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REVIEWS: CURRENT TOPICS

Acetylcarnitine and cellular stress response: roles in nutritional redox homeostasis and regulation of longevity genes

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Abstract

Aging is associated with a reduced ability to cope with physiological challenges. Although the mechanisms underlying age-related alterations in stress tolerance are not well defined, many studies support the validity of the oxidative stress hypothesis, which suggests that lowered functional capacity in aged organisms is the result of an increased generation of reactive oxygen and nitrogen species. Increased production of oxidants in vivo can cause damage to intracellular macromolecules, which can translate into oxidative injury, impaired function and cell death in vulnerable tissues such as the brain. To survive different types of injuries, brain cells have evolved networks of responses, which detect and control diverse forms of stress. This is accomplished by a complex network of the so-called longevity assurance processes, which are composed of several genes termed vitagenes. Among these, heat shock proteins form a highly conserved system responsible for the preservation and repair of the correct protein conformation. The heat shock response contributes to establishing a cytoprotective state in a wide variety of human diseases, including inflammation, cancer, aging and neurodegenerative disorders. Given the broad cytoprotective properties of the heat shock response, there is now a strong interest in discovering and developing pharmacological agents capable of inducing the heat shock response. Acetylcarnitine is proposed as a therapeutic agent for several neurodegenerative disorders, and there is now evidence that it may play a critical role as modulator of cellular stress response in health and disease states. In the present review, we first discuss the role of nutrition in carnitine metabolism, followed by a discussion of carnitine and acetyl-L-carnitine in mitochondrial dysfunction, in aging, and in age-related disorders. We then review the evidence for the role of acetylcarnitine in modulating redox-dependent mechanisms leading to up-regulation of vitagenes in brain, and we also discuss new approaches for investigating the mechanisms of lifetime survival and longevity.

Keywords: Acetylcarnitine; Cellular stress response; Redox homeostasis; Vitagenes; Heme oxygenase; Heat shock response

1. Introduction

There is a considerable evidence that aging is associated with a reduced ability to cope with physiological challenges [1]. Although the mechanisms underlying age-related alterations in stress tolerance are not well defined, many studies support the validity of the oxidative stress hypothesis, which suggests that lowered functional capacity in aged organisms is the result of an increased generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [2]. Increased production of oxidants in vivo can cause damage to intracellular macromolecules such as DNA, proteins and lipids, which can translate into oxidative injury and impaired function of vulnerable tissues, such as the brain [3]. Although several lines of evidence suggest that accumulation of oxidative molecular damage is a primary causal factor in senescence, it is increasingly evident that the mitochondrial genome may play a key role in aging and neurodegenerative diseases [4]. Mitochondrial dysfunction is a characteristic of several neurodegenerative disorders, and evidence for mitochondria being a site of damage in neurodegenerative disorders is partially based on decreases in respiratory chain complex activities in Parkinson's disease, Alzheimer's disease (AD) and Huntington's disease [5,6]. Such defects in respiratory complex activities, possibly associated with oxidant/antioxidant balance perturbation, are thought to underlie defects in energy metabolism

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and induce cellular degeneration [7,8]. However, to survive different types of injuries, brain cells have evolved networks of different responses that detect and control diverse forms of stress [9,10]. Hence, efficient functioning of maintenance and repair processes seem to be crucial for both survival and physical quality of life, and there is now an evidence that this may be accomplished by a complex network of the so-called longevity assurance processes, which are composed of several genes termed vitagenes [9–11]. Among these, chaperones are highly conserved proteins responsible for the preservation and repair of the correct conformation of cellular macromolecules, such as proteins, RNAs and DNA [12]. Chaperone-buffered silent mutations may be activated during the aging process and lead to the phenotypic exposure of previously hidden features and contribute to the onset of polygenic diseases, such as age-related disorders, atherosclerosis and cancer [13]. Recent studies have shown that the heat shock response contributes to establishing a cytoprotective state in a wide variety of human diseases, including ischemia and reperfusion damage, inflammation, metabolic disorders, cancer, infection, trauma and aging [14,15]. Among the various Hsps, Hsp32 also known as heme oxygenase-1 (HO-1), has received considerable attention, as it has been recently demonstrated that HO-1 induction, by generating the vasoactive molecule carbon monoxide (CO) and the potent antioxidant bilirubin, could represent a protective system potentially active against brain oxidative injury [16]. We have recently shown in astrocytes exposed to nitrosative stress that acetylcarnitine, an emerging metabolic and antioxidant modulator, protects against cytokine-mediated mitochondrial chain respiratory complex impairment and the associated increase in protein and lipid peroxidation. The increase in astroglial antioxidative potential observed after acetylcarnitine treatment involves a secondary line of antioxidant defenses, represented by stress responsive genes, such as HO-1, and the mitochondrial Hsp60 and SOD [17]. Thus, given the broad cytoprotective properties of the heat shock response, molecules inducing this defense mechanism appear to be possible candidates for novel cytoprotective strategies. Particularly, manipulation of endogenous cellular defense mechanisms via acetylcarnitine may represent an innovative approach to therapeutic intervention in diseases causing tissue damage, such as neurodegeneration. We hypothesize that maintenance or recovery of the activity of vitagenes may delay the aging process and decrease the risk of age-related diseases.

2. Nutritional implications of carnitine and acetylcarnitine

L-Carnitine is a vitamin-like compound synthesized in the liver, kidney and brain through the conversion of two essential amino acids, lysine and methionine [18,19]. L-Carnitine plays a vital role in cellular energy metabolism. It functions as a transporter of short-, medium- and long-chain fatty acids toward or across the inner mitochondrial membrane, thereby facilitating β-oxidation [20].

Acetyl-L-carnitine (LAC) (Fig. 1) is an amino acid that can be purchased as an individual supplement. Acetyl-L-carnitine is found in lists of nutritional agents promoted as producing cognitive benefits for middle-aged and elderly people [21]. Acetyl-L-carnitine is actively transported across the blood–brain barrier. It is thought to influence the cholinergic system as a cholinergic receptor agonist (facilitator) and may also promote synthesis and release of acetylcholine [22]. More generally, LAC participates in cellular energy production and in maintenance and repair processes in neurons [23].

In addition to its principal function, carnitine and acetylcarnitine also buffer potentially toxic acyl-CoA metabolites and modulates the ratio of acyl-CoA/CoA. The latter regulates the activity of many mitochondrial enzymes involved in the citric acid cycle, the gluconeogenesis, the urea cycle and the fatty oxidation [24]. Although 99% of the carnitine amount is intracellular, the relationship between serum acylcarnitine and free carnitine is highly sensitive to intramitochondrial metabolic alterations. Such alterations occur in different situations both normal and abnormal. A normal situation accompanied with changes in the relationship between acylcarnitine and free carnitine is fasting (a condition associated with a buildup of keto acids and, consequently, to a reduction in plasma-free carnitine and a corresponding increase in acetylcarnitine [25]). Other normal conditions include aging and pregnancy. Pathological situations with abnormal acyl-bound carnitine vs. free carnitine ratio include (a) several inborn errors of metabolism, such as organic acidurias; (b) cirrhosis or chronic renal failure; (c) iatrogenic situations such as treatment with valproate; and (d) all types of diabetes, heart failure and Alzheimer's disease [26]. Under normal conditions, about 80% of serum carnitine is free carnitine, and the normal acyl-conjugated/free carnitine ratio is 0.25; ratios >0.4 are considered as abnormal (carnitine insufficiency) [27].

