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The interaction between the renin-angiotensin system and peroxisome proliferator activated receptors: a hypothesis including the participation of mitochondria in aging

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1. ABSTRACT

The objective of improving health is intimately associated with preventing and delaying age-related diseases. Nutritional and pharmacological approaches aimed at retarding aging are uncovering mechanisms, whose definitive roles in cell and tissue physiology need to be defined. In this article we hypothesize that peroxisome proliferator activated receptor (PPAR)-modulation is a pivotal process that underlies the association between mitochondria and the renin-angiotensin system (RAS) in aging. This hypothesis is based on several lines of evidence suggesting that: a) mitochondrial function and oxidant production are active participants in the aging process; b) PPARs, by regulating mitochondrial function and uncoupling proteins (UCP), seem to play a major role in the age-retarding effects of caloric restriction; c) RAS inhibition delays the deleterious effects of aging and also upregulates PPARs; and d) a number of physiological and molecular events that occur in experimental caloric restriction, and experimental and clinical RAS inhibition, involve changes in mitochondrial functions.

2. INTRODUCTION

The inhibition of the renin-angiotensin system (RAS), a broadly used therapeutic strategy to counteract hypertension, provides ancillary health benefits apparently unrelated to the lowering of blood pressure (1-4). The mechanisms responsible for those beneficial effects are not completely characterized. We have extensively investigated the effects of RAS inhibition on mitochondrial function, mitochondrial oxidant production and oxidative damage to mitochondria (5-8). Based on both our findings and published evidence, in this review we will discuss the hypothesis of a pivotal role of peroxisome proliferator activated receptor (PPAR)-modulation in the preservation of mitochondrial function to explain the beneficial health effects of RAS inhibition. Considering the relevant participation of mitochondria (9-12) and the RAS (13-15) in both, aging and aging-associated diseases, we will focus most of our discussion on aging.

3. OXIDANTS AND MITOCHONDRIA IN THE AGING PROCESS

3.1. Oxidant species, antioxidants, and oxidative stress

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generic terms that include byproducts of oxygen metabolism continuously generated in aerobic organisms (10, 16). ROS and RNS can react with other biologically relevant molecules (*e.g.* lipids, proteins, and nucleic acids) leading to cell and tissue damage (17). Aerobic organisms are endowed with an assorted group of

molecules, referred to as antioxidants, to control oxidant production and prevent cell and tissue damage (18). Antioxidants essentially include both, low molecular weight compounds able to scavenge or deactivate oxidants, and enzymes that catalyze the decomposition of oxidants. In addition, non-enzyme proteins (uncoupling proteins, thioredoxin, histones, etc.) as well as other biomolecules (plant polyphenols, etc.) can afford antioxidant protection to biological systems by mechanisms that do not involve direct oxidant reduction (18). However, under certain circumstances, control over ROS and RNS levels is lost, resulting in oxidative stress. Oxidative stress is a concept defined by Sies (19) as "an imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage".

3.2. Mitochondria as sources and targets of reactive oxygen species and reactive nitrogen species

Mitochondria are essential for the maintenance of aerobic life not only as sources of energy, but also by regulating Ca^{2+} homeostasis (20), tissue O_2 gradients (21), cell apoptosis (22), and intracellular signaling (23). However, the partial reduction of O_2 by the mitochondrial respiratory chain components results in an undesirable production of oxidants. Thus, mitochondria are a major source of ROS, and are themselves targets of ROS-mediated damage. Mitochondria produce superoxide anion (O_2^-) by univalent reduction of O_2 (10) at two sites of the electron transport chain (24, 25), and hydrogen peroxide (H_2O_2) as the product of the enzymatic reduction of O_2^- by superoxide dismutase. Both, O_2^- and H_2O_2, are ROS that can initiate free radical chain reactions leading to the generation of other oxidants, and potentially to a situation of oxidative stress. Further reduction of H_2O_2 to water is catalyzed by the enzymes catalase and glutathione peroxidase. If the targets of ROS are molecules that can propagate free radical reactions (*e.g.* lipids), damage can extend inside the cells leading to the oxidation of other relevant molecules (proteins, nucleic acids, etc.). Nitric oxide (NO) is an essential signaling molecule that is additionally involved in ROS-mediated oxidations. Nitric oxide is produced by the oxidation of L-arginine to L-citrulline, a reaction catalyzed by several nitric oxide synthase (NOS) isoforms (26). By diffusion-limited reaction with O_2^-, NO yields peroxynitrite ($ONOO^-$), a very strong oxidant. Nitric oxide, peroxynitrite, and other oxidant species derived from NO are termed RNS. The existence of a constitutive mitochondrial activity of NOS (mtNOS) (27, 28) underlies the biological relevance of NO generation in mitochondria.

