O_2 production, NOS activity, UCP2 content, and Mn-SOD activity are attenuated by enalapril and losartan treatments

Table 2 shows kidney mitochondrial respiratory control, ADP/O ratios, hydrogen peroxide production, NOS activity, UCP2 protein content, and Mn-SOD activity values. Respiratory control ratios were used as indicators of mitochondrial integrity and ADP/O ratios as an estimation of the capacity for energy production. In kidney mitochondria from the Old group, respiratory control and ADP/O ratios were significantly lower than in mitochondria from the Young, Enal, and Los groups. H_2O_2 production in kidney mitochondria from the Old group was three times higher than in the Young group. Enal and Los mitochondrial H_2O_2 production was approximately two times higher than in the Young group. Mitochondrial NOS activity was significantly lower (27%) in the Old group relative to the Young group. In the Enal and Los groups, mitochondrial NOS activity was similar to that observed in the Young group. Because mitochondrial H_2O_2 generation can be modulated by UCP2 (17), the level of this protein was determined in isolated kidney mitochondria. UCP2 was significantly lower (76%) in the Old group compared with the Enal, Los, and Young groups (Fig. 1). In agreement with a higher expression of UCP2 protein, mitochondrial state 4 oxygen consumption, which is primarily due to H^+ leak, was 30% higher in the Young, Enal, and Los groups relative to that in the Old group. Mitochondrial Mn-SOD activity was significantly higher (70%) in the Old group relative to any of the other groups.

Enalapril and losartan treatments attenuate the age-related oxidation of kidney glutathione, and kidney GSH + GSSG is elevated in losartan-treated rats

GSH+ GSSG content and GSH/GSSG ratio data are presented in Table 3. GSH+ GSSG content in the kidney was similar in the Old, Enal, and Young groups and was significantly higher in the Los group as compared with any of the other groups (54, 47, and 47% vs. Old, Enal, and Young, respectively). In the Old group, kidney GSH/GSSG ratio was significantly lower (80%) than in the Young group. In the Enal and Los groups, GSH/GSSG ratio was significantly higher (132 and 210%, respectively) than in the Old group.

Antioxidant enzymes activities in the kidney

The activities of kidney GPx, GSSG-Rd, CuZn-SOD, and catalase were similar in the four groups studied (Young: 331±24 units GPx/mg protein; 102±11 units GSSG-Rd/mg protein; 25.4±1.9 units CuZn-SOD/mg protein; and 7.18±1.2 pmol catalase/mg protein).

Enalapril and losartan treatments mitigate age-related ultrastructural changes in proximal tubular epithelial cells

Ultrastructural analysis of the kidney was focused on proximal tubular epithelial cells because they have a large number of mitochondria and are highly dependent on mitochondrial energy production for proper function. In proximal tubules from the Young group, the totality of epithelial cells examined showed good preservation of microvilli. In the Old group, the number of epithelial cells showing either the absence of microvilli (Fig. 2A) or altered microvilli was significantly higher than in the Enal or Los groups (Table 4). Accordingly, the percentage of tubular epithelial cells showing well-preserved microvilli was significantly higher in the Enal, Los, and Young groups (Table 4 and Fig. 2B, C, and D) than in the Old group. In the Old group, the number of mitochondria per cell was significantly lower than in the Enal, Los, and Young groups. The number of cristae per unit length of mitochondrial contour was similar in the groups studied. However, the number of mitochondria exhibiting poorly defined cristae was significantly higher in the Old group compared with the Enal, Los, and Young groups (Table 4). In the Young group, mitochondria were mainly located within the basal infoldings of proximal tubular epithelial cells in agreement with the typical mitochondrial distribution for this type of cell (Fig. 2D). In the Old group, mitochondrial distribution was altered, with a preponderance of these organelles localized in the apical cytoplasm (Fig. 2A). In the Enal and Los groups, mitochondria were distributed within the basal infoldings, although they were also present in the apical cytoplasm of the cells (Fig. 2B and C). Apical mitochondria displayed round contours, whereas basal mitochondria showed elongated profiles (Fig. 2A, B, C, and D). In the Old group, 90% of tubular epithelial cells showed osmiophilic bodies enclosed within the basal infoldings of proximal tubular epithelial cells, as opposed to 40% ($P<0.05$) in the Enal and Los groups. In the Young group, osmiophilic bodies were virtually absent from tubular epithelial cells. In addition, in the Old group the number of osmiophilic bodies per cell was significantly higher than in the Enal, Los, and Young groups (Table 4).

