

# From Mitochondria to Disease: Role of the Renin-Angiotensin System

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## Key Words

Aging · Oxidative stress · Angiotensin II · Mitochondria · Hypertension · Renin-angiotensin system · Cardiovascular disease · Kidney · Diabetes · Reactive oxygen species

## Abstract

Mitochondria are energy-producing organelles that conduct other key cellular tasks. Thus, mitochondrial damage may impair various aspects of tissue functioning. Mitochondria generate oxygen- and nitrogen-derived oxidants, being themselves major oxidation targets. Dysfunctional mitochondria seem to contribute to the pathophysiology of hypertension, cardiac failure, the metabolic syndrome, obesity, diabetes mellitus, renal disease, atherosclerosis, and aging. Mitochondrial proteins and metabolic intermediates participate in various cellular processes, apart from their well-known roles in energy metabolism. This emphasizes the participation of dysfunctional mitochondria in disease, notwithstanding that most evidences supporting this concept come from animal and cultured-cell studies. Mitochondrial oxidant production is altered by several factors related to vascular pathophysiology. Among these, angiotensin-II stimulates mitochondrial oxidant release leading to energy metabolism depression. By lowering mitochondrial oxidant production, angiotensin-II inhibition enhances energy production and protects mitochondrial structure. This seems to be one of the mechanisms underlying the benefits of angiotensin-II inhibition in hypertension, diabetes, and aging

rodent models. If some of these findings can be reproduced in humans, they would provide a new perspective on the implications that RAS-blockade can offer as a therapeutic strategy. This review intends to present available information pointing to mitochondria as targets for therapeutic Ang-II blockade in human renal and CV disease.

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## Introduction

Oxidative stress (OxStr), i.e. the sustained increase in the levels of oxidizing species, plays a key role in the pathophysiology of renal damage and cardiovascular disease (CVD). Renal lesions associated with hypertension, diabetes and glomerular diseases are among those that most frequently progress to chronic kidney disease (CKD). In addition, excess generation of reactive oxygen species (ROS), aggravated by the accompanying inflammatory syndrome, is mainly responsible for the acceleration of cardiovascular decay in CKD. Regardless of abundant information documenting the roles of OxStr and inflammatory processes in CKD and CVD, less is known on the involvement of mitochondria, i.e. the main cellular sources of ROS. Most of the evidence supporting a role for mitochondria in disease comes from studies in cultured cells and animal models. Recently, angiotensin-II (Ang-II) was found to stimulate not only cytosolic- but also mitochondrial-ROS generation, indicating that Ang-

II-related tissue damage may involve mitochondrial Ox-Str. This review intends to present available information pointing to mitochondria as targets for therapeutic Ang-II blockade in human renal and CV disease.

### ROS Are Dual Agents

ROS – including superoxide anion radical and hydrogen peroxide ( $H_2O_2$ ) – are continuous subproducts of normal aerobic metabolism that can oxidize nucleic acids, lipids and proteins, leading to the modification and/or loss of their biological functions. Under pathophysiological conditions that exhibit increased levels of ROS, these oxidizing agents contribute to initiate and intensify the injurious events that accompany inflammation, degenerative diseases, hypertension, diabetes and oncogenesis [1, 2]. However, under physiologically low ROS concentrations, these mediators constitute inter- and intracellular signals that, by oxidizing redox-sensitive protein phosphatases or kinases which are activated or inactivated in the process, can modify the phosphorylation status of transcription factors or receptors. This results in activity changes that are vital to maintain proper cellular function. Thus, depending on their cellular levels, ROS can act as either deleterious or crucial biological agents [3, 4].

In this regard, the identification of oxidized molecules in the kidney, and the protective action provided by antioxidants in animal models for human conditions, indicate a role for ROS in glomerular diseases [5].

### The Mitochondrion: A Multi-Task Organelle

Mitochondria are energy-producing organelles that also conduct other key cellular tasks, including the regulation of cytosolic calcium levels [6] and tissue oxygen gradients [7],  $H_2O_2$  signaling [8], and the modulation of apoptosis [9]. Importantly, mitochondria have emerged as organelles that receive, integrate and transmit signals, thus playing a critical role in cellular responses to a variety of stimuli [10]. Hence, it is apparent that mitochondrial damage may lead to the impairment of various aspects of tissue functioning.

