Vitagenes, dietary antioxidants and neuroprotection in neurodegenerative diseases

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1. ABSTRACT

The ability of a cell to counteract stressful conditions, known as cellular stress response, requires the activation of pro-survival pathways and the production of molecules with anti-oxidant, anti-apoptotic or pro-apoptotic activities. Among the cellular pathways conferring protection against oxidative stress, a key role is played by vitagenes, which include heat shock proteins (Hsps) heme oxygenase-1 and Hsp70, as well as the thioredoxin/thioredoxin reductase system. 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 stress responses. Dietary antioxidants, such as curcumin, L-carnitine/acetyl-L-carnitine and carnosine have recently been demonstrated *in vitro* to be neuroprotective through the activation of hormetic pathways, including vitagenes. In the present review we discuss the importance of vitagenes in the cellular stress response and analyse, from a pharmacological point of view, the potential use of dietary antioxidants in the treatment of neurodegenerative disorders in humans.

2. 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 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 lipid (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).
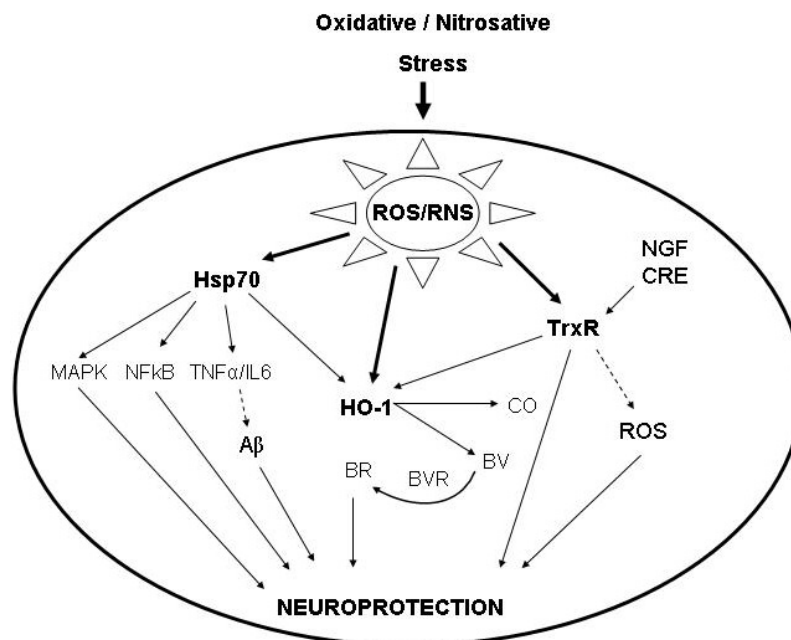
Figure 1. The cellular stress response mediated by vitagenes. Under conditions of oxidative/nitrosative stress, there is an increased formation of reactive oxygen species (ROS) or reactive nitrogen species (RNS). The latter play a key role in the pathogenesis of free radical- induced diseases, such as neurodegenerative disorders. The vitagenes heme oxygenase-1 (HO-1), heat shock protein 70 (Hsp70) and the thioredoxin/thioredoxin reductase system, either individually or by acting in concert, contribute to counteract ROS/RNS-mediated damage thus originating a neuroprotective response.

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 proteins, 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 most studied defense systems active against cellular damage.

In this chapter we describe the recent discoveries about the biochemical changes occurring in the central nervous system (CNS) when brain cells are challenged to activate an integrated network of protective mechanisms which are under control of genes called vitagenes. During times of chronic oxidative insult, the key role played by the heat shock response, particularly the heme oxygenase-1 (Hsp32) and Hsp70 pathways, as potential target for nutritional interventions are also discussed. Although the notion that stress proteins are neuroprotective is broadly accepted, still much work needs to be done in order to associate neuroprotection with specific pattern of stress responses. Emerging evidence underscores the high potential of the Hsp system as target for new neuroprotective strategies, especially those aimed at minimizing deleterious consequences associated to oxidative stress, such as in neurodegenerative disorders and brain aging. We review here also the evidence for the role of some polyphenols and acetylcarnitine in modulating redox-dependent mechanisms leading to up-regulation of vitagenes in brain, and hence potentiate brain stress tolerance.