DISCUSSION

Several health-related effects of RAS inhibitors seem to be independent of their blood pressure lowering action (18, 19). To further advance in the knowledge of how RAS inhibition can affect cell physiology, we investigated the effect of two drugs that inhibit RAS by different mechanisms, i.e., enalapril and losartan, on mitochondrial function and structure. The present results show that long-term inhibition of the RAS can attenuate several age-associated alterations in mitochondrial function and structure.

Enalapril and losartan prevented the age-associated increase in systolic blood pressure, thus evidencing the effectiveness of the treatments used. In addition, and in agreement with other reports (4, 6), both treatments prevented the age-associated decline of kidney function, as evaluated by plasma creatinine and urinary albumin excretion.

The enalapril and losartan treatments preserved kidney mitochondrial function from the effects of aging. This protection was revealed by the ability of both drugs to prevent the age-associated decline in the capacity for energy production and to attenuate the age-associated increase in mitochondrial oxidant production. Mitochondrial respiration is a major subcellular source of $O_2^{\bullet-}$ and H_2O_2 (20), two species that promote oxidative damage to macromolecules. The mitochondrial theory of aging (21) proposes that oxidant-induced mitochondrial degeneration leads to a decrease in their capacity to respond to an energy demand, conducting to a progressive decline of cell function. In this context, enalapril and losartan could protect mitochondria from the effects of aging by attenuating and/or reversing oxidant-induced damage to mitochondrial components, as suggested by the observed lower production of H_2O_2 and the maintenance of an adequate ADP/O ratio in old rats treated with the RAS inhibitors.

Nitric oxide (NO) has been proposed to modulate mitochondrial function (20, 22). Enalapril and losartan can increase endothelial NO generation (23, 24). In this study, we show that both the enalapril and losartan treatments prevented the decrease in NOS activity observed in mitochondria from Old rats. When evaluated in whole kidney, NOS activity was not modified by rat age and/or enalapril and losartan treatments (25). On account of the present experiments, the activity of NOS in mitochondria cannot be univocally ascribed to the recently described mtNOS (26). However, regardless of the site of NO production, an increased activity of NOS is expected to lead to an increased steady-state level of cellular NO, affecting mitochondria, as well as other organelles.

Current evidence points to the concept of UCP2 acting as an oxidant stress compensating mechanism and a role for UCP2 in cellular pathophysiological processes that involve ROS has been suggested (17). Several agents that upregulate NOS also increase UCP expression, possibly to prevent excessive $O_2^{\bullet-}$ production (27). Consequently, the observed modulation of mtNOS activity and UCP2 protein level suggests that the protective action that enalapril and losartan exert on mitochondrial function and structure may rely on a mechanism that involves both NO and UCP2.

Essentially, NO can mediate cell-protective or cell-damaging reactions depending on the relative levels of O_2, NO, $O_2^{\bullet-}$, H_2O_2, and other oxidants (28). Hence, based on the observed preservation of kidney mitochondrial structure and function it can be assumed that in

enalapril- or losartan-treated animals the higher levels of NO, as well as the lower levels of H_2O_2 and $O_2^{\bullet-}$, as compared with untreated Old animals, led to the metabolization of NO through nondamaging routes (28).

Furthermore, the present data may be interpreted considering the role that mitochondria have as active participants in cell signal transduction pathways, particularly with regard to the modulatory role of NO on H_2O_2 production and O_2 metabolism (27). The modulation of mitochondrial respiratory rates and mitochondrial signaling by NO may impact on cell proliferation, growth, and apoptosis, thereby contributing to the maintenance of tissue structure and function. In Old untreated animals, loss of control of NO over respiration and H_2O_2 production may have led to structural and functional changes in the kidney.