#### *Mitochondria and ROS*

Mitochondria utilize more than 90% of cellular oxygen and, while most of it is transformed to water at complex IV of the mitochondrial electron transport chain

(mtETC), approximately 1–2% of the oxygen consumed [11] receive electrons directly from complexes I and III [12], to form superoxide. Other sources of mitochondrial ROS (mtROS) include electrons derived from complex II substrates that can be reverse-transported towards complex I and oxygen [13], matrix enzymes [14, 15], outer membrane monoaminoxidases (MAO) [16], and mitochondrial nitric oxide synthase (mtNOS) uncoupling [17]. Superoxide is released to both the mitochondrial matrix and the space between the inner and outer mitochondrial membrane [18] where it can be converted to  $H_2O_2$  by Mn superoxide dismutase (Mn-SOD) and CuZn-SOD, respectively [19, 20].  $H_2O_2$  can be detoxified to water by mitochondrial glutathione peroxidase, or to water and oxygen by catalase, which is present in cardiac mitochondria. These enzymes belong to a complex multi-leveled mitochondrial defense system, composed of enzymes and non-enzymatic antioxidants, that is involved in ROS detoxification.

Additionally, mitochondria generate other oxidants derived from nitric oxide (NO), collectively known as reactive nitrogen species (RNS), that include peroxynitrite anion, which is formed when superoxide and NO react. Thus, mitochondria are relevant cellular sources of ROS and RNS, and consequently, are themselves major oxidation targets. This eventually leads to mitochondrial dysfunction, i.e. a defective capacity to generate ATP accompanied by increased ROS generation [21].

### Mitochondrial Dysfunction and Disease

Mitochondrial dysfunction is detrimental to cells as a consequence of both the reduction of bioenergetic capacity and the derangement of mtROS-mediated signals. The contribution of dysfunctional mitochondria to disease is emphasized by the recent recognition that, in addition to their well-known roles in energy metabolism, mitochondrial proteins and metabolic intermediates participate in other processes. Thus, succinate dehydrogenase, an enzyme that contributes to mitochondrial oxidative phosphorylation by participating in the citric acid cycle, also seems to modulate mitochondrial  $K^+$  transport by taking part in the formation of an inner membrane multiprotein complex that displays ATP-sensitive  $K^+$ -channel activity [22]. Also, cytochrome c, in addition to its key function as a mitochondrial electron carrier, participates as a signaling molecule in apoptosis [23]. Finally, the citric acid cycle intermediate succinate also acts as a signaling molecule through its binding to G-protein coupled receptors [24].

In this setting, malfunctioning mitochondria are involved in pathological conditions, such as hypertension [25–27], cardiac failure, the metabolic syndrome and obesity [28], diabetes mellitus [29, 30], renal disease [31], atherosclerosis [32], as well as in aging [33].

#### *Mitochondria and Aging*

Oxidative damage to mitochondria, and the associated loss of mitochondrial function, not only contributes to disease, but was also postulated to be instrumental in the aging process. Accordingly, the mitochondrial free radical (FR) theory of aging [34] proposes that in aging cells accumulation of mitochondrial DNA (mtDNA) damage inflicted by FR generated by mitochondria, or at other cell sites, conducts to the progressive impairments of energy production, mitochondrial protein synthesis, and mitochondrial regeneration. In this way degenerative processes are initiated, eventually leading to the senescence-associated loss of functional capacity.

In support of the mitochondrial FR theory of aging, a reduction of mitochondrial number in certain organs is frequently associated with the aging process [35, 36]. Also, tissues obtained from aged animals, display changes in mitochondrial structure [33] associated with increased superoxide and H<sub>2</sub>O<sub>2</sub> generation, and decreased energy production [37, 38]. Notably, as the efficiency of mtATP production declines due to damage by ROS, electron leakage from the mtETC increases, further augmenting FR generation, and giving rise to a self-sustained vicious cycle [39]. In this context, it was recently reported that mitochondria-targeted overexpression of catalase extends median and maximum mouse life-span [40]. This life extension coincided with decreases in mtDNA oxidant damage, mitochondrial H<sub>2</sub>O<sub>2</sub> production, aconitase inactivation, and mtDNA deletion accumulation [40], supporting a link between mtROS generation, mitochondrial damage, and aging.

From another perspective, age-associated diseases, including hypertension, diabetes, cancer, and cardiovascular pathologies, are often accompanied by alterations in lipid metabolism, which is largely modulated by mitochondrial activity.