Current evidence supports a prominent role for ROS and RNS in both, the decline of mitochondrial function and the increase in mitochondrial DNA oxidation that occurs in various tissues upon aging (29-33).

To minimize the production of ROS and RNS, mitochondria drive O_2 reduction to water with very high efficiency (accounting for about 97-98% of the O_2 respired) (10). Additionally, to control the damage inflicted by the partial reduction of the remaining 2-3% of respired O_2, mitochondria are well equipped with a battery of enzymes that metabolize ROS, including manganese superoxide dismutase (Mn-SOD), catalase, and glutathione peroxidase. Other enzymes, such as glutathione reductase and thioredoxin reductase, that catalyze the NADPH-dependent reduction of disulfides, can be also considered part of the mitochondrial antioxidant defenses. Finally, there are other mitochondrial proteins that can limit the production of oxidants, *e.g.* uncoupling proteins (UCP).

Recently, the concept has emerged that mitochondria not only receive signals from elsewhere in the cell, but they also generate signaling molecules (23). For example, it has been demonstrated that H_2O_2 and NO diffuse from the mitochondria into the cytosol modulating redox-sensitive signaling pathways (34, 35).

In summary, mitochondria are not only recognized as the main sources of cellular energy, but also as organelles that by driving ROS and RNS generation play other key roles in the regulation of cell functions and survival.

3.3. Mitochondria and peroxisome proliferator activated receptors

PPARs are nuclear transcription factors that regulate the expression of a number of genes related to lipid metabolism and energy homeostasis (36). PPAR isoforms are members of the nuclear hormone receptor superfamily of transcription factors, and include PPAR-alpha, PPAR-beta, and PPAR-gamma. Upon ligand-binding, PPARs heterodimerize with 9-cis-retinoic acid receptor (RXR), thereby acquiring the ability to recognize and bind peroxisome proliferator-responsive elements (PPRE) in target genes. All three PPAR subtypes bind the same PPRE (37) and can interact with either RXR-alpha, beta, or gamma. Most of the PPAR ligands that have been studied are synthetic, and a limited number are of endogenous origin (38).

PPAR-alpha is expressed highly in liver, heart and skeletal muscle (39). At the mitochondrial level, activation of PPAR-alpha results in an increased expression of many nuclear genes associated with mitochondrial function, including those involved in fatty acid beta-oxidation (38), mitochondrial proton leak (40, 41), and those encoding antioxidant enzymes, *i.e.* Mn-SOD and catalase (42). PPAR-gamma, which is highly expressed in adipocytes, is involved in adipocyte differentiation and controls the expression of lipid storage genes. Another relevant function of PPAR-gamma is the promotion of insulin sensitivity (43). PPAR-delta is involved in the regulation of fatty acid catabolism, metabolic rate and proliferation of mitochondria mainly in skeletal muscles (44).

3.4. Aging, mitochondria, and peroxisome proliferator activated receptors

The participation of mitochondria in the continuous production of ROS and RNS supports the mitochondrial free radical theory of aging as formulated in 1979 by Chance *et. al.* (10) as an extension of the more general free radical theory of aging (9, 12, 45). Concerning aging and mitochondrial ROS generation, it was recently reported that the mitochondria targeted overexpression of catalase, extends the median and maximum lifespan in

mice (46). This life extension concurred with decreases in oxidant damage to DNA, mitochondrial H_2O_2 production, aconitase inactivation, and in the accumulation of mitochondrial DNA deletions (46), suggesting a link between mitochondrial ROS generation, mitochondrial damage, and aging.

In addition, 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. The involvement of PPAR-alpha and PPAR-gamma in the above mentioned diseases is underscored by studies showing the beneficial effects of PPAR-alpha and PPAR-gamma agonist administration (47-51). Aging is associated with a decline in the expression of PPAR-alpha and gamma (52). In addition, in skeletal muscle cells, increased ROS production was shown to downregulate PPAR-alpha mRNA (53). In this setting, a body of evidence support a strong association between PPAR activities and the aging process (54).

Relationships among PPARs, oxidative stress, and aging are supported by studies showing that ligand activated PPAR-alpha can suppress the age-dependent augmentation of both, oxidative stress and oxidant-dependent NF-kappa B activation (55). These results suggested that the prooxidant state displayed by cells of aged animals may be related to the age-related decline in PPAR-alpha expression (34). Furthermore, PPAR-alpha activators upregulate uncoupling protein-2 (UCP-2) expression (41), and treatment with PPAR-gamma agonists increases UCP-2 mRNA levels in adipose and skeletal muscle cells (56, 57).

