CHAPTER 4

Heme Oxygenase as a Therapeutic Funnel in Nutritional Redox Homeostasis and Cellular Stress Response:
Role of Acetylcarnitine

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Abstract

Reduction of cellular expression and activity of antioxidant proteins and the consequent increase of oxidative stress are fundamental causes for both the aging processes and neurodegenerative diseases. Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various pathological states of the brain. Alzheimer's disease (AD) is a progressive disorder with cognitive and memory decline, speech loss, personality changes and synapse loss. Many approaches have been undertaken to understand AD, but the heterogeneity of the etiologic factors makes it difficult to define the clinically most important factor determining the onset and progression of the disease. There is now evidence to suggest that networks of responses exist in the brain to 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. Recent studies have shown that the heat shock response contributes to establish 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 strong interest in discovering and developing pharmacological agents capable of inducing the heat shock response. Acetylcarnitine (LAC) is proposed as a therapeutic agent for several neurodegenerative disorders, and there is evidence that LAC may play a critical role as a modulator of cellular stress response in health and disease states. In the present review we discuss the role of the heme oxygenase pathway in cellular stress response. We then review the evidence for the role of acetylcarnitine in modulating redox-dependent mechanisms leading to up-regulation of vitagenes in brain, and hence potentiate brain stress tolerance.

Introduction

It is well established that living cells are constantly challenged by conditions which cause acute or chronic stress. The brain has a large potential oxidative capacity but a limited ability to

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counteract oxidative stress.[1-3] Within the cell, reactive oxygen species (ROS) are physiologically present at minimal concentration as by-products of aerobic metabolism as well as second messengers in many signal transduction pathways and, in normal conditions, there is a steady-state balance between pro-oxidants and antioxidants which is necessary to ensure optimal efficiency of antioxidant defenses.[4-7] However, when the rate of free radical generation exceeds the capacity of antioxidant defenses, oxidative stress ensues with consequential severe damage to DNA, protein and lipids.[8-10] Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various pathological states of the brain, including neurodegenerative disorders such as Alzheimer's disease (AD).[11-15]

Recently the term "nitrosative stress" has been used to indicate the cellular damage elicited by nitric oxide and its congeners peroxynitrite, N_2O_3, nitroxyl anion and nitrosonium (all can be indicated as reactive nitrogen species or RNS).[16-18]

From a molecular point of view, the cell is able to fight against oxidant stress using many resources, including vitamins (A, C and E), bioactive molecules (glutathione, thioredoxin, flavonoids), enzymes (Heat shock protein-32, superoxide dismutase, catalase, glutathione peroxidases, thioredoxin reductase, etc) and redox sensitive protein transcriptional factors (AP-1, NFkB, Nrf-2, HSF, etc). The heat shock proteins (Hsps) are one of the more studied defense system active against cellular damage.

In this chapter we describe the more recent discoveries about the biochemical changes occurring in the central nervous system (CNS), when brain cells are exposed to chronic oxidative insult as well as the key role played by the heat shock response, particularly the heme oxygenase (Hsp32) and Hsp70 pathways, in modulating the onset and progression of AD. Whether or not stress proteins are neuroprotective is still under debate; however, emerging evidence underscores the high potential of the Hsp system as a target for new neuroprotective strategies, expecially those aimed at minimizing deleterious consequences associated with oxidative stress, such as in neurodegenerative disorders and brain aging. We review here also the evidence for the role of acetylcarnitine in modulating redox-dependent mechanisms leading to up-regulation of vitagenes in brain, and hence potentiate brain stress tolerance.

Heme Oxygenase-1

Heme oxygenase-1 (HO-1), also referred to as heat shock protein-32, is the redox-sensitive inducible isoform of the HO family. Heme oxygenase is a microsomal enzyme and catalyzes the degradation of heme in a multistep, energy-requiring system. The reaction catalyzed by HO is the α-specific oxidative cleavage of heme moieties to form equimolar amounts of ferrous iron, carbon monoxide (CO) and biliverdin. This latter is then reduced by the cytosolic enzyme biliverdin reductase to bilirubin (BR), which is then conjugated with glucuronic acid and excreted.[19]