#### *Mitochondria and Apoptosis*

Present knowledge points to mitochondria as critical players in cell survival, as a consequence of their classical involvement in energy production and their crucial participation in apoptosis. Cell death results from either necrosis, when mtATP fails to be maintained at adequate levels, or apoptosis, when mtATP levels and mitochon-

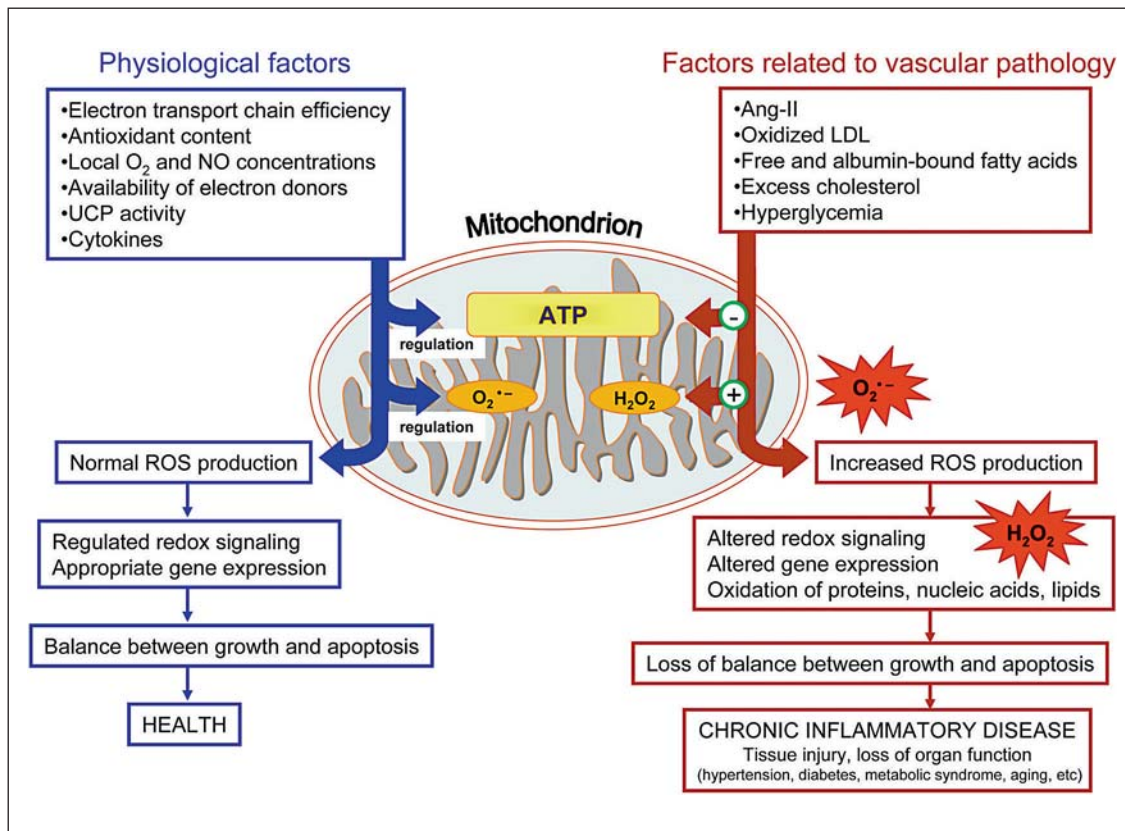
drial membrane potential (mtMP) are sustained, and mitochondria succeed in releasing pro-apoptotic proteins that act as molecular signals that initiate caspase activation [41].

In the kidney, mesangial cell (MC) apoptosis is seriously involved in glomerular remodeling after injury. MC behavior – regarding cell adhesion, replication, and extracellular matrix (ECM) production – is affected by changes in ECM composition associated with disease [42, 43]. Other examples of ECM influence on glomerular structure dynamics come from data showing that cell attachment to the ECM is required to abolish apoptosis [42], and normal ECM composition serves as a signal that prevents MC from undergoing apoptosis [44, 45]. In this context, accumulating evidences show that mitochondria may act as sensors for changes in ECM composition [46, 47].