UCPs are proton gradient-dissipating mitochondrial proteins that seem to act in both, the regulation of fatty acid and glucose oxidation (58, 59), and the control of O_2^- generation by mitochondria (58-60). PPAR-gamma activation increased endothelial NO production in bovine aortic endothelial cells (61). The participation of NO as a downstream effector of PPARs activation has also been observed in animal models. In rat kidney, activation of PPAR-alpha promoted Na^+ excretion by increasing NO generation (62). In hypertensive rats, the cardioprotective effects afforded by PPAR-alpha activation were mediated by an increase in NO production and/or an inhibition of NADPH oxidase activity (63).

The above evidence suggests that PPAR-alpha, through mechanisms involving UCP-2, may lower mitochondrial oxidant production, and, consequently, retard the aging process as well the development of age-associated diseases.

4. THE RENIN-ANGIOTENSIN SYSTEM, MITOCHONDRIA, AND AGING

4.1. The renin-angiotensin system and the cellular generation of ROS and NO

In the classical view, the RAS was exclusively recognized by its circulating actions as a regulator of systemic blood pressure and renal electrolyte balance. After the discovery of RAS gene expression and function in a variety of tissues, a role for RAS as regulator of organ functions (autocrine) was acknowledged. These local tissue effects of RAS are a subject of current research, and appear to be distinct from circulating RAS actions (64). The major components of both, circulating and tissue RAS, include the polypeptides angiotensinogen, angiotensin I (Ang-I) and angiotensin II (Ang-II). Ang-I is the product of enzymatic angiotensinogen cleavage by renin, and Ang-II is the product of Ang-I cleavage by Ang-I-converting enzyme (ACE) (65). Ang-II, the main effector of the RAS, is responsible for vasoconstriction and Na^+ retention (66). Ang-II is also a pro-inflammatory and a pro-fibrotic agent (67, 68).

Abundant evidence supports the notion that both, the increased generation of cellular ROS, and the activation of redox-sensitive signaling cascades are critical events involved in Ang-II actions (69). Many of the functional effects of Ang-II are mediated via the angiotensin-II-receptor 1 (AT1). Ang-II binding to AT1 triggers intracellular O_2^- production by NAD(P)H oxidase activation (70, 71), and also as a result of endothelial NOS (eNOS) uncoupling (72). On account of O_2^- production, cellular NO steady state level may be compromised due to the reaction of NO with O_2^- to generate peroxynitrite. As a final consequence of this series of events, and as it has been observed in a variety of cell types (72, 73), Ang-II promotes the production of both, ROS and RNS, and reduces NO availability. Relevant to endothelial cell NO production, it has been demonstrated that bradykinin, another ACE substrate, binds to BK1 and BK2 plasma membrane receptors, thereby activating endothelial NOS (eNOS) (74).

Under normal physiological conditions, Ang-II-mediated ROS and RNS production, and the resulting stimulation of redox-sensitive signaling pathways, are closely regulated (73). However, under conditions leading to overactivation of the RAS, such as hypertension, diabetes (75, 76) and normal aging (77-80), Ang-II-dependent oxidant generation may become a significant contributor to cell oxidation and tissue damage. In vascular smooth muscle cells from spontaneously hypertensive rats (SHR), Ang-II was shown to enhance the activation of NF-kappa B and AP-1, two transcription factors modulated by the cellular redox status (81). In addition, it was shown that antioxidants and antioxidant enzymes inhibit the regulatory effects of Ang-II on Ras/Raf/ERK and AP-1 signaling pathways (82, 83).

4.2. The renin-angiotensin system and mitochondria

The relationships between RAS and mitochondria started to gain attention when it became evident that, by prompting ROS formation, RAS effectors can activate redox-sensitive transcription factors involved in the modulation of mitochondrial function.

A causative link between RAS activation and alteration of mitochondrial function is supported by the observation that in mice, acute (24 h) and long-term (14 d)

Ang-II infusion leads to decreased cardiac expression of mitochondrial electron transfer chain and Krebs-TCA cycle genes (84). In the short term treatment, Ang-II also promoted the depression of mitochondrial metabolism and Mn-SOD gene, and induced the expression of genes for proteins that protect against oxidative stress, such as thioredoxin, glutaredoxin and transferring receptor 1 (84). These effects are in line with previous observations that indicated the participation of Ang-II in the depression of mitochondrial energy metabolism (85-87). In addition, recent findings show that Ang-II stimulates mitochondrial ROS production associated with the reduction of mitochondrial membrane potential (88).