3. THE VITAGENE FAMILY

The term vitagenes refers to a group of genes which are strictly involved in preserving cellular homeostasis during stressful conditions. The vitagene family is actually composed of the heat shock proteins (Hsp) Hsp32, Hsp70 and by the thioredoxin system (1,19,20). Among these genes heme oxygenase-1 (HO-1), also known as Hsp32, is receiving considerable attention because of its major role in counteracting both oxidative and nitrosative stress. In fact, HO-1 induction is one of the earlier events in the cell response to stress. Heme oxygenase-1 exerts protective role, by degrading the intracellular levels of pro-oxidant heme and by producing biliverdin, the precursor of bilirubin (BR), this latter being an endogenous molecule with powerful antioxidant and antinitrosative features (20-24) (Figure 1).

3.1. Heme oxygenase-1

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 (11,12,25-27). 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 (24,28,29). 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 (30).
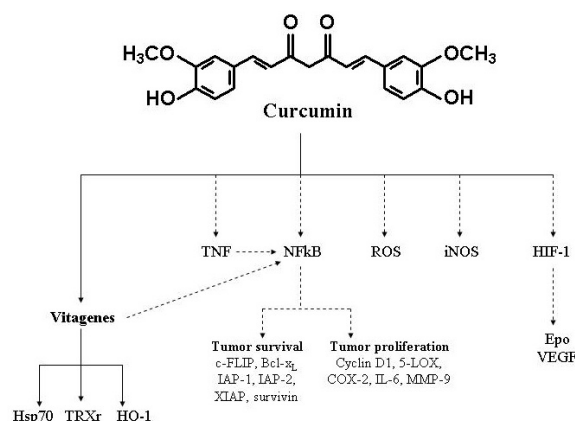
Figure 2. Curcumin-induced activation of vitagenes. The vitagenes heme oxygenase-1 (HO-1), heat shock protein 70 (Hsp70) and the thioredoxin/thioredoxin reductase system, either individually or by acting in concert, contribute to counteract the NFkB-dependent ROS/RNS-mediated damage thus promoting neuroprotection. NFkB inhibition might also have profound impact on cancerogenesis, modulating tumor cell survival and proliferation.

The neuroprotective effects of over-expressed HO-1 has been attributed to several factors such as an increased level of both cGMP and bcl-2 in neurons, the inactivation of the pro-apoptotic transcription factor p53, an increase in both antioxidant sources and in the iron sequestering protein, ferritin. Particularly interesting is the role played by HO-1 in AD, a neurodegenerative disorder which involves a chronic inflammatory response characterized by 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 (31,32). HO-1 increase was not only in association with neurofibrillary tangles, but also co-localized with senile plaques and glial fibrillary acidic protein-positive astrocytes in AD brains (33). 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 carbon monoxide (CO) and BR. The protective role played by HO-1 and its products in AD prompted investigators to propose natural substances, which are able to increase HO-1 levels in vitro, as potential drugs for the treatment of AD. In this light, several *in vitro* studies have been focused on polyphenolic compounds contained in some herbs and spices, e.g. curcumin (34-36). Curcumin (Figure 2) 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 (37). In addition, curcumin has been shown to significantly increase HO-1 in astrocytes and vascular endothelial cells (34,35). 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 (22,23,38,39). An epidemiological study suggested that curcumin, as one of the most prevalent nutritional and medicinal compounds used by the Indian population, is responsible for the reduced (4.4-fold) prevalence of AD in India compared to United States (40). Based on these findings, Lim and colleagues have provided 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 (41). Furthermore, in a human neuroblastoma cell line it has recently been shown that curcumin inhibits NFkB activation, efficiently preventing neuronal cell death (37).

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 and rodent cells exposed to oxidative stress conditions showed a marked HO-1 repression (42-46). The importance of HO-1 repression has been corroborated by the discovery of Bach1/Bach2 as heme-regulated transcription factors for HO-1 gene (47). In fact, Bach1 is broadly expressed in mice and human tissues and, in human cells, it is induced by the same stimuli which are able to repress HO-1 gene (43,48,49). 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 (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 (49).

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, non-adrenergic non-cholinergic gastrointestinal relaxation and vasodilatation, and is currently regarded as a neuromodulator in the peripheral and central nervous system (1,24,50-52). 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 (53-55). 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 two HO substrates, hematin and hemin, were associated with reduced or enhanced CRH release respectively, in two different *in vitro* models (56,57). 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 (24,29). However during the last ten years many studies arose in literature demonstrating that CO signals through the activation of alternative intracellular signal transduction pathways. Studies from our laboratory have 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 prostaglandin (PG) E2 (PGE2) 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 (52,58). The direct evidence about the stimulatory role of CO on PGs production was obtained incubating hypothalami directly in CO saturated solutions and measuring significantly increased PGE2 levels with respect to control tissue (59). Recently Jaggar and coll. have demonstrated that exogenous or endogenously produced CO dilates cerebral arterioles by directly activating large-conductance Ca^{2+}-activated K^+ (KCa) channels primarily by increasing the coupling ratio and amplitude relationship between Ca^{2+} sparks and KCa channels (60,61). Although CO is a potent and effective activator of KCa channels, the gas does not dilate arterioles in the absence of Ca^{2+} sparks. Therefore, CO appears to act by priming KCa channels for activation by Ca^{2+} sparks, and this ultimately leads to arteriole dilation via membrane hyperpolarization (61). Finally, Otterbein and coll. have shown that in organs and tissues different from brain, exogenous CO exerts anti-inflammatory and anti-apoptotic effects dependent on the modulation of the p38 MAPK-signaling pathway (62). By virtue of these effects, CO confers protection in oxidative lung injury models, and perhaps plays a role in HO-1 mediated tissue protection (63).