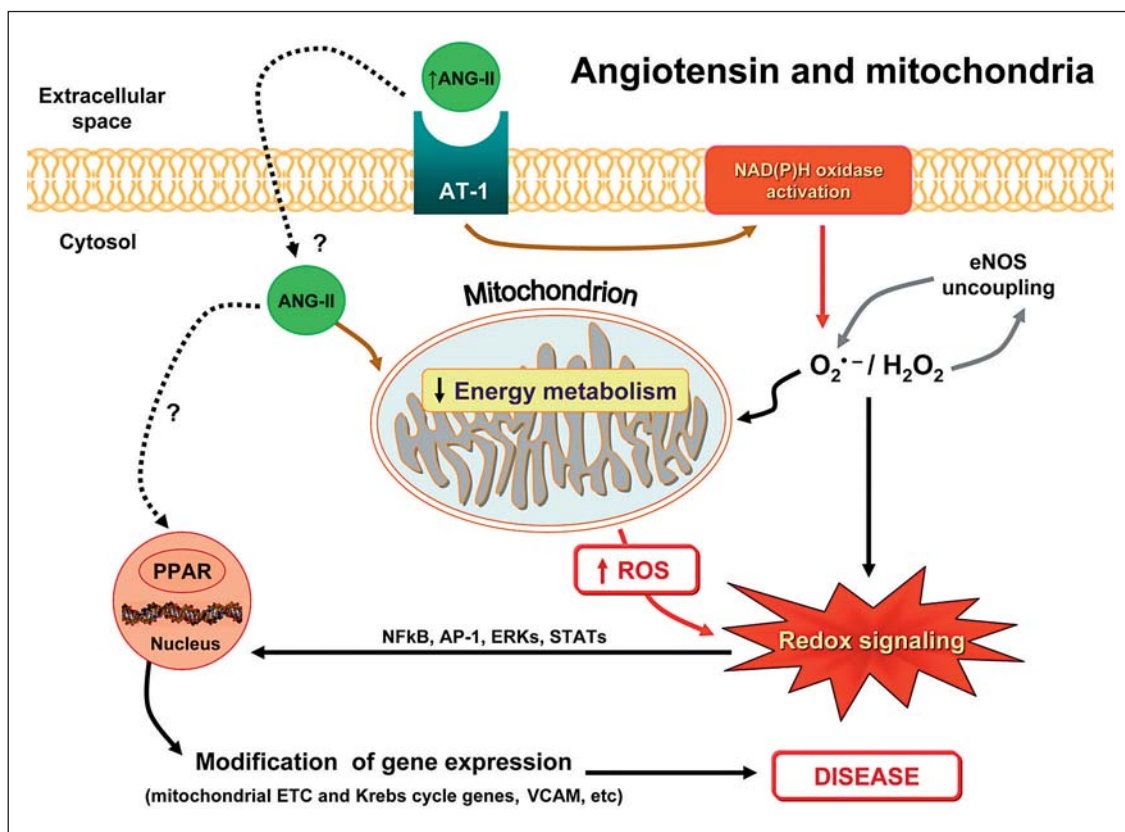
Depending on cell type, receptor subtype, and on interactions with other growth factors, Ang-II can induce differentiation, hypertrophy, proliferation, or apoptosis of vascular, cardiac, and renal cells. These Ang-II cell growth-related effects seem to contribute to the pathophysiology of atherosclerosis, vascular and cardiac remodeling, and progression to CKD [48]. AT<sub>2</sub>-receptor activation was suggested to be responsible for the apoptogenic actions of Ang-II; however, this point remains conflicting because AT<sub>1</sub>-receptor blockade also attenuates apoptosis in certain models [49, 50]. Accordingly, work from our laboratory showed that renin-angiotensin system (RAS)-inhibition prevented the increase in myocardiocyte apoptosis that was observed in untreated aging mice [36], and this was accompanied by attenuation of myocardioclerosis and decreased intracardiac artery wall-to-lumen ratios [51].

#### **mtROS Regulators**

Various factors are known to regulate mtROS production, including mtETC efficiency, mitochondrial antioxidant contents [52], local oxygen [53, 54] and NO concentrations [55–57], the availability of metabolic electron donors [58], uncoupling protein (UCP) activity [59], and cytokines [60] (fig. 1). Additionally, several factors related to vascular pathophysiology can alter mtROS production, such as oxidized LDL [61, 62], free cholesterol [63], free and albumin-bound fatty acids [64–66], hyperglycemia [67], and, pertinent to this review, Ang-II [68, 69] (fig. 1). Interestingly, different therapeutic strategies aimed at modulating these factors – including thiazoli-



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dinediones [70], statins [71], insulin [72], and Ang-II blockers – can attenuate mtROS production. Find below a revision concerning Ang-II inhibitor effects on mitochondrial functions ('Mitochondria and Ang-II inhibitors').