In addition, there is some evidence supporting a direct interaction between Ang-II and nuclear and mitochondrial components. A number of studies have produced evidence for the existence of nuclear AT1-like AngII receptors in rat liver and spleen cells. In studies using ^{125}I-labeled Ang-II, the presence of Ang-II was shown in nuclei and mitochondria of heart, brain and smooth muscle cells (89, 90). Treatment of isolated rat liver nuclei with Ang-II stimulated the transcription of specific genes (91, 92). In addition AT1 receptor blockers (losartan) inhibit Ang-II binding to nuclear receptors in rat hepatocytes (91, 93). The inhibition of Ang-II binding to nuclear receptors by losartan might suggest this possibility. In rat adrenal cortex, immunocytochemical and biochemical evidence indicates the presence of renin, angiotensinogen and ACE within intramitochondrial dense bodies of the zona glomerulosa (94).

Concerning cell signaling modulation by mitochondria, c-Jun NH_2-terminal kinase (JNK) is both, a downstream target of AT1 dependent signaling (95) and a regulator of AP-1 activity. Since AP-1 regulates cytochrome c expression (96), it was suggested that JNK may facilitate changes in the mitochondrial content of cytochrome c in response to Ang-II (97). Adding to previous evidence that supports the participation of the RAS in mitochondrial function responses, the modulation of heart and liver mitochondrial NOS activity and H_2O_2 production by the ACE inhibitor enalapril has been observed in rats (98, 99). Moreover, a recent study showed that the expression of genes related to fatty acid beta-oxidation, mitochondrial proton-electron coupling, and oxidative phosphorylation were up-regulated in captopril-treated diabetic animals, suggesting that RAS inhibition with ACE inhibitors may protect the myocardium by enhancing energy supply (100).

4.3. The renin-angiotensin system and peroxisome proliferator activated receptors

Ang-II infusion was shown to downregulate PPAR-alpha and PPAR-gamma mRNA and protein in apolipoprotein E-deficient (apoE-KO) mice (101). This downregulation occurred in parallel with the activation of NF-kappaB and NF-kappaB-mediated proinflammatory genes (101). Conversely, activators of PPAR-alpha and PPAR-gamma antagonize the proliferative, inflammatory and oxidant-generating actions of Ang-II both, *in vivo* and *in vitro* (43, 102, 103). In this context, it is possible to hypothesize that RAS inhibition lowers oxidant production not only by blocking Ang-II activation of NADPH-oxidase, but also by regulating the expression of PPARs. In this regard, enalapril was shown to upregulate PPAR-alpha and PPAR-gamma while displaying antiatherogenic and anti-inflammatory effects in mice (104). Two AT1-receptor blockers, irbesartan and telmisartan, were identified as activators of PPAR-gamma (105, 106). Recently, a product of the hepatic metabolism of losartan (EXP3179) was identified as a partial PPAR-gamma agonist, suggesting that some of the AT1-receptor blockers can mediate AT1-receptor independent effects (107).

4.4. The renin-angiotensin system and aging

Based on evidence suggesting the involvement of mitochondria in the aging process, we have investigated how the modulation of the RAS can affect mitochondrial dysfunction associated with aging and aging-associated diseases. In one study, we set forth to investigate whether long-term RAS inhibition, either by treatment with an ACE inhibitor (enalapril) or with an AT1 receptor blocker (losartan) might attenuate structural and functional changes that occur in mitochondria upon aging. Supporting our hypothesis, kidney mitochondria from old rats (22 month-old) that were treated for 8 months with enalapril or losartan, showed an improved capacity for energy production, a lower rate of H_2O_2 production, a higher activity of mtNOS, and a higher content of UCP-2, compared to mitochondria isolated from untreated old rats (8) (Figure 1). In the same study we observed a general improvement in mitochondrial number and structure. Proximal tubular epithelial cells from the enalapril- or losartan-treated old rats showed a higher number of mitochondria, a better definition of mitochondrial cristae, and a lower number of osmiophilic bodies (probably derived from lipid oxidation). Furthermore, both treatments (enalapril or losartan) prevented the alteration of mitochondrial distribution inside proximal tubular cells that was observed in untreated old rats, and attenuated glutathione oxidation in renal tissue (8). These results indicate that RAS inhibition, regardless of how it is implemented, protects mitochondrial components and function from certain effects of aging.