Concerning the influence of NO in the modulation of H_2O_2 production, it was reported (29, 30) that NO can increase mitochondrial H_2O_2 generation. This discrepancy with the present data, showing an increase in mtNOS activity in concurrence with a decrease in H_2O_2 production, may stem from the fact that in the studies cited above stimulators of NOS were added to isolated mitochondria, a system where the regulation of UCP2 expression is not possible. Consequently, in vitro the inhibition of respiration by NO brings about an increase of ROS (H_2O_2) production that cannot be compensated by UCP2 upregulation and the consequent attenuation of $O_2^{\bullet-}$ and H_2O_2 formation. In the current study, the in vivo enalapril and losartan treatments increase mtNOS activity in a setting where mitochondrial-nuclear cross-talk is possible and by eliciting UCP2 upregulation can lower H_2O_2 production, possibly leading to a better preservation of mitochondrial function and structure.

We also observed that 8 mo enalapril or losartan treatments did not modify glutathione peroxidase, glutathione reductase, CuZn-SOD, and catalase activities in the kidney. In previous work, we have shown that 11 wk enalapril treatment was associated to an increase in the activities of glutathione peroxidase, glutathione reductase, and CuZn-SOD in the kidney (31, 32). It can be assumed that an 11 wk treatment elicits a response that involves a transient increase of antioxidant enzyme activities, whereas after 8 mo of treatment there is a recomposition of antioxidant defenses. However, in mitochondria, Mn-SOD activity showed an age-related increase that was attenuated by enalapril and losartan treatments, suggesting that in the Old group Mn-SOD activity may have been upregulated to counteract an augmentation in $O_2^{\bullet-}$ generation, as indicated by the observed increase in H_2O_2 production.

The role of glutathione as an antioxidant depends not only on the glutathione pool size but also on its reduction/oxidation status (33). Kidney GSH/GSSG ratios showed that in the Old group glutathione was relatively more oxidized than in the Young group and that both the enalapril and losartan treatments attenuated the age-related oxidation of kidney glutathione. In addition, losartan enhanced total glutathione content. The administration of antioxidants has been shown to protect against several age-associated mitochondrial changes, including oxidant damage to mitochondrial DNA, GSH oxidation, increased peroxide generation, and the impairment of both mitochondrial function and morphology (34, 35). Taken together, these results suggest that exogenously administered antioxidants can either make their way to mitochondria or improve the tissues/cells antioxidant status, thus protecting these organelles from oxidant damage. In the same way as exogenous antioxidants can protect mitochondria

from the effects of aging, the enalapril- and losartan-mediated enhancement of glutathione content and redox status could protect these organelles from oxidants upon aging.

Ultrastructural analysis of kidney tubular epithelial cells showed that both enalapril and losartan treatments can mitigate the age-related decrease in the number of mitochondria, as well as the alterations in mitochondrial structure and distribution, observed in Old rats. Enalapril and losartan treatments also attenuate the age-associated accumulation of osmiophilic bodies in proximal tubular epithelial cells of the kidney. Enhanced osmiophilia probably indicates an increase in the storage of lipids, which is an early event in the process of mitochondrial degeneration. In addition, enalapril and losartan treatments prevented the age-related effacement of proximal tubular cell microvilli. The maintenance of the architecture of a cell depends, at least in part, on an adequate provision of energy. In consequence, the preservation of microvilli by enalapril and losartan treatments might be related to the ability of these drugs to prevent the age-associated decline in the capacity of mitochondria to generate ATP.

The current findings, showing that enalapril or losartan can protect against both age-related mitochondrial dysfunction (decreased capacity for energy production and increased H_2O_2 generation) and mitochondrial ultrastructural changes in the kidney, afford an alternative mechanism to explain the health-related effects of RAS inhibitors. The fact that these two compounds, which act at different levels in the RAS, produce a similar pattern of changes, is indicative of a physiological relationship between the RAS and age-associated cell deterioration. Further experiments will define if the observed association between RAS and mitochondria is related to, or occurs independently of, the modulation of the blood pressure.