Increasing evidence suggested that the HO-1 gene is redox regulated (Fig. 1) and contains in its promoter region the antioxidant responsive element (ARE), similar to other antioxidant enzymes.[20] In fact HO-1 can be induced by several stimuli including oxidative and nitrosative stress, ischemia, heat shock, LPS, hemin and the neuroprotective agent neotrofin.[21-23] HO-1 induction is one of the earlier cellular responses to tissue damage and is responsible for the rapid transformation of the pro-oxidant heme into CO and BR, two molecules with anti-inflammatory and anti-oxidant activity.[24-27]

The HO-1 gene is induced by other factors (Fig. 2), including metallophorphyrins and hemin, as well as ultraviolet A (UVA) irradiation, hydrogen peroxide, pro-oxidant states or inflammation.[28,29] This characteristic inducibility of HO-1 gene strictly relies on its configuration: the 6.8-kilobase gene is organized into 4 introns and 5 exons. A promoter sequence is located approximately 28 base pairs upstream from the transcriptional site of initiation. In addition, different transcriptional enhancer elements, such as heat shock element and metal regulatory element reside in the flanking 5' region. Also, inducer-responsive sequences have been identified in the proximal enhancer located upstream the promoter and, more distally, in two enhancers located 4kb and 10 kb upstream the initiation site.[30] The molecular mechanism

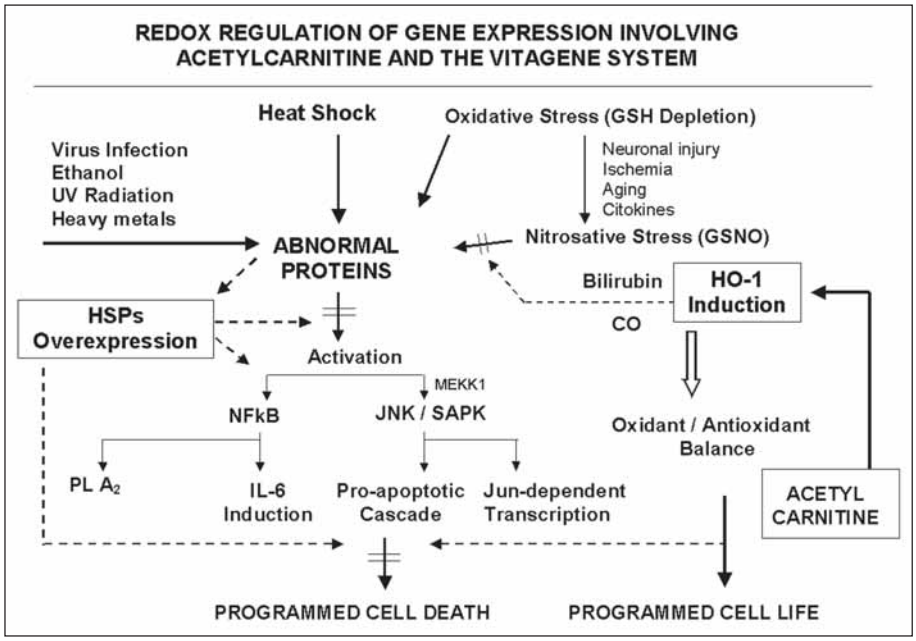

Figure 1. Redox regulation of gene expression involving Acetylcarnitine and the Vitagene system. Proposed role for acetylcarnitine and the *vitagene* member HSPs, in modulation cellular redox state and cell stress tolerance. Various proteotoxic (or genotoxic) conditions cause depletion of free HSPs that lead to activation of stress kinase and proinflammatory and apoptotic signaling pathways. HSP70 prevents stress-induced apoptosis by interfering with the SAPK/JNK signaling and by blocking caspase proteolytic cascade. Nitrosative-dependent thiol depletion triggers HO-1 induction, and increased HO-1 activity is translated into augmented production of carbon monoxide and the antioxidant bilirubin. Exogenous non toxic inducers, such as acetylcarnitine or polyphenols can counteract increased NOS activity and NO-mediated cytotoxicity through up-regulation of the HO system. HO-1 may directly decrease NO synthase protein levels by degrading the cofactor heme. (PLA_2: phospholipase A_2; IL-6: interleukin-6; AP-1: activator protein-1; SAPK: stress-activated protein kinase; JNK: c-jun N-terminal kinase; NFκB: nuclear factor kappa-B; GSNO: S-nitrosoglutathione; HO-1: heme oxygenase-1).