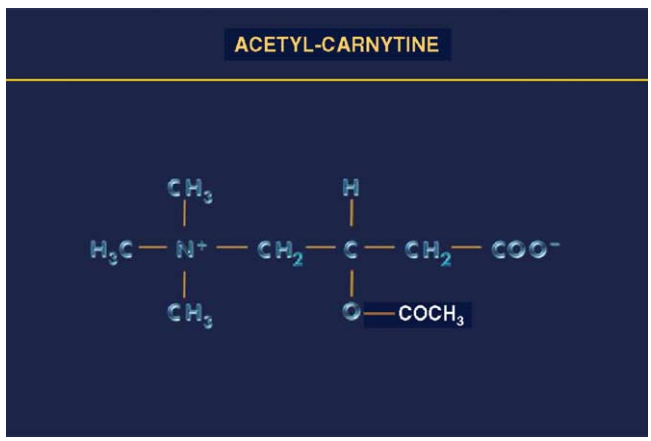

Fig. 1. Chemical structure of acetylcarnitine.

3. Carnitine: biosynthesis, physiological roles and nutritional aspects

Carnitine is a nonessential nutrient, first described in the early beginnings of the 20th century. It was initially considered to be an essential vitamin; it was later discovered that it could be manufactured in the liver, kidney and brain from amino acid precursors (lysine and methionine). Human skeletal muscle, heart, liver, kidney and brain are able to synthesize γ-butyrobetaine, an immediate precursor of carnitine, but only liver, kidney and brain have the ability to convert this substrate to carnitine [28]. The reaction is catalyzed by γ-butyrobetaine hydroxylase. Due to the very low activity of γ-butyrobetaine hydroxylase in fetuses and neonates, carnitine is considered as a vitamin for newborn babies [28]. In humans, 75% of carnitine intake comes from dietary sources. These include most animal foods; however, due to losses in food cooking and preparation, there are few data on the total content of the diet. Carnitine ingested or synthesized by humans is in the L-isoform and is carried for storage via blood, predominantly in heart and skeletal muscle. During fasting, there is an increased production of ketones with a subsequent reduction in plasma-free carnitine and a corresponding increase in acylcarnitine. This is due to an enhanced production of acetyl-CoA via β-oxidation. The supplementation of fasting persons with carnitine should further accelerate β-oxidation and increase acetyl-CoA production. Increased acetyl-CoA concentration stimulates the first step of gluconeogenesis (conversion of pyruvate to oxaloacetate by pyruvate carboxylase). Moreover, the ratio of acetyl-CoA to CoA is increased. This relation is crucial in mitochondrial metabolism, modulating the activity of many mitochondrial enzymes, including among others pyruvate dehydrogenase (PDH), a very important enzyme in glucose metabolism [29]. A high ratio of acetyl-CoA to CoA reduces the activity of PDH with subsequent reduction of carbohydrate breakdown. L-Carnitine supplementation has also protein sparing effects; the activation of the β-oxidation pathway, mediated through carnitine, reduces the breakdown of branched chain amino acids via an internal feedback mechanism (inhibition of the branched chain keto-dehydrogenase) and promotes protein synthesis resulting in a leaner body [30]. Furthermore, a supplement of L-carnitine improves recycling of CoA by shuttling the short-chain acyl groups from the inside of the mitochondria to the cytosol, thus, raising the levels of mitochondrial-free CoA. The increased availability of mitochondrial-free CoA supports the continuation of β-oxidation. The released CoA is made available to the Krebs cycle linked to the electron transport chain where energy is ultimately produced. The increased availability of amino acids also supports hepatic gluconeogenesis, enhancing the glucose supply to the brain [31]. The involvement of L-carnitine in the excretion of an excess of acyl-*S*-CoA groups to prevent a possible systemic acidosis, as well as hormonal changes and a reduction of L-carnitine biosynthesis, plays significant roles in the variations in L-carnitine metabolism encountered in patients with several inborn errors of fatty acid metabolism [32]. Fetuses and neonates do not have the ability to convert γ-butyrobetaine to carnitine. Therefore, in contrast to adulthood, L-carnitine supplementation is essential for fetuses and neonates whose source of carnitine is placenta and breast milk, respectively [33]. Soy-based and cow's milk-based infant formulas also contain carnitine.

Carnitine deficiency is also observed in aging. Animal experiments have shown that the aging process is accompanied by alterations in carnitine metabolism in brain myocardium and skeletal muscle [34]. Experimental evidence indicates that the aging process impairs the homeostatic regulation of serum lipid through a "lipid-carnitine" system and that thyroid hormone plays a contributory role in the activation of this system. The clinical correlation of all these changes is not yet clarified. Acetylcarnitine, however, as a carnitine derivative with a better ability to cross the blood brain barrier, seems to be more promising in elderly patients.

3.1. Acetylcarnitine

Acetyl-L-carnitine (Fig. 1) is an ester of the trimethylated amino acid, L-carnitine, and is synthesized in the brain, liver and kidney by the enzyme LAC transferase. Acetyl-L-carnitine facilitates the uptake of acetyl-CoA into the mitochondria during fatty acid oxidation, enhances acetylcholine production and stimulates protein and membrane phospholipid synthesis [35]. At present, studies have shown that LAC is a compound of great interest for its wide clinical application in various neurological disorders: it may be of benefit in treating Alzheimer's dementia, chronic fatigue syndrome, depression in the elderly, HIV infection, diabetic neuropathies, ischemia and reperfusion of the brain, cognitive impairment of alcoholism and aging [36–40]. The neuroprotective benefits of this compound have been observed in the hippocampus, prefrontal cortex, substantia nigra and muscarinic receptor portions of the brain [41]. These include antioxidant activity, improved mitochondrial energetics [42,43], stabilization of intracellular membranes and cholinergic neurotransmission. Promising therapeutic applications of LAC are derived from observations that this compound crosses the blood–brain barrier through a saturable process in a sodium-dependent manner and improves neuronal energetic and repair mechanisms, while modifying acetylcholine production in the CNS [44]. Acetyl-L-carnitine treatment restores the altered neurochemical abnormalities, cerebral energy metabolites in ischemia and aging and, in particular, ammonia-induced cerebral energy depletion [45]. In addition, it increases the responsiveness of aged neurons to neurotrophic factors in the CNS, and it has a preventive and corrective effects on diabetic neuropathology. Its beneficial effects have been also observed in aged animals on the brain redox state [46] as well as on EEG, evoked potentials and synaptic long-term potentiation [47]. Moreover, LAC is commonly used also for the treatment of

painful neuropathies: it exerts a potent analgesic effect by up-regulating metabotropic glutamate receptors [48]. There are experimental data that LAC improves memory function in Alzheimer's patients, and it influences attention, learning and memory in the rat [49]. Chronic treatment enhances spatial acquisition in a novel environment of rats with behavioral impairments and has a slight effect on retention of the spatial discrimination in a familiar environment [50]. More recently, it has been observed that LAC produces sustained changes of nonassociative learning of sensitization and dyshabituation type in the invertebrate *Hirudo medicinalis*, and it has been suggested that LAC might exert its effects by means of new protein synthesis, through qualitative and quantitative changes of gene expression. In addition, recent evidences have reported that it influences expression of glyoxylase 1, a gene involved in the detoxification of metabolic by-products, and increases p75-mRNA in Alzheimer's disease mutant transgenic mouse model Tg2576 [51]. Recently, by using suppressive subtractive hybridization strategy, a PCR-based cDNA subtraction procedure particularly efficient for obtaining expressed transcripts often obscured by more abundant ones, it has reported that LAC modulates specific genes in the rat CNS, such as the hsp72 gene, the gene for the isoform of 14-3-3 protein and that encoding for the precursor mitochondrial P3 of ATP synthase lipid-binding protein [52].