A body of evidence supports the concept that vascular Ang-II presence and responsiveness increase with aging (78-81), and that the RAS may contribute to the development of age-related tissue damage (108). Inhibition of the RAS, either with ACE inhibitors or AT1 receptor blockers, can attenuate several effects of aging in rodents (109, 110). Numerous studies have shown that RAS inhibition prevents different aspects of renal, cardiac, vascular, and behavioral changes that are associated with the aging process in rodents (111).

The potential associations between RAS inhibition and PPAR modulation in aging are congruent with: i) the relatively high levels of Ang-II present in aged organisms (77-80); and ii) the prevention of age-dependent decreases in UCP content that occur secondary to RAS inhibition (8). Furthermore, the stimulation of PPAR-alpha dependent transcription of nuclear genes involved in

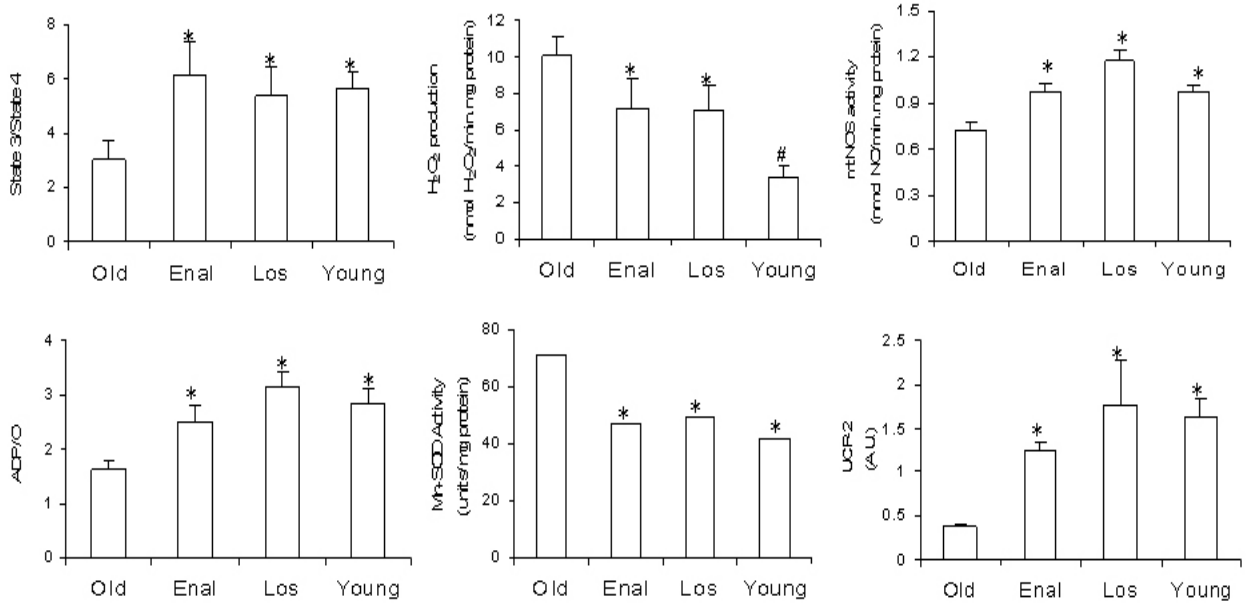

Figure 1. Enalapril and losartan treatments make mitochondrial functions in old rats resemble those of young rats. Respiratory control (respiratory state 3/ respiratory state 4), ADP/O, H_2O_2 production, Mn-SOD activity, nitric oxide synthase (mtNOS) activity, and UCP-2 content, in kidney mitochondria from 22-month old untreated rats (Old); 22-month old rats treated with enalapril (Enal) or losartan (Los) during 8 months; and 4-month old untreated rats (Young). *p< 0.05 vs. Old; #p< 0.01 vs Old, Enal, and Los. Data taken from (8).

mitochondrial fatty acid oxidation would enhance the generation of electron donors for the respiratory chain, thus suppressing the age-dependent deficit of ATP production. This is supported by our observation of an increase in mitochondrial ADP/O ratios following RAS inhibition in aging rats (8).

4.5. Renin-angiotensin system inhibition in hypertension and diabetes

Pharmacological RAS inhibition, either with ACE inhibitors or AT1 receptor blockers, is widely used in patients with hypertension, cardiovascular disease and diabetes (112-114). In these pathological states, the cardiac and renal benefits of RAS inhibition go beyond its blood pressure lowering effects (112, 113, 115) suggesting that ACE inhibitors and AT1 receptor blockers can exert tissue actions that are not associated with their hemodynamic effects.