3.2. 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) (20,21).

Only recently, the availability of transgenic animals and gene transfer allowed us 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 (64,65). 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 (66). It has been suggested that this neuronal expression of Hsp70 outside an infarct can be

used to define the ischemic penumbras, which means the zone of protein denaturation in the ischemic areas (66).

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 (67-69). Recently Kakimura *et al.* 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 (70) (Figure 1). The same authors hypothesize that Hsps could activate microglial cells through NFkB and p-38 MAPK-dependent pathways (70) (Figure 1).

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 (71,72).

3.3. Thioredoxin/Thioredoxin reductase

The thioredoxin (Trx) system, originally identified in *Escherichia coli*, in 1964, as a hydrogen donor for ribonucleotide reductase required for DNA synthesis, plays a key role in cell function by limiting oxidative stress directly via antioxidant effects and indirectly by protein-protein interactions (73). It is well established that, in mammals, cellular redox regulation of many processes is provided by the cooperation between the Trx and glutathione systems (74). In fact, thioredoxin and reduced glutathione (GSH) systems are involved in a variety of redox-dependent pathways such as supplying reducing equivalents for ribonucleotide reductase, and peptide methionine sulfoxide reductase, the latter being involved in antioxidant defence and regulation of the cellular redox state (75). Therefore, Trx and GSH form a powerful system controlling redox regulation of gene expression, signal transduction, cell proliferation, protection against oxidative stress, anti-apoptotic functions, growth factor and co-cytokine effects, as well as regulation of the redox state of the extracellular environment (76). The promoter of the Trx gene contains a series of stress-responsive elements, various transcription factor binding sites, such as SP1, AP-1, NFkB, and the antioxidant-response element (ARE) (77-79). Importantly, induction of thioredoxin reductase and glutathione has been demonstrated to occur in parallel with other ARE-dependent phase 2 cytoprotective genes in several experimental systems, e.g., in cortical astrocytes (80), in human hepatoma cells (81) and in human keratinocytes (82). Similarly to induction of HO-1 gene expression, the ARE-mediated Trx-1 induction involves transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) (83).

Importantly, it has been reported that Trx is constitutively present as a surface-associated sulfhydryl protein in plasma membrane of a wide range of cells (84). Many physicochemical stimuli, such as UV irradiation and hydrogen peroxide, have been shown to induce Trx expression and secretion, as a redox-sensitive molecule with cytokine-like and chemokine-like activities to prevent cell injury against oxidative stress (75). In addition to UV irradiation, treatment of cell culture with phorbol esters, hydrogen peroxide, hypoxia, the cancer drug cisplatin and hemin has been reported to cause the translocation of Trx from the cytoplasm to the nucleus, where it regulates the redox-activation and DNA binding activity of critical transcription factors (Jun, Fos, p53, CREB, PEBP2/CBF, Myb), all involved in fundamental processes, such as gene expression, cell growth and apoptosis (84). Thioredoxin plasma levels in normal individuals vary between 20 and 30 ng/mL (85,86) and increase in certain human diseases including HIV infection and cancer (85,87). Noteworthy, several studies reported increased Trx-1 expression in many human primary cancers and tumor cell lines, including astrocytic brain tumors (88,89). Elevated Trx levels may contribute to increased cancer cell proliferation and resistance to chemotherapy by several mechanisms as the stimulation of DNA synthesis and the activation of redox-modulated transcription factors (84,90). Recent work suggests that Trx-1 is involved in nerve growth factor (NGF) signaling pathways (91). NGF, a neurotrophic factor regulating development, maintenance and function of the CNS, has been shown to activate Trx-1 expression via cyclic AMP (cAMP)-response elements (CREB) present in the Trx-1 gene promoter, and also to induce nuclear translocation of Trx1 (92) (Figure 1). Recent data suggest that, beyond its ability to regulate the function of proteins through thiol-disulfide exchange reactions, Trx and its substrates may also have beneficial effects during oxidative stress by upregulating HO-1 (Figure 1), with important cytoprotective pleiotropic effects deriving from heme degradation and bilirubin formation (93,94). Besides the role as a source of reducing equivalents, Trx *per se* acts as antioxidant or ROS scavenger. In fact, Trx eliminates singlet oxygen, hydroxyl radical and hydrogen peroxide (95).