4. The carnitine system

4.1. Carnitine acyltransferases

The carnitine acyltransferases consist of mitochondrial carnitine palmitoyltransferases (CPT), peroxisomal carnitine octanoyltransferase (COT) and mitochondrial/peroxisomal carnitine acetyltransferase (CAT). Carnitine palmitoyltransferase and COT transesterify medium- and long-chain fatty acyl chains, whereas CAT transesterifies short-chain acyl chains. There are three genes in the human genome known to be responsible for mitochondrial CPT activity: *CPT1A* encodes liver-type (L-)CPT I, *CPT1B* encodes muscle-type (M-)CPT I and *CPT2* (formerly annotated as *CPT1* because it was the first known gene for a CPT enzyme) encodes CPT II. The CPT I and CPT II couple mediate the transport of long-chain fatty acids by transesterification of long-chain acyl-CoA into long-chain acylcarnitine in the cytosol and vice versa in the mitochondrial matrix. The acylcarnitine shuttle composed by these enzymes is possible through the transport over the mitochondrial inner membrane via the carnitine/acylcarnitine translocator CACT [53]. Overall, the net mitochondrial uptake of activated long-chain fatty acids, that is, long-chain acyl-CoA, is facilitated through the temporary replacement of the CoA group by carnitine. The CPT I and CPT II enzymes therefore have a clear-cut function that is essential to allow mitochondrial β-oxidation of long-chain fatty acids. The product of this process, acetyl-CoA, is the central substrate for many processes, most importantly, the Krebs cycle and, in the liver, ketogenesis. Apart from mitochondrial β-oxidation, mitochondrial acetyl-CoA can also come from carbohydrate oxidation. The acetyl-CoA/CoA ratio is known to be one of the many determinants of the activity of the PDH complex through the action of acetyl-CoA on the PDH inactivating kinase PDK4. Therefore, CAT is a potentially important mitochondrial enzyme because it determines the equilibrium between acetyl-CoA (plus carnitine) and acetylcarnitine (plus free CoA), and thereby modulates the acetyl-CoA/CoA ratio. Thus, a buffer is formed when acetyl-CoA production from fatty acids or pyruvate is high, and circulatory acetylcarnitine could possibly facilitate replenishment of acetyl-CoA elsewhere. This buffer may explain many of the beneficial effects of carnitine and propionylcarnitine for cardiac performance in models of ischemia and hypertrophy [54], and of acetylcarnitine for the aging heart [55]. Carnitine acetyltransferase is also active in the peroxisome where it acts on acetyl-CoA and propionyl-CoA, which result from oxidation (chain shortening) of branched-chain and very long-chain fatty acids. Because mammalian peroxisomes do not fully β-oxidize medium-chain fatty acid intermediates, the medium- and long-chain acyl-CoA molecules resulting from chain shortening are further metabolized elsewhere, that is, in the mitochondria. The first enzyme needed to allow transport of medium- and long-chain acyl-CoA out of the peroxisome is COT. Although medium-chain fatty acids can traverse membrane systems without being esterified to carnitine, peroxisomal transesterification is beneficial because the activated state of medium-chain fatty acids as acyl-CoA is energetically preserved as acylcarnitine. Therefore, medium-chain acylcarnitines and also long-chain acylcarnitines are formed from their corresponding CoA esters to be transported to the cytosol and mitochondria. This transport is most probably mediated by the same carrier as the mitochondrial CACT.

4.2. The CPT system

Both CPT I isoforms are located in the mitochondrial outer membrane and are sensitive for inhibition by malonyl-CoA, which makes these enzymes important sites for metabolic regulation [56]. Malonyl-CoA is the product of a reaction catalyzed by acetyl-CoA carboxylases. This reaction is the first step in fatty acid biogenesis from acetyl-CoA and is preserved in nonlipogenic tissues such as skeletal and heart muscle [57]. The formation of malonyl-CoA may therefore serve as a main control of fatty acid catabolism through the inhibition of CPT I. The tissue distribution of the two CPT I isoforms and their level of sensitivity for malonyl-CoA inhibition are markedly different, the muscle isoform being more sensitive than the liver isoform, thus providing the body the possibility to modulate the degree of control over fatty acid β-oxidation in organs that express both CPT I isoforms, such as the heart [58]. Carnitine palmitoyltransferase II is also expressed in the

heart, as it is in many tissues. Recent progress in the structural knowledge of CPT I has pointed out that two transmembrane domains anchor the enzyme and that the active site in the C-terminal domain is at the cytosolic side of the mitochondrial outer membrane [59]. It is now generally accepted that the CPT I reaction is carried out at the cytosolic face of the mitochondrion [60]. For uptake of acylcarnitine into the intermembrane space, Fraser and Zammit [61] proposed a diffusion process through porin, and Kerner and Hoppel [62] point at the involvement of porin as well. Notably, the cytosolic formation of acylcarnitine resides at the surface of the *mitochondria*, and it is possible that transesterification of long-chain acyl moieties, which perhaps only temporarily are removed from the membrane by CPT I, is followed by flipping of acylcarnitine and channeling toward CPT II by CACT.

4.3. Genomics and promoter sequences

Five human carnitine acyltransferase genes have been cloned [63]. Four of these genes (*CPT1A*, *CPT1B*, *CPT2* and *CAT*) have been localized to their chromosomal positions by different methods. Hybrid panel mapping was used to localize *CPT1A*, a location confirmed by fluorescent in situ hybridization (FISH) [62]. *CPT1B* was localized by FISH and by other means [64]. The gene for CPT1B is located in a telomeric region of chromosome 22 [64]. Because metabolic diseases are known to be caused by deficiencies in L-CPT I and CPT II and possibly CAT [56], the localization of *CPT1A* and *CPT2* and possibly also *CRAT* is of direct clinical relevance. Carnitine palmitoyltransferase II deficiency is the most common carnitine transferase disorder [65]. The nonredundant database revealed the full genomic structure of *CROT* and several exons of the flanking genes *P53TG* and *PGY3*. The latter encodes a multidrug resistance *P*-glycoprotein of the ABC transporter family and was localized at 7q21.1 [66]. The knowledge of genomic sequences is of clinical relevance because it allows the design of primers for PCR analysis on genomic DNA.

Human *CROT* and *CRAT* promoter sequences have been investigated for potentially important transcription factor binding sites. Because peroxisome proliferator-activated receptors are important for both constitutive and regulated expression of many genes of fat metabolism and establish a molecular connection among the principal pathways of energy metabolism, putative peroxisome proliferator response elements (PPREs) were sought and were readily identified only in the promoter of *CPT1B*. This PPRE, also known as fat-activated response element, is present in both ubiquitous and muscle-specific human *CPT1B* promoters [64]. No PPREs were found in the promoters of human *CROT*, mitochondrial *CRAT* and *CPT2*, nor in the rodent orthologues [64]. All exon–intron boundaries of *CROT* comply with the GT-AG rule, as do all boundaries of the other genes as well, with one functional exception (*CRAT*).