that confers inducible expression of *ho-1* in response to numerous and diverse conditions has remained elusive. One important clue has recently emerged from a detailed analysis of the transcriptional regulatory mechanisms controlling the mouse and human *ho-1* genes. The induction of *ho-1* is regulated principally by two upstream enhancers, E1 and E2.[31] Both enhancer regions contain multiple stress (or antioxidant) responsive elements (StRE, also called ARE) that also conform to the sequence of the Maf recognition element (MARE)[32] with a consensus sequence (GCnnnGTA) similar to that of other antioxidant enzymes.[33] There is evidence to suggest that heterodimers of NF-E2-related factors 2 (Nrf2) and one or another of the small Maf proteins (i.e., MafK, mafF and MafG) are directly involved in induction of *ho-1* through these MAREs.[32] A possible model, centered on Nrf2 activity, suggests that the *ho-1* locus is situated in a chromatin environment that is permissive for activation. Since the MARE can be bound by various heterodimeric basic leucine zipper (bZip) factors including NF-E2, as well as several other NF-E2-related factors (Nrf1, Nrf2, and Nrf3), Bach, Maf and AP-1 families,[31] random interaction of activators with the *ho-1* enhancers would be expected to cause spurious expression. This raises a paradox as to how cells reduce transcriptional noise from the *ho-1* locus in the absence of metabolic or environmental stimulation. This problem could be

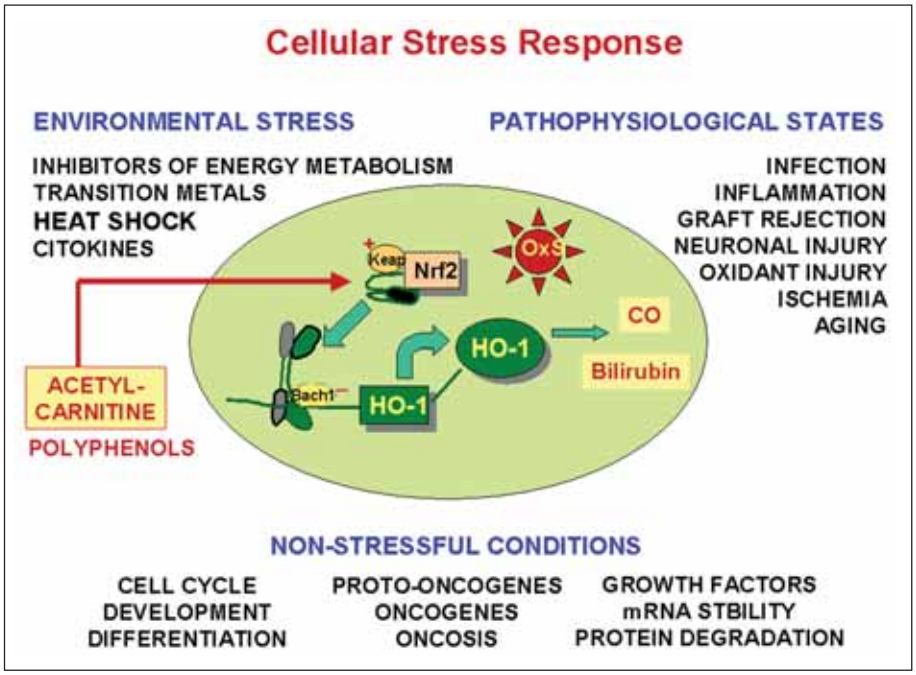

Figure 2. Physiological and pathophysiological conditions inducing cellular stress response. Environmental stress factors, such as heavy metals, cytokines, heat shock or energy metabolism inhibitors or pathophysiological conditions of oxidant antioxidant balance perturbation such as inflammation, graft rejections, neuronal damage, ischemia and brain aging are all situations associated with induction of cellular stress response. Hsp response is also involved in cellular homeostasis during various physiological conditions, such as during brain development and differentiation, cell cycle, apoptosis and oncosis, oncogene and growth factors action, as well as mRNA and protein half-life. The heme oxygenase system represents a therapeutic funnel for cellular stress tolerance and can be activated by non noxious stimuli, such as nutritional antioxidants or acetylcarnitine. Acetylcarnitine, through activation of the redox sensitive transcription factor Nrf-2, by up-regulating HO-1 may counteract nitrosative stress and NO-mediated neurotoxicity. In the same figure are described the respective roles of protein factors Bach-2 (positive) and Keap (negative) in Nrf2 activation.