With regard to the interaction between NO and the Trx system, it has been reported that some of the neuroprotective effects of S-nitrosoglutathione (GSNO) on beta-amyloid- or ferrous citrate- induced toxicity in rat cortical neurons or in rat *substantia nigra* can be due to the activation of multiple signalling pathways including thioredoxin (96,97). Interestingly, the interaction between Trx and GSNO seems to involve both the activation of soluble guanylate cyclase (sGC) and the following cGMP generation and a direct S-nitrosylation reaction (97). Finally, NO-dependent expression of Trx has been shown to be involved in the neuroprotection against oxidative stress mediated by estrogens (98).

4. NATURAL ANTIOXIDANTS AND NEURODEGENERATIVE DISORDERS

4.1. Curcumin

Curcumin (Figure 2) is a poliphenol quite stable at acidic pH and upon ingestion almost 40-80% of this compound remains in the gastrointestinal tract (99). However, curcumin undergoes a marked first-pass metabolism which limits its systemic bioavailability (~ 60%) as demonstrated in humans and rodents (100-102). Interestingly, in order to increase its bioavailability, the co-administration of curcumin with piperine or its complexation with phospholipids to form a curcumin-phospholipids complex have been proposed (100,103,104). Preclinical studies have shown that administration of 1 g/kg of curcumin to the rat allows the polyphenol to reach plasma concentrations around 1.3 μM; on the other hand, patients affected by malignant or pre-malignant conditions of the bladder, skin, cervix, stomach or oral mucosa, treated with high dose curcumin (0.5-8 g/day for 3 months) had a plasma concentration of this compound of 1.75 ± 0.8 μM (100,105). In the rat, the volume of distribution of curcumin is around 190 liters thus suggesting that this polyphenol may accumulate in many organs including colorectal tissue and liver (100,103,106). Studies in rodents and humans demonstrated that, after oral dosing, curcumin is conjugated to curcumin glucuronide and curcumin sulfate as well as reduced into dihydrocurcumin (DHC), tetrahydrocurcumin (THC), hexahydrocurcumin, octahydrocurcumin and hexahydrocurcuminol (99,197,108); curcumin, DHC and THC can be further converted in monoglucuronide conjugates (107,109). These metabolic changes seem to occur not only in the liver, the main organ deputed to biotransformation, but also in the intestinal tract (99,108). Interestingly, the metabolism of curcumin generates products such as THC which retains anti-inflammatory activity comparable to that of the parental compound (99,108). In rodents and humans curcumin inhibits cytochrome P450 enzymes, glutathione S-transferase and UDP-glucuronosyltransferases, therefore the ingestion of this spice may alter the metabolism of drugs thus increasing their plasma concentrations and initiating potential toxic effects (110-113). In the rat, curcumin is mainly excreted into the bile and eliminated in the feces, only a small amount is excreted in the urine (101,102) with a half-life of elimination of ~ 1.5 hours (103). The urinary elimination of curcumin and its metabolites seems to increase if curcumin is administered at large doses (for example 3.6 g/day for up to 4 months) (100,114). With regard to the toxicity profile of curcumin, studies in rodents and primates have shown that doses of up to 3.5 g/kg body weight administered for up to 3 months were well tolerated by the animals (100). In humans, curcumin at doses ranging from 2.1 and 8 g/day for up to 3 months did not originate any toxic effect (105,115). However, patients affected by advanced colorectal cancer treated with curcumin (3.6 g/day) developed diarrhea whereas a dose of 0.9 g/day was associated with nausea, which resolved spontaneously. In the same patients, blood test abnormalities related to curcumin administration were a rise in serum alkaline phosphatase and lactate dehydrogenase, but the possibility that they resulted from the progression of cancer rather than curcumin toxicity can not be excluded (100,114).