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Table 1

Biological outcome of 22-month-old rats treated with Enal or Los during 8 months, and 4-month-old untreated rats

Parameter	Old	Enal	Los	Young
Body weight (g)	669 ± 14	629 ± 7	662 ± 10	370 ± 7#
Kidney weight (g)	1.53 ± 0.08	1.59 ± 0.10	1.55 ± 0.09	1.54 ± 0.12
Plasma creatinine (mg%)	0.65 ± 0.05	0.52 ± 0.06*‡	0.55 ± 0.04*‡	0.41 ± 0.02*
UAE (mg/day)	261 ± 42	108 ± 39*‡	62.1 ± 14.9*‡	6.20 ± 0.81#
SBP (mmHg)	141 ± 2	110 ± 1*	111 ± 1*	110 ± 2*

Values are means ± SE of 8 animals. UAE, urinary albumin excretion; SBP, systolic blood pressure. #P < 0.01 vs. Old, Enal, and Los; * P < 0.05 vs. Old; ‡P < 0.05 vs. Young.

Table 2

Respiratory control, ADP/O, H_2O_2 production, NOS activity, UCP2 content, and Mn-SOD activity in kidney mitochondria from 22-month-old rats treated with Enal or Los during 8 months and 4-month-old untreated rats

	Old	Enal	Los	Young
State 3/state 4	3.04 ± 0.68	6.13 ± 1.25*	5.37 ± 1.08*	5.62 ± 0.64*
ADP/O	1.64 ± 0.15	2.50 ± 0.31*	3.14 ± 0.26*	2.83 ± 0.28*
H_2O_2 (nmol/min/mg protein)	10.1 ± 1.0	7.18 ± 1.61*	7.10 ± 1.32*	3.39 ± 0.68$^{\#}$
NOS (nmol NO/min/mg protein)	0.72 ± 0.06	0.98 ± 0.05*	1.18 ± 0.07*	0.98 ± 0.04*
UCP2 (arbitraty units)	0.38 ± 0.01	1.25±0.10*	1.77±0.51*	1.63±0.20*
Mn-SOD (units/mg protein)	71.0 ± 0.1	47.2 ± 0.1*	49.0 ± 0.22*	41.8 ± 0.18*

Values are means ± SE of 8 animals. State 3/state 4, respiratory control. *$P < 0.05$ vs. Old; $^{\#}P < 0.01$ vs. Old, Enal, and Los.

Table 3

Total glutathione content and glutathione redox status in kidneys from 22-month-old rats treated with Enal or Los during 8 months and 4-month-old untreated rats

	Old	Enal	Los	Young
GSH+GSSG	0.81 ± 0.11	0.85 ± 0.15	1.25 ± 0.12†	0.85 ± 0.02
(nmol GSH equivalents/g wet tissue)				
GSH/GSSG	3.1 ± 1.4	7.2 ± 2.2*‡	9.6 ± 3.9*‡	15.3 ± 1.6$^{\dagger\#}$
(nmol GSH/g wet tissue)/(nmol GSSG/g wet tissue)				

Values are means ± SE of 8 animals. GSH+GSSG, total glutathione content; GSH/GSSG, glutathione redox status. $^{\dagger}P < 0.05$ vs. Old, Enal, and Young; *$P < 0.05$ vs. Old; $^{\ddagger}P < 0.05$ vs. Young; $^{\#}P < 0.01$ vs. Old.

Table 4

Ultrastructural analysis of proximal tubular epithelial cells in the kidneys from 22-month-old rats treated with Enal or Los during 8 months and 4-month-old untreated rats

Morphological Trait	Old	Enal	Los	Young
No microvilli (%cells)	36 ± 2	10 ± 1*‡	7 ± 1*‡	0#
Altered microvilli (%cells)	43 ± 3	21 ± 1*‡	14 ± 1*‡	0#
Preserved microvilli (%cells)	21 ± 1	69 ± 3*‡	79 ± 3*‡	100#
Osmiophilic bodies/cell	12 ± 1	4.1 ± 0.8*	6.6 ± 0.9*	5.7 ± 1.4*
Mitochondria/cell	59 ± 10	180 ± 15*	126 ± 16*	194 ± 11*
Cristae/μm mitochondrial contour	13 ± 1	14 ± 2	19 ± 1	16 ± 2
Mitochondria with poorly defined cristae/cell (%)	45 ± 5	17 ± 3*‡	11 ± 2*‡	0#

Morphometric analysis was carried out on 6 micrographs from different sections of a tissue sample. Values are means ± SE of samples from 8 animals. *$P < 0.05$ vs. Old; ‡$P < 0.05$ vs. Young; #$P < 0.01$ vs. Old.