5. Mitochondrial decay and aging: role of acetylcarnitine

Harman [67] in 1972 first proposed that mitochondria may have a central role in the process of aging. According to this theory, free radicals generated through mitochondrial metabolism can act as causative factor of abnormal function and cell death. Mitochondria are the cell's most significant source of oxidants, and in vitro studies have indicated that approximately 1–2% of electron flow through the ETC results in the univalent generation of superoxide [68]. Moreover, various toxins in the environment can injure mitochondrial enzymes, leading to increased generation of free radical that over the life span would eventually play a major role in aging [67]. Ultrastructural changes have been also reported to occur in mitochondria with age. They become larger and less numerous with vacuolization, cristae rupture and accumulation of paracrystalline inclusions. Cardiolipin, an acidic phospholipid that occurs only in mitochondria, has been shown to decrease with age [69]. This inner membrane lipid is known to have optimal electrical insulating properties, thereby contributing significantly to the transmembrane potential that drives the formation of ATP via ATP synthase. Indeed, a decrease in membrane potential in mitochondria from older animals has been demonstrated [70].

Several age-related disorders have been shown to be linked to higher levels of mtDNA mutations than age-matched controls. In the CNS, 17 times higher levels of the common deletion in the striatum of patients with Parkinson's disease have been demonstrated, compared to age-matched controls. Evidence also exists indicating higher levels of this deletion in patients with Alzheimer's disease, which parallel increased levels in the oxidized nucleotide 8-OH-dG [71]. A major feature of mtDNA disease in humans is the presence of cells with low cytochrome *c* oxidase activity, and evidence exists, which indicates that the mechanism for these changes is likely to be a clonal expansion of individual mtDNA deletions within single cells [72]. Complex IV-deficient cells, which occurred only sporadically earlier than the sixth decade of life, were present regularly after this age, with the loss of enzyme activity being always confined to single randomly distributed cells. Similarly, cytochrome *c* oxidase-negative neurons have been demonstrated to exist in abundance in the CNS of patients with a mitochondrial disorder [73]. These findings establish the relationship between age-associated accumulation of mtDNA mutations and bioenergy degradation as a key feature of the aging process, at least in tissues predominantly composed by postmitotic cells, such as CNS and skeletal muscle. Relevant to mitochondrial bioenergetics, in fact, is the finding of a significant decrease in state 3/state 4 ratio, which has been observed to occur in brain as function of age [74]. Because this ratio relates to the coupling efficiency between electron flux through the electron transport chain and ATP production, an increase in state 4 would result in a more reductive state of

mitochondrial complexes and, consequently, to an increase in free radical species production. A decrease in state 3/state 4 respiration during aging has been found associated with a significant decrease in cardiolipin content in brain mitochondria [75,76]. This loss could play a critically important role in the age-related decrements in mitochondrial function and appears to be associated with both quantitative and qualitative region-specific protein changes that are parallel to structural changes, such as decrease of the inner membrane surface, smaller as well as sparser cristae, decreased fluidity and increased fragility. Modifications in cardiolipin composition are recognized to accompany functional changes in brain mitochondria, which include all proteins of the inner mitochondrial membrane that generally require interaction with cardiolipin for optimal catalytic activity [77]. Acetylcarnitine fed to old rats increased cardiolipin levels to that of young rats and also restored protein synthesis in the inner mitochondrial membrane, as well as cellular oxidant/antioxidant balance [78], suggesting that administration of these compounds may improve cellular bioenergetics in aged rats. Interestingly, caloric restriction, a dietary regimen that extends life span in rodents, maintains the levels of 18:2 acyl side chains and inhibits the cardiolipin composition changes [79]. In addition, caloric restriction was shown to retard the aging-associated changes in oxidative damage, mitochondrial oxidant generation and antioxidant defenses observed during aging [80].

6. Oxidative stress and cellular stress tolerance

There is now an evidence to suggest that reduction of cellular expression and activity of antioxidant proteins and the resulting increase of oxidative stress are fundamental causes for both the aging processes and neurodegenerative diseases. However, to survive different types of injuries, brain cells have evolved networks of different responses, which detect and control diverse forms of stress. Efficient functioning of maintenance and repair process seems to be crucial for both survival and physical quality of life. This is accomplished by a complex network of the so-called longevity assurance processes, which are composed of several genes termed vitagenes [9–11] Signaling mechanisms adopted by regulatory proteins to control gene expression in response to alterations in the intracellular redox status are very common in prokaryotes. Gene activation by oxidative stress was first described in bacteria where regulatory proteins such as OxyR was discovered as an activator of antioxidant and stress responsive genes. As the cytoprotective mechanism triggered by SoxR in *Escherichia coli* includes the expression of critical antioxidant defensive proteins, such as superoxide dismutase, the emerging concept is that analogous system might operate in mammalian cells [81]. In eukaryotes, typical examples are genes such as heme oxygenase (HO) and hsp genes, thioredoxin and detoxificant enzymes (Mn-SOD, glutathione *S*-transferase, NADPH: quinone reductase), cytokine, immunoreceptors and growth factors.

7. Redox regulation of gene expression

Signaling mechanisms adopted by regulatory proteins to control gene expression in response to alterations in the intracellular redox status are very common in prokaryotes. Gene activation by oxidative stress was first described in bacteria where regulatory proteins such as OxyR was discovered as an activator of antioxidant and stress responsive genes. The OxyR is a homotetramer that is activated by hydrogen peroxide and *S*-nitrosothiols. The protein contains six cysteine residues, one of each is absolutely necessary for activity and two are required for maximal activation. Recent studies suggested that oxidation of a single thiol to a sulfenic acid may represent a sensor mechanism, whereas the activation mechanism can be ascribed to formation of an intramolecular disulfide or, alternatively, to *S*-nitrosylation of a single cysteine residue, with Cys 199 being a likely candidate site of posttranslational modification [82,83]. The expression of these protective genes renders the bacteria more resistant to oxidant damage [84]. As the cytoprotective mechanism triggered by SoxR in *E. coli* includes the expression of critical antioxidant defensive proteins, such as superoxide dismutase [86], the emerging concept is that analogous system might operate in mammalian cells. In eukaryotes, typical examples are genes such as HO gene, thioredoxin and detoxificant enzymes (Mn-SOD, glutathione *S*-transferase, NADPH: quinone reductase), cytokine, immunoreceptors and growth factors. That the antioxidant protein HO could “sense” NO and, thus, protect against ROS and RNS insults is supported by the following findings: (a) NO and NO-related species induce HO-1 expression and increase HO activity in human glioblastoma cells, hepatocytes and aortic vascular cells; (b) cells pretreated with various NO-releasing molecules acquire increased resistance to H_2O_2-mediate cytotoxicity at the time HO is maximally activated; and (c) bilirubin, one of the end products of heme degradation by HO, protects against the cytotoxic effects caused by strong oxidants H_2O_2 and $ONOO^-$ [85,86]. The conception that NO and RNS can be directly involved in the modulation of HO-1 expression in eukaryotes is based on the evidence that different NO-releasing agents can markedly increase HO-1 mRNA and protein, as well as HO activity, in a variety of tissues, including brain cells [87]. In rat glial cells, treatment with lipopolysaccharide (LPS) and interferon-γ results in a rapid increase in both iNOS expression and nitrite levels [88], followed by enhancement of HO-1 protein, whereas addition of NOS inhibitors suppressed both nitrite accumulation and HO-1 mRNA expression. Modulation of HO-1 mRNA expression by iNOS-derived NO following stimulation with LPS has also been reported in different brain regions, particularly in the hippocampus and substantia nigra in vivo rat model of septic shock [87]. Moreover, the early increase in iNOS protein levels observed in endothelial cells exposed to low oxygen tension seems to precede the stimulation of HO-1 expression and activity, an effect that appears to be finely regulated by redox reactions