### Mitochondria and Ang-II

Ang-II can promote OxStr by stimulating the generation of both NO [73] and NAD(P)H oxidase-derived superoxide [74], thereby enhancing peroxynitrite formation. Also, Ang-II can induce endothelial nitric oxide synthase (eNOS) to switch from NO to superoxide production [75]. In addition, a recent report indicates that Ang-II stimulates mtROS production in vascular smooth muscle cells and in rat aorta in vivo [69]. In bovine aortic endothelial cells, Ang-II prompts mitochondrial superoxide production as a result of vascular NAD(P)H oxidase activation [76] (fig. 2). A link between Ang-II-related ROS/RNS production and mitochondrial function was suggested by a report showing that antioxidants inhibit the regulatory effects of Ang-II on the AP-1 signaling pathway [77]. Since AP-1, whose activity responds to oxidation/reduction, regulates cytochrome c expression [78], it was suggested that Ang-II may facilitate changes in mitochondrial cytochrome c content [79].

Of note, in endothelial cells, Ang-II enhances mtROS generation, thus activating redox-sensitive NF-kappaB, which is followed by stimulation of vascular cell adhesion molecule-1 expression, a key molecule in atherosclerotic lesion initiation [68]. In mice, acute and chronic Ang-II infusion led to decreased cardiac mtETC and Krebs cycle gene expression [80], supporting previous observations indicating a role for Ang-II in the depression of mitochondrial energy metabolism [81–83]. Moreover, recent

findings show that Ang-II lowers mtMP as a result of stimulation of mtROS production [69, 84].

Also, there are evidences indicating a direct interaction between Ang-II and mitochondrial components. In studies using <sup>125</sup>I-labeled Ang-II, Ang-II was detected in heart, brain and smooth muscle cell mitochondria and nuclei [85, 86]. In rat adrenal zona glomerulosa, renin, angiotensinogen and angiotensin-converting enzyme (ACE) were detected within intramitochondrial dense bodies [87]. Ang-II immunoreactivity was also observed in rat cerebellar cortex mitochondria [88]. Hence, it is possible to speculate that Ang-II may have direct actions on mitochondria, independent of AT1-receptor signalling (fig. 2).

Under normal physiological conditions, Ang-II-mediated ROS- and NO-derived oxidant production, and the resulting stimulation of redox-sensitive signaling pathways, are closely regulated [89]. However, under conditions associated with RAS overactivation, including hypertension, diabetes [90, 91] and aging [92–95], dysregulation of Ang-II-dependent ROS generation may become a significant contributor to cell oxidation and tissue damage (fig. 2). In this context, studies from our laboratory showed that in rodent models of hypertension, diabetes and aging, Ang-II blockade not only attenuates oxidant production, but also improves mitochondrial function [26, 27, 30, 33].

### Mitochondria and Ang-II Inhibitors

The renal and cardiac benefits of ACE-inhibitors and Ang-II type 1 receptor (AT1) blockers in hypertension, cardiovascular disease and diabetes patients [96–98] seem to be – at least partly – independent from their blood pressure lowering actions [96, 98–100], suggesting that these drugs can execute direct tissue effects that do not result from their hemodynamic actions. In this regard, RAS-inhibitors were proposed to act as a magic bullet against OxStr [101].

Considering that (a) Ang-II inhibitors are indicated for the treatment of hypertension and cardiac failure, (b) international guide recommendations exist for the use of Ang-II inhibitors as first-line drug therapy for kidney protection in diabetic patients, even in the absence of hypertension [102], (c) the cellular mechanisms responsible for Ang-II inhibitors protective effects are poorly understood, and (d) malfunctioning mitochondria seem to be involved in the pathogenesis of a variety of disease conditions, we set forth to investigate Ang-II inhibitors effects on mitochondria. The first study showed that ACE-inhi-

**Fig. 1.** Factors that influence mitochondrial ROS generation.

**Fig. 2.** mtROS are involved in redox cell signaling, but are also potential mediators of oxidative stress. Ang-II can promote oxidative stress by activating NAD(P)H oxidase-derived superoxide production, and/or by inducing eNOS to switch from NO to superoxide production. Also, Ang II-mediated enhancement of mtROS generation leads to the depression of mitochondrial energy metabolism, and a direct interaction between Ang-II and mitochondrial components by-passes activation of NAD(P)H oxidase. Thus, Ang-II can lead to changes in gene expression that may result in disease. Ang-II inhibition is expected to lower mtROS release, increasing the efficiency of the respiratory chain and protecting mitochondrial structure.

bition (enalapril) in aging mice prevented the lowering of mitochondrial number [35], and attenuated age-related mitochondrial structure changes in myocytes and hepatocytes [unpubl. results]. These protective effects of enalapril were associated with a significant increase in animal survival, suggesting that natural aging mechanisms had been altered in enalapril-treated animals. The latter action on mice survival is in agreement with recent data in aging rats [103].