reconciled by the activity of repressors that prevent nonspecific activation. One possible candidate is the heme protein Bach1, a transcriptional repressor endowed with DNA binding activity, which is negatively regulated upon binding with heme. Bach1-heme interaction is mediated by evolutionarily conserved heme regulatory motifs (HRM), including the cysteine-proline dipeptide sequence in Bach1. Hence, a plausible model accounting for the regulation of *ho-1* expression by Bach1 and heme, is that expression of *ho-1* gene is regulated through antagonism between transcription activators and the repressor Bach1 (Fig. 2). While under normal physiological conditions expression of *ho-1* is repressed by Bach1/Maf complex, increased levels of heme displace Bach1 from the enhancers and allow activators, such as heterodimer of Maf or Keap with NF-E2 related activators (Nrf2), to the transcriptional promotion of *ho-1* gene[31] (Fig. 2). To our knowledge, the Bach1- *ho-1* system is the first example in higher eukaryotes that involves a direct regulation of a transcription factor for an enzyme gene by its substrate. Thus, regulation of *ho-1* involves a direct sensing of heme levels by Bach1 (by analogy to *lac* repressor sensitivity to lactose), generating a simple feedback loop whereby the substrate affects repressor-activator antagonism.

The promoter region also contains two metal responsive elements, similar to those found in metallothionein-1 gene, which respond to heavy metals (cadmium and zinc) only after recruitment of another fragment located upstream, between -3.5 and 12 kbp (CdRE). In addition, a 163-bp fragment containing two binding sites for HSF-1, which mediates the HO-1 transcription are located 9.5 kb upstream of the initiation site.[34] The distal enhancer regions are important in regulating HO-1 in inflammation, since, as has been demonstrated, they are responsive to endotoxin. In the promoter region also resides a 56 bp fragment which responds to the STAT-3 acute-phase response factor, involved in the down-regulation of HO-1 gene induced by glucocorticoid.[35,36]

HO-1, Oxidative Stress and Neurodegenerative Disorders

The mechanisms responsible for neuronal death are not completely elucidated, even if many studies suggest that ROS are primarily involved in the genesis of neurodegenerative disorders.[11-15,37-39] Due to its strong antioxidant properties and wide distribution within the CNS HO-1 has been proposed as a key enzyme in the prevention of brain damage.[21,22,40] Recently, Panahian et al using transgenic mice over-expressing HO-1 in neurons, demonstrated the neuroprotective effect of this enzyme in an experimental model of ischemic brain damage.[41] The neuroprotective effects of over-expressed HO-1 can be attributed to: (i) increase in cGMP and bcl-2 levels in neurons; (ii) inactivation of p53, a protein involved in promoting cell death; (iii) increase in antioxidant sources and (iv) increase in the iron sequestering protein, ferritin.[41] Particularly interesting is the role played by HO-1 in AD, a neurodegenerative disorder which involves a chronic inflammatory response associated with both oxidative brain injury and β-amyloid associated pathology. Significant increases in the levels of HO-1 have been observed in AD brains in association with neurofibrillary tangles and also HO-1 mRNA was found increased in AD neocortex and cerebral vessels.[42,43] The HO-1 increase was not only in association with neurofibrillary tangles, but also colocalized with senile plaques and glial fibrillary acidic protein-positive astrocytes in AD brains.[44] It is plausible that the dramatic increase in HO-1 in AD may be a direct response to an increase in free heme concentrations, associated with neurodegeneration, and can be considered as an attempt of brain cells to convert the highly toxic heme into the antioxidants CO and BR.

The protective role played by HO-1 and its products in AD raised new possibilities regarding the possible use of natural substances, which are able to increase HO-1 levels, as potential drugs for the treatment of AD. In this light, very promising are the polyphenolic compounds contained in some herbs and spices, e.g., curcumin.[45-47] Curcumin is the active anti-oxidant principle in *Curcuma longa*, a colouring agent and food additive commonly used in Indian culinary preparations. This polyphenolic substance has the potential to inhibit lipid peroxidation and to effectively intercept and neutralize ROS and RNS.[48] In addition, curcumin has been shown to significantly increase HO-1 in astrocytes and vascular endothelial cells.[46,49] This latter effect on HO-1 can explain, at least in part, the anti-oxidant properties of curcumin, in particular keeping in mind that HO-1-derived BR has the ability to scavenge both ROS and RNS.[24,27,50,51] Epidemiological studies suggested that curcumin, as one of the most prevalent nutritional and medicinal compounds used by the Indian population, is responsible for the significantly reduced (4.4- fold) prevalence of AD in India compared to United States.[52] Based on these findings, Lim and colleagues have provided convincing evidence that dietary curcumin given to an Alzheimer transgenic APPSw mouse model (Tg2576) for 6 months resulted in a suppression of indices of inflammation and oxidative damage in the brain of these mice.[53] Furthermore, in a human neuroblastoma cell line it has recently been shown that curcumin inhibits NFkB activation, efficiently preventing neuronal cell death.[48]