involving glutathione [86,89,90]. Taken together, these findings point to the central role of the NO as a signaling molecule, which, by triggering expression of cytoprotective genes such as HO-1, may lead to adaptation and resistance of brain cells to subsequent, eventually more severe, nitrosative and oxidative stress insults [89,91]. Thus, a direct interaction of NO with selective chemical sites localized in transcription proteins that can be activated through nitrosative reactions could effectively contribute to the enhancement of both HO-1 gene expression and stress tolerance. Recent knowledge concerning the modulation by thiol redox state of the activity of several transcription factors that recognize specific binding sites within the promoter and distal enhancer regions of the *HO-1* gene include Fos/Jun [activator protein-1 (AP-1)], nuclear factor-kB (NFkB) and the more recently identified Nrf2 proteins [92–94]. Importantly, both AP-1 and NFkB contain cysteine residues whose interaction with oxidant or nitrosant species might be crucial for determining the binding activity to DNA [86]. Data in the literature show that NO can either activate or inhibit these transcription factors, and that in many circumstances, activation depends on the reversibility of the posttranslational modification elicited by the various RNS [86,95].

Recent data from our laboratory have provided experimental evidence that acetylcarnitine treatment of astrocytes exposed to cytokine-induced nitrosative stress restored GSH/GSSG ratio and complex IV inhibition, an effect associated with up-regulation of HO and nuclear translocation of the transcription factor Nrf-2 [17]. In in vivo experiments, we have also demonstrated that the redox glutathione status is a critical factor for induction of cytoprotective Hsps [96,97].

8. The heat shock pathway of cellular stress tolerance

It is well known that living cells are continually challenged by conditions that cause acute or chronic stress. To adapt to environmental changes and survive different types of injuries, eukaryotic cells have evolved networks of different responses that detect and control diverse forms of stress. One of these responses, known as the heat shock response, has attracted a great deal of attention as a universal fundamental mechanism necessary for cell survival under a wide variety of toxic conditions. In mammalian cells, Hsp synthesis is induced not only after hyperthermia but also after alterations in the intracellular redox environment, exposure to heavy metals, amino acid analogs or cytotoxic drugs. Although prolonged exposure to conditions of extreme stress is harmful and can lead to cell death, induction of Hsp synthesis can result in stress tolerance and cytoprotection against stress-induced molecular damage. Furthermore, transient exposure to elevated temperatures has a cross-protective effect against sustained normally lethal exposures to other pathogenic stimuli. Hence, the heat shock response contributes to establish a cytoprotective state in a variety of metabolic disturbances and injuries, including stroke, epilepsy, cell and tissue trauma, neurodegenerative disease and aging [9,11,14]. This has opened new perspectives in medicine and pharmacology, as molecules activating this defense mechanism appear as possible candidates for novel cytoprotective strategies [94]. In mammalian cells, the induction of the heat shock response requires the activation and translocation to the nucleus of one or more heat shock transcription factors that control the expression of a specific set of genes encoding cytoprotective heat shock proteins. Some of the known Hsps include ubiquitin, Hsp10, Hsp27, Hsp32 (or HO-1), Hsp47, Hsp60, Hsc70, Hsp70 (or Hsp72), Hsp90 and Hsp100/105. Most of the proteins are named according to their molecular weight: *Hsp70*. The 70-kDa family of stress proteins is one of the most extensively studied. Included in this family are Hsc70 (heat shock cognate, the constitutive form), Hsp70 (the inducible form, also referred to as Hsp72) and GRP75 (a constitutively expressed glucose-regulated protein found in the endoplasmic reticulum). After a variety of central nervous system (CNS) insults, Hsp70 is synthesized at high levels and is present in the cytosol, nucleus and endoplasmic reticulum. Denaturated proteins are thought to serve as stimulus for induction [2,3,98]. These denaturated proteins activate heat shock factors (HSFs) within the cytosol by dissociating other Hsps that are normally bound to HSF [99]. Freed HSF is phosphorylated and forms trimers, which enter the nucleus and bind to heat shock elements within the promoters of different heat shock genes leading to transcription and synthesis of Hsps. After heat shock, for instance, the synthesis of Hsp70 increases to a point to where it becomes the most abundant single protein in a cell. Once synthesized, Hsp70 binds to denaturated proteins in an ATP-dependent manner. The N-terminal end contains an ATP-binding domain, whereas the C-terminal region contains a substrate-binding domain [99]. Heat shock proteins serve as chaperones that bind to other proteins and regulate their conformation, regulate the protein movement across membranes or through organelles, or regulate the availability of a receptor or activity of an enzyme [3,94].

In the nervous system, Hsps are induced in a variety of pathological conditions, including cerebral ischemia, neurodegenerative disorders, epilepsy and trauma. Expression of the gene encoding Hsps has been found in various cell populations within the nervous system, including neurons, glia and endothelial cells [100]. Hsps consist of both stress-inducible and constitutive family members. Whether stress proteins that are neuroprotective has been the subject of much debate, as it has been speculated that these proteins might be merely an epiphenomenon unrelated to cell survival. Only recently, however, with the availability of transgenic animals and gene transfer, it has become possible to overexpress the gene encoding Hsp70 to test directly the hypothesis that stress proteins protects cells from injury, and it has been demonstrated that overproduction of Hsp70 leads to protection in several different models of nervous system

injury [100–102]. Following focal cerebral ischemia, mRNA encoding Hsp70 is synthesized in most ischemic cells except in areas of very low blood flow, because of limited ATP levels. Hsp70 protein is produced mainly in endothelial cells, in the core of infarcts in the cells that are most resistant to ischemia, in glial cells at the edges of infarcts and in neurons outside the areas of infarction. It has been suggested that this neuronal expression of Hsp70 outside an infarct can be used to define the ischemic penumbras, the zone of protein denaturation in the ischemic areas [94]. A number of in vitro studies show that both heat shock and Hsp overproduction protect CNS cells against both necrosis and apoptosis. Mild heat shock protects neurons against glutamate-mediated toxicity and protects astrocytes against injury produced by lethal acidosis [102]. Transfection of cultured astrocytes with Hsp70 protects them from ischemia or glucose deprivation [103]. Hsp70 has been demonstrated to inhibit caspase-3 activation caused by ceramide and also affect JUN kinase and p38-kinase activation [104]. In addition, Hsp70 binds to and modulates the function of BAG-1, the bcl-2 binding protein, thus, modulating some type of apoptosis-related cell death.

A large body of evidence now suggests a correlation between mechanisms of oxidative and/or nitrosative stress and Hsp induction [9,11,16,105]. Current opinion holds also the possibility that the heat shock response can exert its protective effects through inhibition of NFkB signaling pathway [2,14]. We have demonstrated in astroglial cell cultures that cytokine-induced nitrosative stress is associated with an increased synthesis of Hsp70 stress proteins. Increase in hsp70 protein expression was also found after treatment of cells with the NO generating compound sodium nitroprusside, thus, suggesting a role for NO in inducing Hsp70 proteins. The molecular mechanisms regulating the NO-induced activation of heat shock signal seems to involve cellular oxidant/antioxidant balance, mainly represented by the glutathione status and the antioxidant enzymes [88,89,96].