Incidentally, both, mitochondrial dysfunction and RAS have been independently implicated in hypertension (116) and diabetes (117). These findings led us to investigate whether RAS inhibition might protect mitochondria from damage related to both pathological conditions. In spontaneously hypertensive rats (SHR), treatment with losartan prevented the alterations in kidney mitochondrial membrane potential, UCP-2 content, H_2O_2 production rate, and in the activities of mtNOS, Mn-SOD, and cytochrome oxidase that occurred in untreated SHR (118). In rats rendered diabetic by streptozotocin injection, losartan protected kidney mitochondria against changes in membrane potential, H_2O_2 production rate, and pyruvate content, without lowering plasma glucose content (119). In both studies, the administration of amlodipine, a Ca^{2+} channel blocker, reduced blood pressure to an extent similar to losartan, but showed no beneficial effects on kidney mitochondria alterations. The results obtained in these two rat models of hypertension and diabetes, indicate: a) a dissociation between blood pressure lowering and the improvement of mitochondrial function; and b) the involvement of Ang-II-AT1 receptor interaction as a relevant step for mitochondrial dysfunction.

5. CALORIC RESTRICTION, AGING, OXIDATIVE STRESS, MITOCHONDRIA, AND PEROXISOME PROLIFERATOR ACTIVATED RECEPTORS

Caloric restriction, without malnutrition, is one of the most consistent interventions to reduce the rate of aging in different species. Caloric restriction increases mean and maximum lifespan, and retards both, the decay of physiological functions, and the appearance of diseases associated with aging, such as hypertension, diabetes, nephropathy, cardiovascular disease, and cancer (120-124).

Reduction in metabolic rate represents one explanation for the beneficial effects of caloric restriction observed in different animal species. This reduction leads to lower O_2 consumption, ROS generation (125), and oxidatively damaged proteins (126-128), lipids (129-131), and DNA (32, 33, 132, 133) than do *ad libitum* fed animals. With regard to its effects on mitochondria, long-term caloric restriction lowers the rate of mitochondrial H_2O_2 production and decreases the levels of mitochondrial DNA oxidative damage in rat liver, heart, skeletal muscle and brain (32, 33, 133, 134). In addition, caloric restriction was