Early studies have shown that curcumin and related products such as THC, have strong antioxidant

activity. In fact, these compounds have been able to reduce free radical- or copper- induced lipid peroxidation in several experimental systems (116-118). Furthermore, structure-activity studies clearly demonstrated the importance of the beta-diketone moiety and, especially, the phenolic hydroxyl group, for the antioxidant activity of curcumin and its analogues (116,119). Very recently, many papers have appeared in the literature demonstrating that curcumin and its metabolites interact with several intracellular systems such as the transcription factor NFkB, inducible nitric oxide synthase (iNOS), hypoxia-inducible factor-1 (HIF-1) and members of the vitagene family (see below). This complex array of interactions is in agreement with the well known ability of curcumin to serve not only as an antioxidant but also as anti-inflammatory and anticarcinogenic molecule.

Reyes-Gordillo *et al.* have shown that curcumin reduced the CCl4-induced liver toxicity in the rat; in particular, curcumin reduced the CCl4-related increase in pro-inflammatory cytokines and blocked the nuclear translocation of NFkB (120). Similarly, curcumin prevented the dinitrochlorobenzene-induced colitis in the rat by down-regulating both NFkB and iNOS (121). In lung epithelial cells, curcumin exerted anticarcinogenic activity and prevented the cigarette smoke-induced NFkB activation through inhibition of IkBα kinase activation, IkBα phosphorylation and degradation (122). The inhibition of the NFkB activation was paralleled by the suppression of many NFkB-related genes, including cyclin D1, cyclooxygenase-2 and matrix metalloproteinase-9 (122). Comparable results have been found in a macrophage cell line (RAW 264.7) challenged with bacterial endotoxin. In these cells, curcumin and its reduced metabolites blocked the activation of NFkB, and the downstream activation of iNOS, *via* inhibition of the IkB kinases 1 and 2, thus providing further evidence about the importance of the effects on NFkB in the anti-inflammatory and anticarcinogenic activity of this phenolic compound (123). Through interaction with NFkB, curcumin exerts protective function also in the regulation of T-cell-mediated immunity. In fact, overexpression of NFkB in T-cells confers protection against tumor-induced apoptosis, whereas when NFkB is inhibited, the cell becomes much more vulnerable and undergoes apoptosis (124). By so doing, NFkB plays an important role in the regulation of T-cell apoptosis and the related thymic atrophy which occurs during carcinogenesis. In this experimental model, curcumin prevented the tumor-induced apoptosis and the following thymic atrophy by restoring the activity of NFkB (124). Another transcription factor involved in the anticarcinogenic effect of curcumin is HIF-1. Hypoxia-inducible factor-1 is composed of two proteins, HIF-1α and the aryl hydrocarbon receptor nuclear translocator (ARNT) and plays a major role in the development of hypoxic tumors (125). Curcumin has been demonstrated to inactivate HIF-1 in several cell lines and this effect has been related to its ability to promote ARNT degradation (125). As a consequence of HIF-1 inactivation, several proteins downstream to HIF-1 were downregulated, such as erythropoietin and the vascular endothelial growth factor (125). Particularly interesting is the interaction of curcumin