Ubiquitin is one of the smallest Hsps and is expressed throughout brain in response to ischemia. It is involved in targeting and chaperoning of proteins degraded in proteasomes, which include NFkB, cyclins, HSFs, hypoxia-inducible factor, some apoptosis-related proteins, tumor necrosis factor and erythropoietin receptors [106].

Hsp27 is synthesized mainly in astrocytes in response to ischemic situations or to kainic acid administration. It chaperones cytoskeletal proteins, such as intermediate filaments, actin or glial fibrillary acidic protein following stress in astrocytes. It also protects against Fas-Apo-1, staurosporine, TNF and etoposide-induced apoptotic cell death as well as H_2O_2-induced necrosis [107,108]. *Hsp47* is synthesized mainly in microglia following cerebral ischemia and subarachnoid hemorrhage [106].

Hsp60, glucose-regulated protein 75 (GRP75) and Hsp10 chaperone proteins within mitochondria. GRP75 and GRP78, also called oxygen-regulated proteins, are produced by low levels of oxygen and glucose. These protect brain cells against ischemia and seizures in vivo after viral-induced overexpression [109,110]. Hsp60 form the chaperonin complex, which is implicated in protein folding and assembly within the mitochondria under normal conditions [109]. Most mitochondrial proteins are synthesized in the cytosol and must be imported into the organelles in an unfolded state [111]. During translocation, the proteins interact with Hsp70 ATP-dependent binding, and release of Hsp70 provides the major driving force for complete transport of polypeptides into the matrix. Although most imported polypeptides are released from soluble Hsp70, however, a subset of aggregation-sensitive polypeptides must be transferred from Hsp70 to Hsp60 for folding [111]. Owing to the close functional interaction between this chaperonin system and the Hsp70 system, it is likely that up-regulation of Hsp60 may be a fundamental site targeted by LAC action, with consequent restoration of complex IV function, under conditions of nitrosative stress-dependent perturbation of mitochondrial function [17].

HSP32 or HO is the rate-limiting enzyme in the production of bilirubin. There are three isoforms of HO, HO-1 or inducible isoform, HO-2 or constitutive isoform and the recently discovered HO-3 [112–117].

9. Heme oxygenase system: a putative vitagene target for neuroprotection

Hsp32 or HO is the rate-limiting enzyme in the production of bilirubin. In the last decade, the HO system has been strongly highlighted for its potential significance in maintaining cellular homeostasis. It is found in the endoplasmic reticulum in a complex with NADPH cytochrome *c* P450 reductase. It catalyzes the degradation of heme in a multistep, energy-requiring system. The reaction catalyzed by HO is the α-specific oxidative cleavage of the heme molecule to form equimolar amounts of biliverdin and CO. Iron is reduced to its ferrous state through the action of NADPH cytochrome *c* P450 reductase. Carbon monoxide is released by elimination of the α-methene bridge of the porphyrin ring. Further degradation of biliverdin to bilirubin occurs through the action of a cytosolic enzyme, biliverdin reductase. Biliverdin complexes with iron until its final release [14,94,113]. Heme oxygenase is present in various tissues with the highest activity in the brain, liver, spleen and testes. There are three isoforms of HO, HO-1 or inducible isoform [112,113], HO-2 or constitutive isoform [114–116] and the recently discovered HO-3, cloned only in rat to date [117,118]. They are all products of different genes, and, unlike HO-3, which is a poor heme catalyst, both HO-1 and HO-2 catalyze the same reaction (i.e., degradation of heme) but differ in many respects and are regulated under separate mechanisms. The most relevant similarity between HO-1 and HO-2 consists in a common 24 amino acid domain (differing in just one residue) called the “HO signature,” which renders both

proteins extremely active in their ability to catabolize heme [119]. They have different localization, similar substrate and cofactor requirements while presenting different molecular weight. They also display different antigenicity, electrophoretic mobility, inducibility as well as susceptibility to degradation. The proteins for HO-1 and HO-2 are immunologically distinct, and in humans, the two genes are located on different chromosomes, that is, 22q12 for HO-1 and 16q13.3 for HO-2, respectively [116].

Various tissues have different amounts of HO-1 and HO-2. Brain and testes have a predominance of HO-2, whereas HO-1 predominates in the spleen. In the lung not subjected to oxidative stress, more than 70% of HO activity is accounted for by HO-2, whereas in the testes, the pattern of HO isoenzyme expression differs according to the cell type, although HO-1 expression predominates after heat shock. This also occurs in brain tissue, where HO isoforms appears to be distributed in a cell-specific manner, and HO-1 distribution is widely apparent after heat shock or oxidative stress. Although previous reports from our and other groups have not found detectable levels of HO-1 protein in normal brain [105,115], we have recently demonstrated that HO-1 mRNA expression is physiologically detectable in the brain and shows a characteristic regional distribution, with high level of expression in the hippocampus and the cerebellum [112,120]. This evidence may suggest the possible existence of a cellular reserve of HO-1 transcript quickly available for protein synthesis and a posttranscriptional regulation of its expression.

Heme oxygenase isoenzymes are also seen to colocalize with different enzymes dependently on the cell type. In the kidney, HO-1 colocalizes with erythropoietin, whereas in smooth muscle cells, HO-1 colocalizes with nitric oxide synthase. In neurons, HO-2 colocalizes with NOS, whereas endothelium exhibits the same isoform to colocalize with NOS III. The cellular-specificity of this pattern of colocalization lends further support to the concept that CO may serve as a function similar to that of NO. Furthermore, the brain expression pattern shown by HO-2 protein and HO-1 mRNA overlaps with distribution of guanylate cyclase, the main CO functional target [121].

Heme oxygenase-3, the third isoform of HO, shares a high homology with HO-2, both at the nucleotide (88%) and protein (81%) levels. Both HO-2 and HO-3, but not HO-1, are endowed with two Cys-Pro residues considered the core of the heme-responsive motif (HRM), a domain critical for heme binding but not for its catalysis [122].

Although the biological properties of this isoenzyme still remains to be elucidated, the presence of two HRM motifs in its amino acidic sequence might suggest a role in cellular heme regulation [122]. Studying HO-3 mRNA sequence (GenBank accession no.: AF058787), we have observed that its 5′ portion corresponds to the sequence of a L-1 retrotransposon, a member of a family of retrotransposons recently involved in evolutionary mechanisms [123]. Based on the close similarity to a paralogous gene (HO-2) and the preliminary data from our group demonstrating no introns in the HO-3 gene [110], it is possible that this last could have originated from the retrotransposition of the HO-2 gene. In addition, this genetic mutation in rat may represent a *specie*-specific event because no other sequence in the public databases match the rat HO-3.

Induction of HO-1 gene could be used clinically. However, the GT length polymorphism in the promoter of the gene encoding HO-1 that regulates the magnitude of the HO-1 response to a given stress signal can render this approach difficult for those individuals with the long GT repeats that are associated with low HO-1 responsiveness. This polymorphism appears to be of functional significance in that short repeats, which are associated to high responsiveness, seem to be also associated with lesser likelihood of restenosis after angioplasty [124].