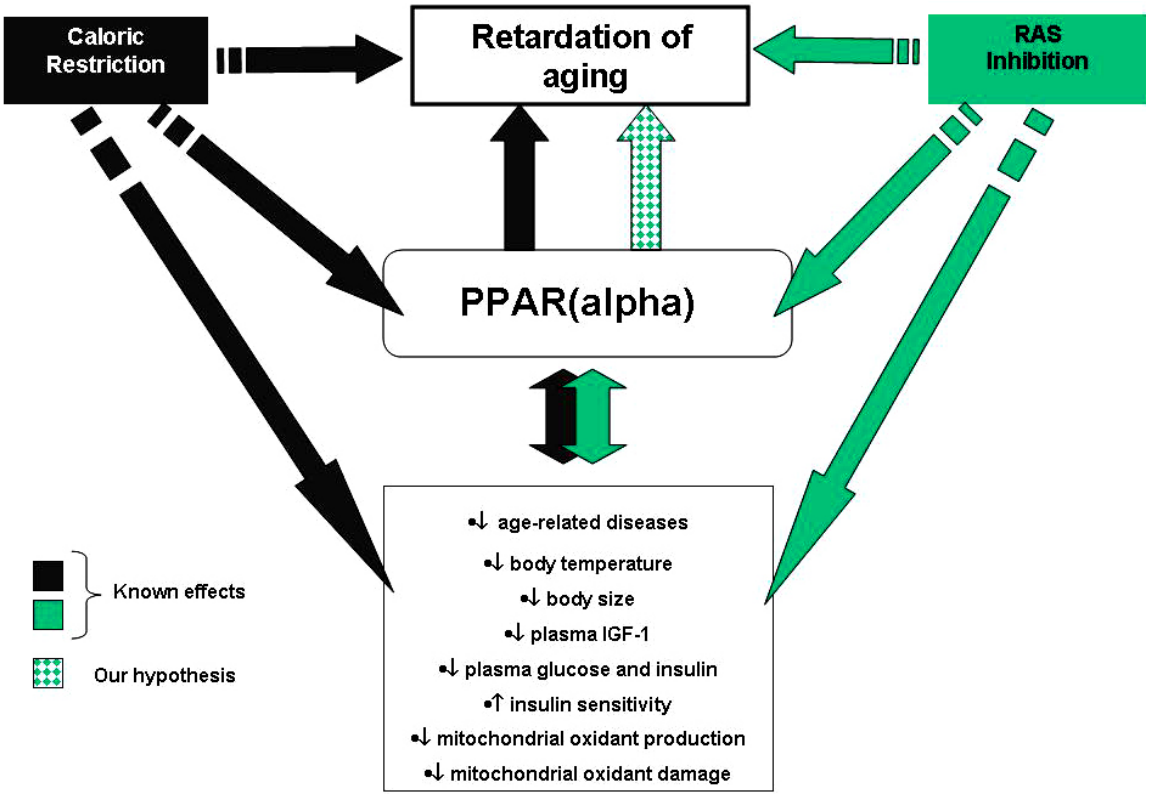

Figure 2. Scheme of the physiological actions elicited by caloric restriction and RAS inhibition and their impact on aging, having peroxisome proliferator activated receptors (PPARs) as central players. Our hypothesis of PPAR participation in the RAS-mediated retardation of aging is developed based on the converging effects of caloric restriction and RAS inhibition, and on the effects of RAS inhibition on mitochondrial function and PPAR modulation.

shown to increase the expression of UCP-2 in mice and in humans (135, 136), which may explain the effects of dietary manipulation on the reduction of mitochondrial H_2O_2 production. Analogous antioxidant and protective effects were observed in different animal species as well as in different tissues and cells (31). These observations emphasize the relevance of mitochondria and oxidative stress in the aging process.

Recent evidence suggests that PPARs may play an important role in the delay of aging caused by dietary restriction (54). In this context, PPAR nuclear protein, mRNA level, and DNA binding activity were shown to decrease with age, whereas caloric restriction blunted these reductions (52). Alternatively, using microarray technology, PPAR target genes were shown to be upregulated early and intensely in the livers of calorie-restricted mice (137).

6. RENIN-ANGIOTENSIN SYSTEM INHIBITION AND CALORIC RESTRICTION, TWO STRATEGIES CONVERGING AT BIOLOGICAL EFFECTS THAT INCLUDE PEROXISOME PROLIFERATOR ACTIVATED RECEPTORS

Considering that PPARs seem to play a major role in the age-retarding effects of caloric restriction, and that RAS inhibition has been shown to retard aging and to upregulate PPARs, we hypothesize that the converging effects displayed by both interventions are mediated by the maintenance of adequate levels of PPAR protein/activity (Figure 2). This concurrence is supported by a number of physiological and pathological conditions that are affected in a similar manner when RAS inhibition or caloric restriction, are studied in both humans and animal models. The converging effects include: i) retarding the manifestations of hypertension (121), diabetes (123, 138), nephropathy (120, 139), cardiovascular disease (112, 122), and cancer (124, 140, 141); ii) increasing body temperature (142, 143) and loss of body weight (144, 145); iii) lowering of insulin-like growth factor-I (IGF-1) plasma levels (146-148); iv) reduction of plasma glucose and insulin levels in hypertensive patients (149), and rats (143, 150-152); v) improvement of insulin sensitivity in hypertensive patients (153-156), which is in agreement with several lines of evidence that point to a role for Ang-II in the development of insulin resistance (153, 157-159); vi) diminution of protein oxidation (126-128), lipid oxidation (129-131), and DNA oxidation (32, 33, 132, 133); vii) diminution of the rate of mitochondrial H_2O_2 production in concurrence with a decrease of mitochondrial DNA oxidative damage in rat liver, heart, skeletal muscle, and brain (32, 33, 133, 134) and increased expression of UCP-2 in mice and in humans (8, 135, 136).