Although it is generally agreed that HO-1 over-expression is a common feature during oxidative stress, recent papers demonstrated that HO-1 can be repressed following oxidant conditions. In particular human cells exposed to hypoxia, thermal stress and interferon-γ treatment showed a marked HO-1 repression and this effect seems to be peculiar for human,

because rodent cells over-expressed HO-1 when exposed to the same stimuli.[54-57] The importance of HO-1 repression has been corroborated by the discovery of Bach-1/Bach-2 as heme-regulated transcription factors for the HO-1 gene.[58] In fact, Bach-1 is broadly expressed in mice and human tissues and, in human cells, it is induced by the same stimuli which are able to repress the HO-1 gene.[54,59-61] The reason why the cell should react to an oxidant stress by repressing HO-1 gene is strictly related to the maintenance of a good metabolic balance during stressful conditions. The current hypothesis suggests that HO-1 repression is useful for the cell because this (i) decreases the energy costs necessary for heme degradation; (ii) reduces the accumulation of CO and BR, which can become toxic if produced in excess; and (iii) increases the intracellular content of heme necessary for the preservation of vital functions such as respiration and defense.[60]

Carbon Monoxide and Stress Response

Carbon monoxide (CO) is the gaseous products of HO and it has been found to play a role in several biological phenomena, including hippocampal long-term potentiation, nonadrenergic noncholinergic gastrointestinal relaxation and vasodilatation, and is currently regarded as a neuromodulator in the peripheral and central nervous system (for an extensive review on CO and its functions in the nervous system see ref. 40). Evidence from in vitro and in vivo studies suggests that the HO-CO pathway is involved in the modulation of the neuroendocrine mechanism of stress. Thus, increased CO generation is clearly associated with the inhibition of K^+ stimulated arginine vasopressin (AVP) and oxytocin release from rat hypothalamic explants, whereas the inhibition of HO activity significantly potentiates the LPS-induced increase in AVP circulating levels while reducing the hypothalamic content of this neuropeptide.[62-64] With regards to corticotropin-releasing hormone (CRH), the effects of CO on the release of this hormone are contradictory, since increases in CO generation induced by the HO substrates, hematin and hemin, were associated with reduced or enhanced CRH release respectively, in two different in vitro models.[65,66] As far as the intracellular mechanism(s) by which CO exerts its biological functions, it is generally agreed that this gas activates the cytosolic form of guanylyl cyclase (sGC), which in turn increases intracellular cGMP levels.[22] However during the last ten years many studies have appeared in literature demonstrating that CO signals through the activation of alternative intracellular signal transduction pathways. Studies from our laboratory suggested that the activation of another hemoprotein, cyclooxygenase (COX), plays a significant role in CO signaling in the rat hypothalamus. In these studies we demonstrated that hemin, the precursor of CO via HO, dose-dependently increases PGE_2 production from rat hypothalamus in vitro and this effect is specifically due to CO because it is counteracted by the HO inhibitor Sn-mesoporphyrin-IX and oxyhemoglobin, the latter being a well known scavenger for CO.[67] The direct evidence about the stimulatory role of CO on PGs production was obtained by incubating hypothalami directly in CO saturated solutions and measuring significantly increased PGE_2 levels with respect to control tissue.[25] Recently Jaggar et al,[68] in a very elegant paper, demonstrated that exogenous or endogenously produced CO dilates cerebral arterioles by directly activating large-conductance Ca^{2+}-activated K^+ (K_{Ca}) channels primarily by increasing the coupling ratio and amplitude relationship between Ca^{2+} sparks and K_{Ca} channels. Although CO is a potent and effective activator of K_{Ca} channels, the gas does not dilate arterioles in the absence of Ca^{2+} sparks. Therefore, CO appears to act by priming K_{Ca} channels for activation by Ca^{2+} sparks, and this ultimately leads to arteriole dilation via membrane hyperpolarization.[68] Finally, Otterbein et al[26] have shown that in organs and tissues different from brain, CO exerts anti-inflammatory and anti-apoptotic effects dependent on the modulation of the p38 MAPK-signaling pathway. By virtue of these effects, CO confers protection in oxidative lung injury models, and likely plays a role in HO-1 mediated tissue protection.[69]

Heat Shock Protein-70

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 GRP-75 (a constitutively expressed glucose-regulated protein found in the endoplasmic reticulum).