10. Heme oxygenase-1 and Hsp70 as a therapeutic funnel

It is becoming increasingly clear that genetic factors are prominently involved in aging, the major lines of empirical evidence being (a) the life span in human populations shows significant heritability; (b) different species have different intrinsic life spans due to genomic differences; (c) human populations possess inherited progeroid disorders, such as Werner's syndrome, a disease characterized by premature age-related disorders, including atherosclerosis, type II diabetes, osteoporosis and cancers; (d) clear evidence of genetic effects on life span has been demonstrated in invertebrate model system, such as *Drosophila melanogaster* and *Caenorhabditis elegans* [125].

At least three categories of genes are predicted to affect aging and longevity: (1) genes that regulate levels of somatic maintenance and repair; (2) pleiotropic genes, whose expression involves trade-offs between early-life fitness benefits and late-life fitness disadvantages, which do not encompass somatic maintenance; (3) late-acting deleterious mutations that have escaped elimination as consequence of the decline in force of natural selection at old ages [125].

From an evolutionary perspective, aging is a nonadaptive phenomenon because it limits the reproductive potential of an individual. A clear prediction is that the actual mechanisms of senescence are stochastic, involving most likely processes such as random accumulation of somatic mutations or oxidative damage to macromolecules.

Increase in longevity in mammals may be seen as a combination of two main factors: a reduction in the rates of growth and reproduction, the so-called essential life [10], and, concomitantly, an increase in the accuracy of synthesis of macromolecules. This theory can be tested by measuring accuracy in germ line and somatic cells and also by comparing somatic cells from mammals with different longevities. Notably, the HO gene is evolutionarily different in birds and mammals, with the biliverdin reductase-bilirubin step present in the latter case, but absent in the

former group. Consistent with this notion, the organism sacrifices the potential for indefinite survival in favor of earlier and more prolific fecundity.

Efficient functioning of maintenance and repair process seems to be crucial for both survival and physical quality of life. This complex network of the so-called longevity assurance processes is composed of several genes, termed vitagenes. [9,10,11]. The homeodynamic property of living systems is a function of such a vitagene network. Because aging is characterized by the failure of homeodynamics, a decreased efficiency and accuracy of the vitagene network can influence gerontogenic processes [10]. It is not clear how various components of the vitagene network operate and influence each other in a concordant or a discordant manner. Because aging is characterized by a progressive failure of maintenance and repair, it is reasoned that genes involved in homeodynamic repair pathways, such as the HO-1 or Hsp70 genes, are the most likely candidate vitagenes [9,11].

A promising approach for the identification of critical vitagene-related processes is represented by the hormesis-like positive effect of stress, including regular muscle exercise [9,11,14] caloric restriction, which can result in activation of the Hsp signal pathway and, consequently, in stress tolerance. In particular, there is a strong evidence that the HO/CO and biliverdin–bilirubin redox system might work critically as a *therapeutic funnel* in a number of physiopathological situations where the sensing of redox active events is coupled to acquirement of major resistance to the effect of stressful and pathogenic conditions (Fig. 2). Heme oxygenase-1 activity seems to be required for the action of several other therapeutic molecules. In each case, the expression of HO-1 or administration of one of its metabolic products substitutes for the actions of the other protective molecule [124].

In many inflammatory situations, the ability of IL-10 to suppress TNFα expression in macrophages requires the presence of HO-1 and the generation of CO; HO-1 expression or CO administration has the same effects as IL-10 [126]. In concert with this conceivable possibility, the protective effect of IL-10 in a lethal endotoxic shock mice model is strongly dependent on the expression of HO-1 and the generation of CO [124]. Moreover, rapamycin appears not to exert its antiproliferative effects on smooth muscle cells unless HO-1 is present [127], and it has been proven that in order for NO to protect mice livers from hepatitis induced by TNFα and galactosamine, up-regulation of HO-1 seems to be essential. Also, alcohol has anti-inflammatory effects in that TNFα is suppressed and IL-10 is increased. However, protection is lost when HO-1 is blocked [128]. In addition, the anti-inflammatory effect of 15-deoxy-Delta 12, 14-prostaglandin J2 has been shown to require the activity of HO-1. Notably, during heat shock, which leads to up-regulation of several heat shock proteins endowed with cytoprotective actions, entire cytoprotection is lost if HO-1 is blocked with SnPPIX. Last, relevant to brain physiopathology, dietary and medicinal phytochemicals that can inhibit, retard or reverse the multistage pathogenic events associated with degenerative damage, particularly polyphenols such as curcumin, caffeic acid and ferulic acid, all capable of

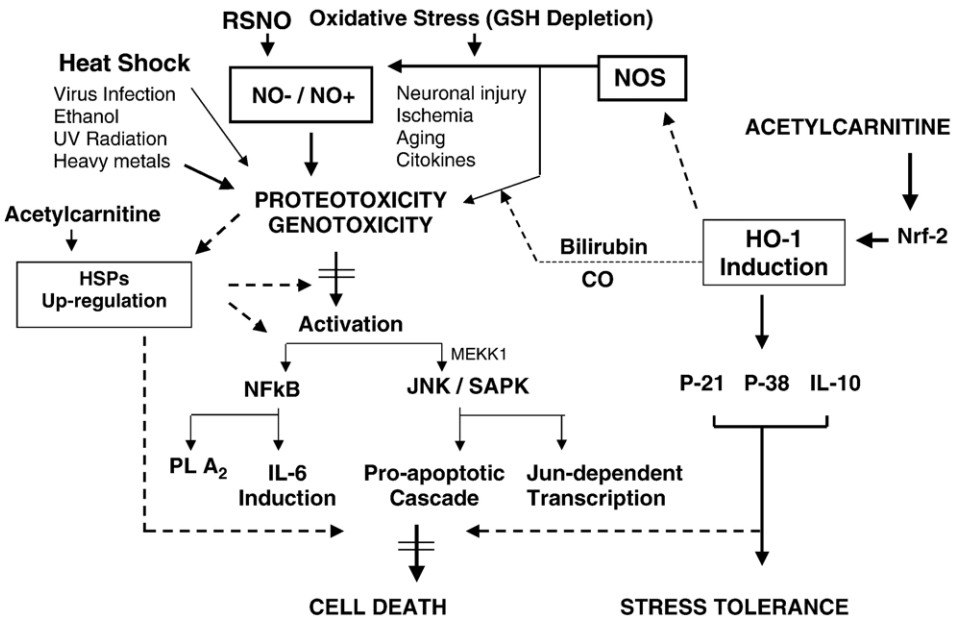

Fig. 2. Redox regulation of gene expression involving the Vitagene system: role of acetylcarnitine. Proposed role for the *vitagene* member HSPs in modulation of the cellular redox state and the role of acetylcarnitine in cell stress tolerance. Various proteotoxic (or genotoxic) conditions cause depletion of free HSPs that lead to activation of stress kinase and pro-inflammatory and apoptotic signaling pathways. HSP70 prevents stress-induced apoptosis by interfering with the SAPK/JNK signaling and by blocking caspase proteolytic cascade. Acetylcarnitine, through activation of Nrf-2 transcription factor, by up-regulating HO-1 and Hsp60, may counteract nitrosative stress and NO-mediated neurotoxicity. In addition, HO-1 may directly decrease NO synthase protein levels by degrading the cofactor heme (PLA_2: phospholipase A_2; IL: interleukin; SAPK: stress-activated protein kinase; JNK: c-*Jun* N-terminal kinase; GSNO: *S*-nitrosoglutathione).

exerting powerful anti-inflammatory action, have been shown to function by up-regulation of HO-1 [94,129–131]. The fact that in all these situations, specific molecules or biological phenomena appear to lose most, if not all, of their effect when HO-1 is absent, represent a compelling evidence that HO-1 system may represent a final common mediator of many biological events associated to cell stress response and, as such, working as a critical *vitagene*, which links redox-dependent pathways of stress tolerance to a versatile biological program of cell life.