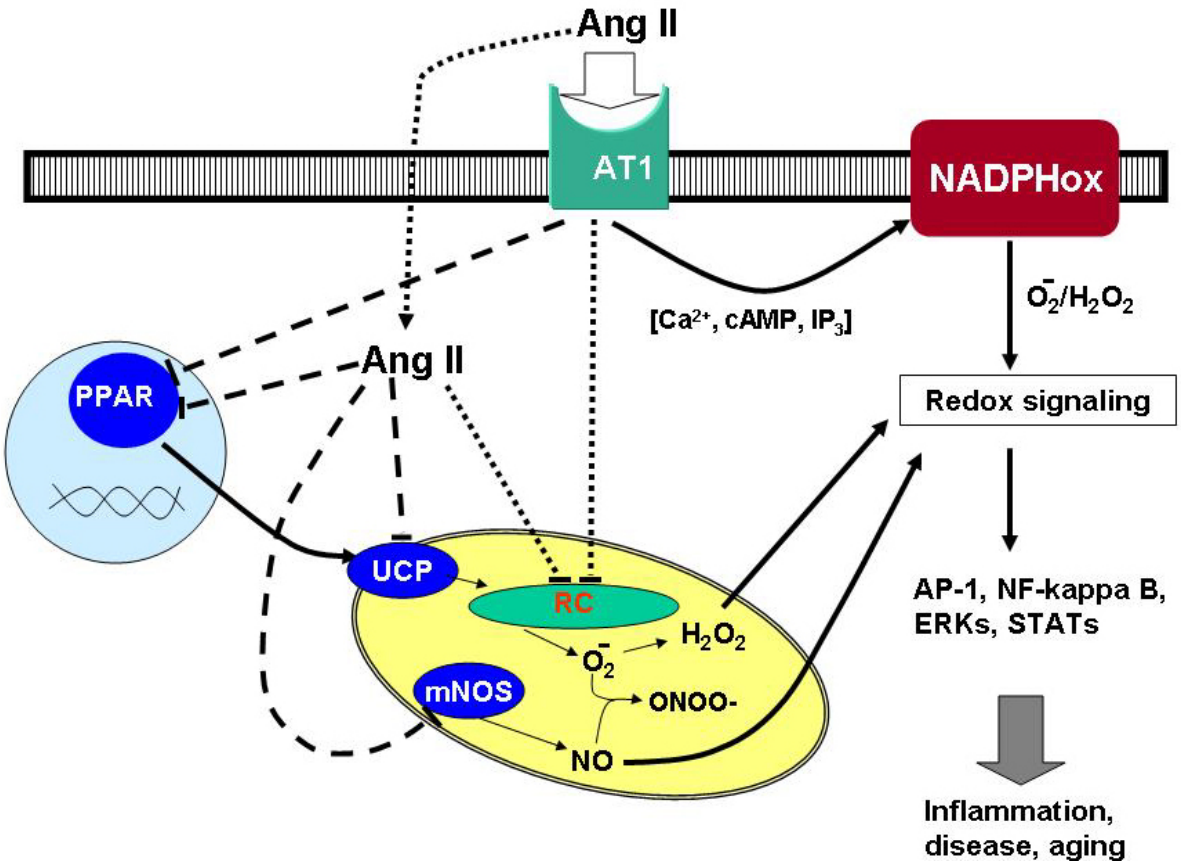

Figure 3. Scheme of the molecular pathways relating Ang-II with aging, having PPARs and mitochondria as central players. Solid arrows indicate accepted molecular pathways; dashed lines, pathways with experimental support; and dotted lines, hypothetical pathways. The mitochondrion is shown in yellow and the nucleus in light blue. Ang-II, angiotensin II; PPAR, peroxisome proliferator activated receptor; UCP, uncoupling proteins; mtNOS, mitochondrial nitric oxide synthase; RC, respiratory chain; AT1, angiotensin II-receptor 1; NADPHox, NADPH oxidase; TF, transcription factors. For the sake of clarity oxidant damage by NO, O_2^-, H_2O_2, and $ONOO^-$, as well as other agents in addition to Ca^{2+}, cAMP, and IP_3, that are generated downstream of AT1 activation are not described in the scheme.

Regarding the effects of RAS inhibition and caloric restriction on weight loss, we must add that a positive correlation between plasma angiotensinogen levels and body mass index was reported in humans (160). However, the fact that Ang-II can produce anorexia (145) may afford an alternative interpretation for the Ang-II-mediated weight loss.

7. CONCLUSIONS AND PERSPECTIVES

By integrating current knowledge in the areas of mitochondrial metabolism, RAS, caloric restriction, and aging, we have developed a hypothesis in which PPARs serve an integrative role as central players. Figure 3 illustrates the potential molecular mechanisms underlying this hypothesis. The proposed mechanisms are based on the known activation of the AT1 receptor by Ang-II that leads to changes in Ca^{2+} homeostasis, and cAMP- and inositol triphosphate-mediated events, and the subsequent activation of NADPH-oxidase which, by generating O_2^-, activates redox-sensitive signaling pathways. The changes driven by the activation of the AT1 receptor could also lead to the uncoupling of mitochondrial respiration with the subsequent increase in O_2^- generation, and/or to the downregulation of PPARs activity, which could result in UCP deactivation and increased O_2^- generation. If Ang-II or any of its metabolites could reach the cytosol and the mitochondria they might interact with: a) PPARs or UCPs, leading to their inactivation; b) mitochondrial components, resulting in an increased production of O_2^- and a decreased of NO bioavailability; and/or c) NOS leading to a decreased NO generation.

The present hypothesis relating PPARS, mitochondria, and the RAS, provides a possible explanation for the molecular events that could be involved in the well documented effects ascribed to RAS inhibition in delaying the onset of age-associated pathologies.

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