Later, we found that long-term RAS inhibition during aging, with enalapril or AT1-blocker (losartan), improved kidney mitochondria mtATP production, lowered H<sub>2</sub>O<sub>2</sub> generation, and enhanced mtNOS activity and UCP-2 content, when compared with mitochondria isolated from untreated old rats [33]. In the same study, a general improvement in mitochondrial number and structure was observed, indicating that RAS inhibition, regardless of how it is implemented, protects mitochondrial components and function from certain effects of aging.

Evidence pointing to mitochondrial dysfunction as a contributing factor to the pathophysiology of hypertension [104] led us to address the possibility that, in addition to the results obtained in aging rats, RAS inhibition might protect mitochondria from hypertension-related damage.

In spontaneously hypertensive rat (SHR) kidneys, chronic Ang-II blockade prevented the decreases in mtMP, NOS, UCP-2 content, Mn-SOD and cytochrome oxidase activities, and the increase in H<sub>2</sub>O<sub>2</sub> production observed in untreated SHR [26, 27]. The mitochondrial protective action displayed by losartan treatment was absent in amlodipine-treated SHR. Furthermore, in untreated and in amlodipine-treated SHR, mitochondrial impairment was accompanied by renal damage. Taken together, the above findings indicate that AT1-blockade attenuates mitochondrial dysfunction in SHR, and this may underlie the beneficial actions of AT1-blockade on kidney structure and function [26].

Recent data indicating that the kidneys are main targets of mitochondrial impairment at the onset of and throughout streptozotocin-induced diabetes – a model of type-1 diabetes – and that insulin treatment is unable to restore normal mitochondrial function [105], led us to investigate whether AT1-blockade might protect mitochondria against the effects of diabetes. In streptozotocin-diabetic rats, losartan protected kidney mitochondria against changes in mtMP, H<sub>2</sub>O<sub>2</sub> production and pyruvate content, without normalizing plasma glucose. Conversely, amlodipine was equally potent as losartan as an hypertensive agent, but showed no beneficial effects on kidney mitochondrial changes [30]. It can be proposed

that the contrasting effects displayed by losartan and amlodipine on kidney mitochondrial function may be, at least partly, a consequence of losartan-mediated blockade of Ang-II actions (see above ‘Mitochondria and Ang-II’). In agreement with our findings in the kidney, work by other groups support the notion that Ang-II inhibition improves cardiac mitochondria energy production [106–108]. In this line, a recent study showed that the expression of genes related to energy production were up-regulated in captopril-treated diabetic rats [109].

Concerning the potential factor(s) that may mediate the effects of Ang-II inhibitors on mitochondrial function, a study showed that in normotensive enalapril-treated rats, kidney mitochondrial electron transfer activities were lower, and UCP-2 content significantly higher, than in untreated controls. These changes were accompanied by a higher production/bioavailability of kidney NO, and were prevented by co-treatment with L-NAME (a NOS inhibitor). L-NAME abolished mtNOS activity, but failed to inhibit extra-mitochondrial kidney NOS, underscoring the relevance of mitochondrial NO in mediating those effects of enalapril that were suppressed by L-NAME co-treatment [110].

## Summary and Conclusion

Mitochondria are main sources of both of cellular energy and ROS. mtROS are involved in cell signaling, but are also potential mediators of OxStr. Available evidences suggest that Ang-II enhances mtROS generation leading to the depression of mitochondrial energy metabolism (fig. 2). Consequently, Ang-II inhibition lowers mtROS release, increasing the efficiency of mtETC and protecting mitochondrial structure. This seems to be one of the mechanisms underlying the beneficial effects of Ang-II inhibition in rodent models of hypertension, diabetes, and normal aging. If some of these findings can be reproduced in humans, they would provide a new perspective on the implications that RAS blockade can offer as a therapeutic strategy.

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