Fig. 1

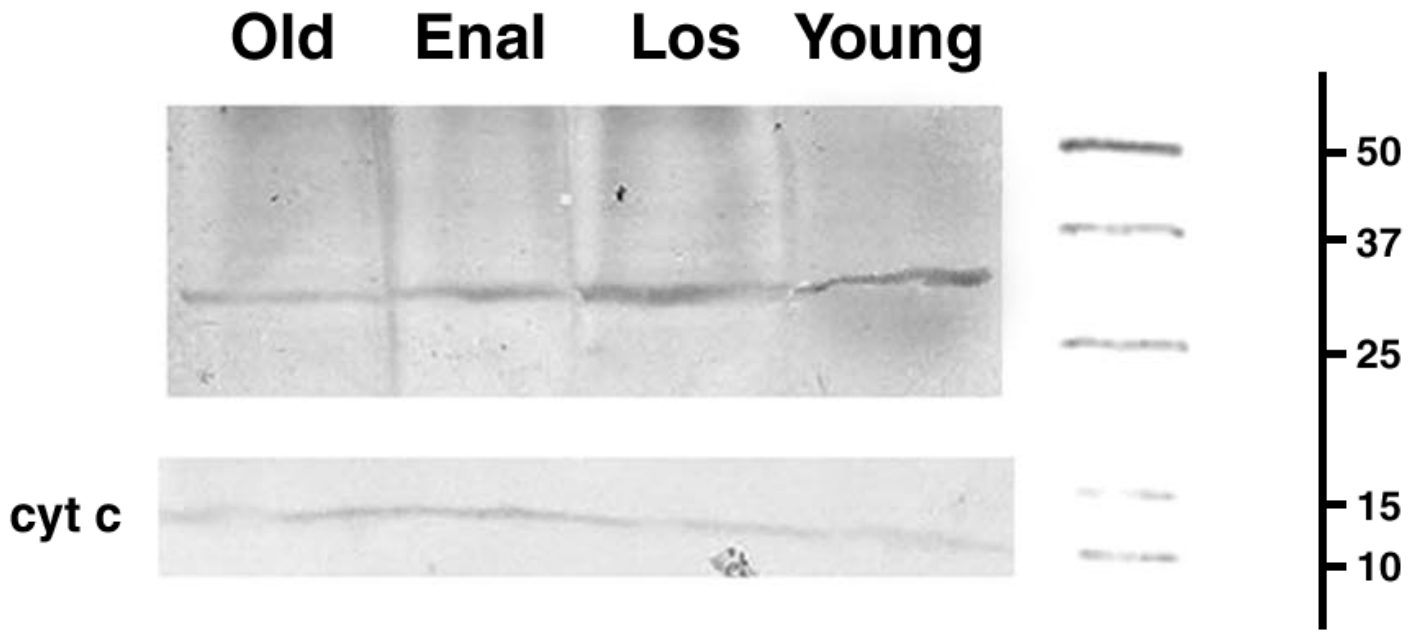

Figure 1. Western blot analysis of UCP2 in kidney mitochondria from 22-month-old rats treated with enalapril or losartan for 8 months and from untreated 4-month-old rats. Mitochondrial proteins (200 μg) were loaded on SDS-12.5% PAGE and revealed with UCP2 antibodies. UCP2 migrated at 32 kDa. Mitochondrial proteins from rat brown fat were used as positive controls (not shown).