with the vitagene system. Notably, curcumin increased the expression of HO-1 in human cardiac myoblasts, hepatocytes, monocytes and endothelial cells (126-129), rat neurons and astrocytes (34) as well as porcine endothelial cells (130). In several rodents and human cells, the curcumin-induced HO-1 overexpression was correlated with production of mitochondrial ROS, activation of transcription factors Nrf2 and NFkB, induction of MAPK p38 and inhibition of phosphatase activity (129,131,132). Moreover, curcumin up-regulated Hsp70 in human colorectal carcinoma cells, proximal tubule cells (133-136) and rat glioma cells (137). Quite different is the effect of curcumin on thioredoxin reductase (TrxR), as it has been shown that curcumin irreversibly inhibits TrxR activity. As a consequence, there was increased NADPH oxidase activity which, in turn, produced an abundance of ROS (138). This latter paradoxical effect may explain, at least in part, the cancer chemopreventive activity of curcumin (Figure 2) (138). The activation of transcription factor Nrf2 by curcumin deserves a special consideration because of the widely recognized role of this transcription factor in cellular protection against oxidants and electrophiles. Curcumin has two Michael acceptor centers and induces the gene expression of several cytoprotective proteins in a process that is often referred to as "the phase 2 response" (139). There are three essential components in the general scheme of induction: (i) the antioxidant response element (ARE), (ii) transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2), and (iii) the sensor protein Keap1. The first component was provided by the scientific efforts of the laboratories of Pickett, Daniel, Fahl, and Jaiswal (139). The ARE is represented by upstream regulatory sequences that are present on all genes encoding for phase 2 proteins in either single or multiple copies, and contain the consensus core sequence TGACNNNGC. More recently, a detailed analysis of the enhancer of the mouse NQO1 in the Hayes laboratory has shown that certain nucleotides thought to be redundant in the ARE function have essential roles for its activity, while others that were previously considered essential, were in fact dispensable (140). Nonetheless, the concept of the existence of a common enhancer element provided the basis for the common transcriptional regulation of phase 2 genes. Furthermore, the identification of its sequence and function led to the addition of new members to the family of phase 2 proteins, e.g., HO-1 (141), that were previously not considered part of the phase 2 response. The second component essential for both basal and inducible expression of phase 2 genes was identified by Yamamoto and his colleagues. They recognized that Nrf2, a 66-kDa transcription factor discovered in the laboratory of W. Kan (142), is the principal transcription factor that binds to the ARE (143). Nrf2 belongs to the cap'-n'-collar (CNC) family of transcription factors that share a highly conserved basic region-leucine zipper (bZIP) structure (144). It forms heterodimers with members of the small Maf family, the resultant dimeric complex binds to the ARE and subsequently recruits the general transcriptional machinery to activate transcription of phase 2 genes (143-145). There is now an overwhelming amount of evidence that Nrf2 serves as a master regulator of the ARE-driven cellular defense systems against electrophiles and oxidants (146). Tissues and embryonic fibroblasts obtained from *nrf2*
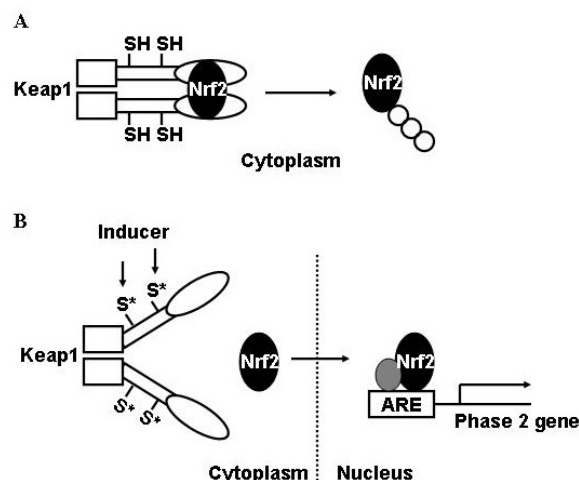
Figure 3. Mechanism of induction of cytoprotective phase 2 genes. Under basal conditions (A) transcription factor Nrf2 is bound to its cytoplasmic repressor partner Keap1 and actively targeted for ubiquitination and proteasomal degradation via association with the Cullin 3-based E3 ubiquitin ligase complex. When an inducer enters the cell (B), it modifies highly reactive cysteine residues of the sensor Keap1 which loses its repressor function. Consequently, Nrf2 is stabilized and undergoes a nuclear translocation where it binds to the ARE (in heterodimeric combinations with members of the small Maf family of transcription factors), and activated transcription of cytoprotective phase 2 genes. SH, reactive cysteine; S*, modified cysteine.

knockout mice have low and uninducible levels of glutathione and phase 2 enzymes compared to their wild type counterparts (143,147). *Nrf2* knockout mice exhibit enhanced sensitivity to the pulmonary toxicity of butylated hydroxytoluene and hyperoxia, the hepatotoxicity of acetaminophen, the neurotoxicity of 3-nitropropionic acid, the carcinogenicity of benzo(*a*)pyrene, diesel exhaust, and N-nitrosobutyl-(4-hydroxybutyl)-amine (148). Chronic exposure of *nrf2* knockout mice to cigarette smoke leads to enhanced development of lung emphysema (149). Disruption of *Nrf2* also increases susceptibility to severe airway inflammation and asthma following an allergen challenge and to septic shock (150,151). Importantly, while phase 2 inducers protect wild type mice against environmental challenges, they have no effect on phase 2 enzymes and show no protective effects in *nrf2* knockout mice. Taken together, these *in vivo* studies provided powerful genetic evidence about the essential role of Nrf2 in: (i) regulating phase 2 gene expression, and (ii) mediating the protective effect of phase 2 inducers. The role of the small Maf proteins in induction of the phase 2 response was also established in a series of genetic experiments (145). Although the complete absence of all small Maf proteins (MafG, MafK, and MafF) resulted in embryonic lethality before E11.5, it was possible to obtain fibroblasts from embryos at E11.0. Detailed analysis using these cells revealed a complete loss of induction in nearly all Nrf2-dependent genes that were examined.