Only recently, the availability of transgenic animals and gene transfer allowed to over-express the gene encoding for Hsp70, thus demonstrating that overproduction of this protein leads to protection in several different models of nervous system injury.[70,71] Following focal cerebral ischemia, Hsp70 mRNA is synthesized in most ischemic cells except in areas of very low blood flow, due to scarce ATP levels. Hsp70 proteins are 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[72] It has been suggested that this neuronal expression of Hsp70 outside an infarct can be used to define the ischemic penumbras, i.e., the zone of protein denaturation in the ischemic areas.[72]

As mentioned above, Hsps are induced in many neurodegenerative disorders mainly in the view of its cytoprotective function. Hsp72 was overexpressed in post-mortem cortical tissue of AD patients and an increase in Hsp70 mRNA was found in cerebellum, hippocampus and cortex of AD patients during the agonal phase of the disease.[73-75] Recently Kakimura et al[76] demonstrated that Hsp70 induces IL-6 and TNF-α in microglial cells, and this event is associated with an increased phagocytosis and clearance of Aβ peptides. The same authors hypothesize that Hsps could activate microglial cells through NFkB and p-38 MAPK-dependent pathways.[76]

A large body of evidence now suggest a correlation between mechanisms of nitrosative stress and Hsp induction. We have demonstrated in astroglial cell cultures that cytokine-induced nitrosative stress is associated with an increased synthesis of Hsp70 stress 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.[77,78]

Acetylcarnitine

Mitochondria are cellular organelles involved in many metabolic processes such as pyruvate oxidation, the tricarboxylic acid cycle, fatty acid β-oxidation and are the common final pathway of oxidative phosphorylation, which generates most of the cellular energetic source, ATP. It has been proposed that accumulation of mitochondrial DNA (mtDNA) during life is a major cause of age-related disease and this is because of its high mutagenic propensity. The lack of introns and protective histones, limited nucleotide excision and recombination DNA repair mechanisms, location in proximity of the inner mitochondrial membrane which exposes mtDNA to an enriched free radical milieu, are all factors contributing to a 10-fold higher mutation rate occurring in the mtDNA than in the nuclear DNA. 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.[79] Since this respiratory control 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.[80] 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, which 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.[81]

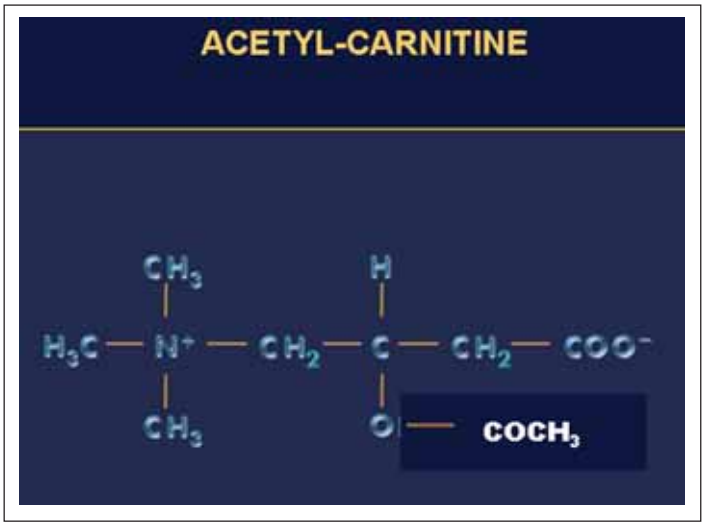

Figure 3. Chemical structure of Acetyl-L-carnitine.