11. Caloric restriction and endogenous oxidative stress: relevance to aging and cell survival

Caloric restriction in mammals has been recognized as the best characterized and most reproducible strategy for extending maximum survival, retarding physiological aging and delaying the onset of age-related pathological situations. The overwhelming majority of studies using caloric restriction has used short-lived rodent species, although current work using monkeys should reveal whether this paradigm is also relevant to manipulating the rate of primate aging. The mechanisms by which restricted calorie intake modifies the rate of aging and cellular pathology have been the subject of much controversy, although an attenuation of accumulating oxidative damage appears to be a central feature [132]. A major effect of calorie-restricted feeding now appears to be on the rate of production or leak of free radicals from mitochondrial sites, although the details of the adaptation and the signaling pathway that induces this effect are currently unknown. General consensus, however, has been achieved that caloric restriction feeding regimes reduces the rate of accrual of oxidative damage as measured by lipid peroxidation, nuclear and mtDNA damage, and protein carbonyl formation. An analysis of published studies that used a degree of food restriction in the range of 40–50% ad libitum intake revealed a significant positive correlation between survival parameters, such as mean, maximum and average survival time, and duration of caloric restriction. The longer the animals are maintained on low-calorie intake during the postweaning period of the life span, the greater is the survival [133]. It is unclear whether caloric restriction protects against random oxidative damage per se or is protective for those vulnerable proteins of key pathways, such as those containing iron–sulfur centers of the ETC or DNA-binding signaling proteins. This is directly related to the question whether oxidative damage in genomic and mtDNA is primarily random as a function of age or whether there is a specific pattern of distribution of ROS, which may vary dependent on the tissue or the state of the cell cycle within any particular cell. It is generally accepted that age-related accrual of ROS-induced damage represents a balance between generation and defenses, such as antioxidant enzymes, repair systems and turnover. It has been demonstrated that caloric restriction reduces cellular injury and improves heat tolerance of old animals by lowering radical production and preserving cellular ability to adapt to stress through antioxidant enzyme induction and translocation of these proteins to the nucleus [134]. It has been also demonstrated that mitochondria from calorie-restricted animals produce less ROS per nanomole of O_2 during state 4 respiration, and recent work on ETC complexes suggests a modification in the Km for complex III associated with a retention of high-affinity binding sites for complex IV as a possible mechanism operating in reducing superoxide generation [135]. It is conceivable that low-calorie-induced changes in unsaturated fatty acid composition of the mitochondrial membranes not only may protect against ROS-induced lipid peroxidation but also may influence the binding properties of ETC proteins embedded in the membrane, and the related transport processes. However, several questions need to be addressed, such as the signaling pathway underlying the adaptive responses triggered by caloric restriction, or the effect of chronic caloric restriction on either the bioenergetic of individual mitochondria, or mitochondrial number and turnover rate. High-density oligonucleotide arrays studies have recently provided compelling evidence that aging results in a differential gene expression pattern indicative of a marked stress response associated with lower expression of metabolic and biosynthetic genes, and also, these alterations are either completely or partially prevented by caloric restriction. In addition, the transcriptional patterns of calorie-restricted animals suggests that caloric restriction retards the aging process by causing a metabolic shift toward increased protein turnover and decreased macromolecular damage [136].

12. Conclusion

Modulation of endogenous cellular defense mechanisms via the stress response signaling represents an innovative approach to therapeutic intervention in diseases causing tissue damage, such as neurodegeneration. Efficient functioning of maintenance and repair processes seems to be crucial for both survival and physical quality of life. This is accomplished by a complex network of the so-called longevity assurance processes, which are composed of several genes termed vitagenes [9–11]. By maintaining or recovering, the activity of vitagenes can possibly delay the aging process and decrease the occurrence of age-related diseases with resulting prolongation of a healthy life span. As one of the most important neurodegenerative disorders, AD is a progressive disorder with cognitive and memory decline, speech loss, personality changes and synapse loss. With the increasingly aging population of the United States, the number of AD patients is predicted to reach 14 million in the mid-21st century in the absence of effective interventions [8,13]. This will pose an immense economic and personal burden on the people of this country. Similar considerations apply worldwide, except in sub-Sahara

Africa, where HIV infection rates seem to be leading to decreased incidence of AD [137]. There is now a strong evidence to suggest that factors such as oxidative stress and disturbed protein metabolism and their interaction in a vicious cycle are central to AD pathogenesis. Brain-accessible antioxidants, potentially, may provide the means of implementing this therapeutic strategy of delaying the onset of AD, and more in general, all degenerative diseases associated with oxidative stress [138,139]. As one potentially successful approach, potentiation of endogenous secondary antioxidants systems can be achieved by interventions that target the HO-1/CO and/or Hsp70 systems. In this review, the importance of the stress response signaling and, in particular, the central role of HO-1 together with the redox-dependent mechanisms involved in cytoprotection are outlined (Fig. 2). The beneficial effects of HO-1 induction result from heme degradation and cytoprotective regulatory functions of biliverdin/bilirubin redox cycling. Thus, HO-1 can amplify intracellular cytoprotective mechanisms against a variety of insults. Consequently, induction of HO-1, by increasing CO and/or biliverdin availability, can be of clinical relevance.

Very importantly, HO-1 and CO can suppress the development of atherosclerotic lesions associated with chronic rejection of transplanted organs [140]. Interestingly, the recent discovered role of acetylcarnitine as a molecule endowed with the capability of potentiating cellular stress response pathways appear to afford similar protective action, thereby providing an alternative therapeutic approach for those pathophysiological conditions where stimulation of the HO pathway is warranted [141]. Although clinical application of compounds potentiating the action of stress responsive genes should be fully considered, a better understanding of how HO mediates its action will guide therapeutic strategies to enhance or suppress HO effects. Remarkably, the recent envisioned role of Hsp70 as a vehicle for intracytoplasmic and intranuclear delivery of fusion proteins or DNA to modulate gene expression [142], along with the evidence that binding of HO protein to HO-1 DNA modifies HO expression via nonenzymatic signaling events associated to CO and p-38-dependent induction of Hsp70 [143]. This opens intriguing perspectives, as it is possible to speculate that synergy between these two systems might impact cell proliferation and apoptotic processes during oxidative stress, hence, contributing to programmed cell life or programmed cell death, depending on the relative extent of activation.

Presented here is a strong evidence that a cross talk between stress response genes is critical for cell stress tolerance, highlighting compelling reasons for a renewed effort to understand the central role of this most extraordinary defense system in biology and medicine. All of the above evidence supports also the notion that stimulation of various maintenance and repair pathways through exogenous intervention, such as mild stress, or compounds targeting the heat shock signal pathway, such as acetylcarnitine, may have biological significance as a novel approach to delay the onset of various age-associated alterations in cells, tissues and organisms. Hence, the activity of vitagenes can possibly delay the aging process and decrease the occurrence of age-related diseases with resulting prolongation of a healthy life span.

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