The third essential component in the scheme of induction of the phase 2 response was discovered in the Yamamoto laboratory (143). This is Keap1, a dimeric cytosolic repressor protein that binds and actively targets Nrf2 for ubiquitination and subsequent proteasomal degradation via its association with Cullin 3 to form an E3 ubiquitin ligase complex (152,153). Inducers of the phase 2 response react with Keap1 delivering a chemical signal that results in loss of its ability to repress Nrf2 which can then undergo nuclear translocation and activate the transcription of phase 2 genes. Because all phase 2 inducers are sulfhydryl reagents, it is perhaps not surprising that Keap1 is a cysteine-rich protein with 25 and 27 cysteine residues among the 624 amino acids of the murine and human homologues, respectively. Detailed analysis of the ability of Keap1 to react with inducers revealed that although the specific cysteine thiols that are modified by inducers appear to depend on the type of inducer, as well as on the experimental conditions, the most reactive cysteine residues are located in the central (intervening) domain of Keap1 (154). Importantly, mutagenesis analysis showed that amino acid replacements of C273 and C288, either individually or in combination, but not of any other cysteine residues, result in complete loss of the repressor function of Keap1 establishing that the same cysteine residues that are targets for phase 2 inducers are essential for the ability of Keap1 to repress Nrf2 (153).

Overall, the current model for the general mechanism of induction of cytoprotective phase 2 genes can be summarized as follows (Figure 3). In the absence of inducing stimuli the cysteine rich sensor dimeric metalloprotein Keap1 binds and targets transcription factor Nrf2 for ubiquitination and proteasomal degradation via association with the Cullin 3-based E3 ubiquitin ligase complex. Inducers (e.g., curcumin) react and chemically modify specific highly reactive cysteine residues of the sensor Keap1. Consequently, Keap1 loses its ability to repress transcription factor Nrf2. This leads to increased stabilization of Nrf2, its nuclear translocation, binding to the ARE (in heterodimeric combinations with members of the small Maf family of transcription factors), and ultimately transcriptional activation of cytoprotective phase 2 genes (153-155).

Because both HO-1 and the thioredoxin/ thioredoxin reductase system can be upregulated in an Nrf2/ARE-dependent manner, the questions arise whether: (i) the third member of the vitagene family, Hsp70, is also inducible by other phase 2 inducers, and (ii) there could be a common regulatory mechanism. Indeed, in addition to curcumin, several other inducers of Nrf2-dependent genes have been shown to increase the protein levels of Hsp70. Among them are the cyclopentenone prostaglandin 15-deo-$\Delta^{12,14}$-prostaglandin J_2 (15dPGJ$_2$), and the vicinal dithiol reagent phenylarsine oxide (156,157). Importantly, all of these compounds react with sulfhydryl groups and the transcriptional activation of both Nrf2, the major transcription factor responsible for phase 2 gene expression, and heat shock factor 1 (HSF1), the major activator of Hsp70 gene expression, depend on cysteine

modification either within the Nrf2 regulator Keap1 (147,154), or within HSF1 itself (158).

Neurodegenerative disorders, AD and Parkinson's disease (PD), belong to the family of the "protein conformational diseases" and affect a large portion of our aging population (159). In general, conformational diseases are conditions that arise from the dysfunctional aggregation of proteins in non-native conformations. It is known that the beta conformation in proteins is particularly susceptible to perturbations in the quality control system and that ROS play an important role in the development and/or pathogenetic progression in aging and neurodegenerative diseases (160-162). Chaperones can rescue misfolded proteins by breaking up aggregates and assisting in the refolding process (161,163). Proteins that cannot be rescued by refolding can be delivered to the proteasome by chaperones to be recycled (163). If the cell is not able to eliminate misfolded proteins multiple metabolic derangements resulting in the excessive production of ROS and RNS occur (164). The ability of a cell to deal with oxidative and nitrosative stress requires functional chaperones, antioxidant production, protein degradation and a cascade of intracellular events collectively known as the "unfolded protein response", a form of cell stress response (165,166). As the cell's quality control system becomes overwhelmed, conformational changes occur to amyloid polypeptide intermediates, generating stable oligomers with an anti-parallel crossed beta-pleated sheet structure that eventually accumulate as space-occupying lesions within neurons (162). Although it is clear why mutant proteins form amyloid, it is harder to rationalize why a wild-type protein adopts a native conformation in most individuals, but it misfolds in a minority of others, in what should be a common extracellular environment. This discrepancy suggests that another event likely triggers misfolding in sporadic amyloid disease. One possibility is that an abnormal metabolite, generated only in some individuals, covalently modifies the protein or peptide and causes it to misfold. Candidate metabolites are suggested by the recently recognized links between AD and atherosclerosis, in which known chronic inflammatory metabolites, may play a critical pathogenic role. If this holds true, then new targets are disclosed for a prevention strategy brought about through nutritional antioxidants.