Acetylcarnitine (LAC) (Fig. 3) is an ester of the trimethylated amino acid, L-carnitine, and is synthesised in the brain, liver, and kidney by the enzyme LAC-transferase. LAC facilitates the uptake of acetyl-CoA into the mitochondria during fatty acid oxidation, enhances acetylcholine production, and stimulates protein and membrane phospholipid synthesis.[82] 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, aging.[83-85] The neuroprotective benefits of this compound have been observed in the hippocampus, prefrontal cortex, substantia nigra and muscarinic receptor portions of the brain.[86] These benefits include antioxidant activity, improved mitochondrial energetics, stabilization of intracellular membranes and cholinergic neurotransmission.[87] 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.[88] LAC treatment restores the altered neurochemical abnormalities, cerebral energy metabolites in ischemia and aging and, in particular, ammonia-induced cerebral energy depletion.[87] In addition, it increases the responsiveness of aged neurons to neurotrophic factors in the CNS and it has preventive and corrective effects on diabetic neuropathology. Its beneficial effects have been also observed on EEG, evoked potentials and long-term synaptic potentiation in aged animals.[89] Moreover, LAC is commonly used also for the treatment of painful neuropathies: it exerts a potent analgesic effect by up-regulating metabotropic glutamate receptors.[90] There are experimental data that LAC improves memory function in Alzheimer's patients and it influences attention, learning and memory in the rat.[91] 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.[92] 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 exerts its effects by means of new protein synthesis, through qualitative and quantitative changes of gene expression. Furthermore, recent evidences have reported that LAC 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.[93] Recently, by using suppressive subtractive hybridisation (SSH) strategy, a PCR-based cDNA subtraction procedure particularly efficient for obtaining expressed transcripts often obscured by more abundant ones, it was 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.[94]

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, suggesting that administration of this compound may improve cellular bioenergetics in aged rats.[95] Fascinatingly, 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.[96] In addition, caloric restriction was shown to retard the aging associated changes in oxidative damage, mitochondrial oxidant generation and antioxidant defenses observed during aging.[97,98]

Interestingly, we have recently demonstrated that acetylcarnitine treatment of astrocytes induces HO-1 in a dose and time dependent manner and that this effect was associated with up-regulation of other Hsps as well as high expression of the redox-sensitive transcription factor Nrf2 in the nuclear fraction of treated cells.[82] In addition, we showed that addition of LAC to astrocytes, prior to LPS and INFγ-induced nitrosative stress, prevents changes in mitochondrial respiratory chain complex activity, protein nitrosation and antioxidant status induced by inflammatory cytokine insult.[82] Very importantly, this new envisioned role of LAC as a molecule endowed with the capability of potentating the cellular stress response pathways appear to provide an alternative therapeutic approach for those pathophysiological conditions where stimulation of the HO pathway is warranted.[7] 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 intra-cytoplasmic and intra-nuclear delivery of fusion proteins or DNA to modulate gene expression[99,100] along with the evidence that binding of HO protein to HO-1 DNA modifies HO expression via nonenzymatic signaling events associated to CO and p38-dependent induction of Hsp70,[101] open intriguing perspectives, as it is possible to speculate that synergy between these two systems might represent a possible important target for the acetylcarnitine action, with possible impact on cell survival during times of oxidative stress, hence contributing to activation of cell life programs and to the extent of cellular stress tolerance.

Conclusions and Perspectives

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*. Consistently, by maintaining or recovering the activity of vitagenes it can be possible to delay the aging process and decrease the occurrence of age-related diseases with resulting prolongation of a healthy life span.[6,7,61] 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.[2,45] This will pose an immense economic and personal burden on the people of the U.S.A. Similar considerations apply worldwide, except in sub-Sahara Africa, where HIV infection rates seem to be leading to decreased incidence of AD. There is now 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.[47,102] As one potentially successful approach, potentiation of endogenous secondary antioxidant systems can be achieved by interventions which 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. 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.[103] Consistently, LAC, as a molecule endowed with the capability of potentating the cellular stress response pathways, appears to afford similar protective action, thereby providing an alternative therapeutic approach valuable for all those pathophysiological conditions where stimulation of the HO pathway becomes a primary target.

Presented here is strong evidence that a crosstalk 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 also supports 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 LAC, may have biological significance as a novel approach to delay the onset of various age-associated alterations in cells, tissues and organisms. Hence, by maintaining or recovering the activity of vitagenes it can be possible to delay the aging process and decrease the occurrence of age-related diseases with resulting prolongation of a healthy life span.

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