Fig. 2

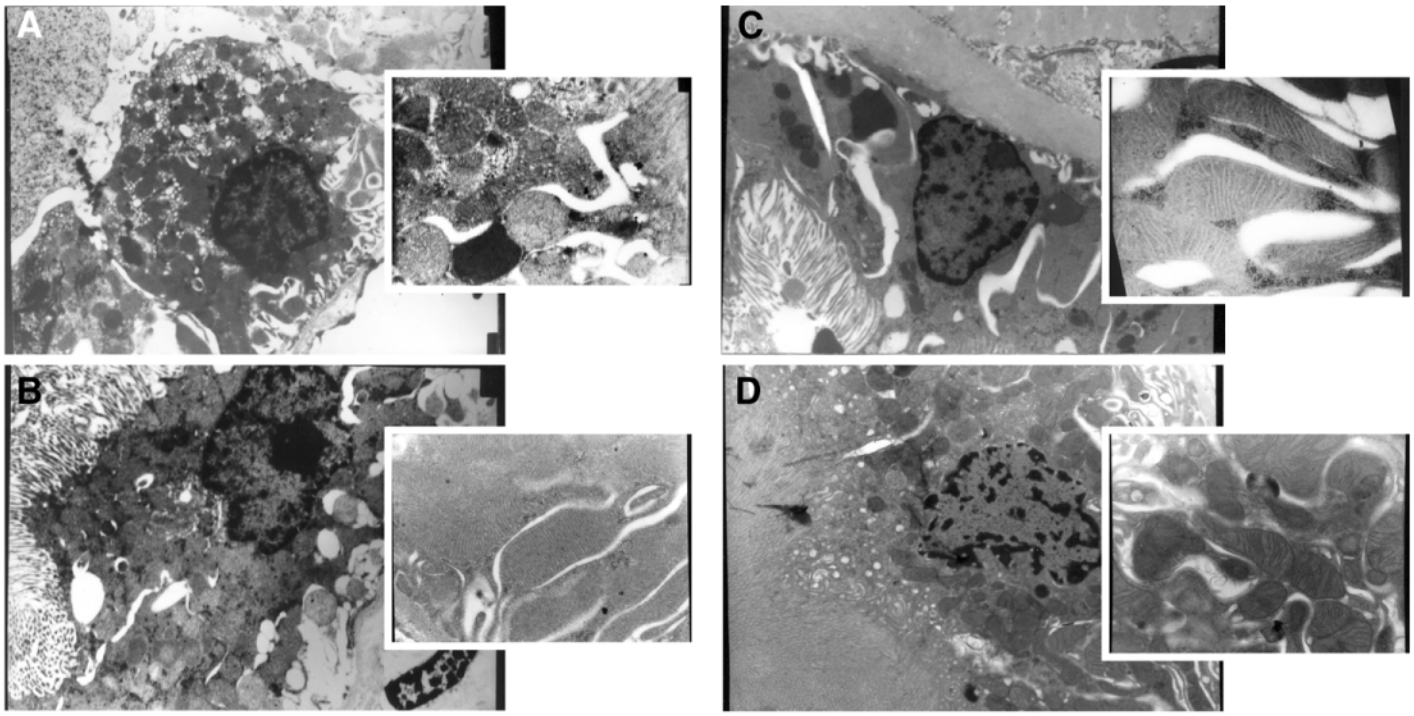

Figure 2. Electron micrographs of proximal tubular epithelial cells in kidneys from 22-month-old rats treated with enalapril or losartan for 8 months and from untreated 4-month-old controls. A) Old group: shows effacement of microvilli, round mitochondria mainly localized in the apical cytoplasm. Inset) Mitochondrial cristae are poorly defined. **B)** Enal group: microvilli are well preserved, elongated mitochondria located within the basal infoldings and round mitochondria in the apical cytoplasm. Inset) Mitochondrial cristae are well defined. **C)** Los group: shows good preservation of microvilli, elongated mitochondria within the basal infoldings, a few round mitochondria in the apical cytoplasm and several osmiophilic bodies. Inset) Mitochondrial cristae are clearly defined. **D)** Young group: showing abundant microvilli, numerous elongated mitochondria within the basal infoldings, a few round mitochondria in the apical cytoplasm. Inset) Clear definition of mitochondrial cristae. (**A**, **B**, **C**, **D** = x6,000; Insets = x20,000).