Alzheimer disease is characterized by a subtly impaired cognitive function or a disturbance of behaviour. With time there is a gradual memory loss and disorientation which eventually progress into dementia. Although, most cases are sporadic, 5-10% or more are familial. Gross examination of the brain in AD shows a variable degree of cortical atrophy with narrowed *gyri* and widened *sulci* most apparent in the frontal, parietal and temporal lobes. Microscopically, the features include neurofibrillary tangles, neurite (senile) plaques, the central core of which is amyloid-beta peptide, derived from the transmembrane amyloid precursor protein (APP), amyloid angiopathy, granulovacuolar degeneration and Hirano bodies. Importantly, all of these changes are present in the brains of non demented older individuals but to a much lesser extent

(167,168). Several lines of evidence now support a fundamental role for oxidative and nitrosative stress in the pathogenesis of this disease (20,167,169). As mentioned above, the only evidence of a protective role of curcumin in the onset of AD was provided by Ganguli *et al.* who demonstrated that Indian populations consuming curcumin-rich diet have reduced prevalence of AD compared to United States (40). Following this observation, many basic studies were conducted and the neuroprotective role of curcumin was corroborated. *In vitro* studies have shown that curcumin protects neuron-like PC12 cells from β-amyloid toxicity and, interestingly, the polyphenol displayed a neuroprotective effect greater than a well known antioxidant such as α-tocopherol (170). In addition, in cultured human keratinocytes it has been found that low concentrations (1 μM) of curcumin activated the proteasomal activity, whereas high concentrations (10 μM) were inhibitory (171). By using an Alzheimer transgenic APPSw mouse model (Tg2576), Lim and colleagues have shown that dietary curcumin suppressed inflammation and oxidative damage in the brain of these mice (41). More recently, Garcia-Alloza *et al.* in transgenic APPswe/PS1dE9 mice demonstrated that curcumin, given intravenously for 7 days, crosses the blood-brain-barrier, binds to β-amyloid deposits in the brain and accelerates their rate of clearance (106). These latter results are in good agreement with previous findings which demonstrated that curcumin disaggregates and inhibits β-amyloid aggregation (171,172).

Parkinson's disease, whose cardinal features include tremor, slowness of movement, stiffness and poor balance, is attributed to a profound deficit in dopamine that follows the loss of dopaminergic neurons in the *substantia nigra pars compacta* and dopaminergic nerve terminals in the *striatum* (20,173). Although the mechanisms leading to PD are still uncertain, a large amount of experimental evidence implicates oxidative and nitrosative stress as one of the crucial factors in the pathogenesis of PD (174,175). Considerable insights into the pathogenesis of PD, indeed, have been achieved by use of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is commonly used to induce an experiment model of PD (174,176). Excessive free radical formation or antioxidant deficiency and the resulting oxidative stress are all mechanisms involved in MPTP neurotoxicity (177). Rajeswari has shown that curcumin protects rat brain from MPTP-induced neurotoxicity by virtue of its scavenger activity (178). On the other hand, curcumin has been shown to protect PC12 cells from MPP^+ (the active metabolite of MPTP) by inducing the antiapoptotic protein bcl-2, preventing the dissipation of mitochondrial membrane potential and reducing the intracellular iNOS levels (179). The importance of mitochondria in the neuroprotective effect of curcumin has been also stressed by Mythri *et al.* (180) who demonstrated that curcumin prevents the formation of peroxynitrite which is responsible for the complex I damage which is a common feature in PD.

Transient forebrain ischemia is a common cause of stroke and occurs in people suffering from cardiovascular diseases (181). As a consequence of
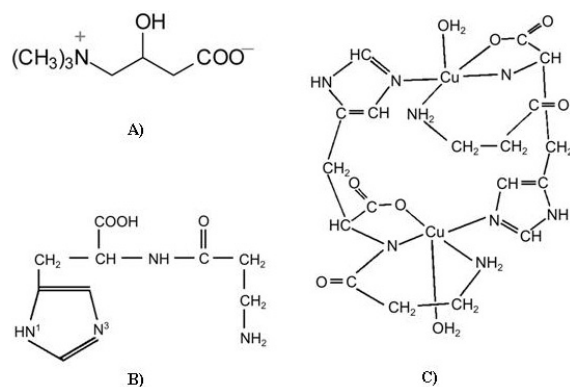
Figure 4. Chemical structures of carnitine (4A) and carnosine (4B). The structure of carnosine, as a bi-functional metal ion complex is also illustrated (4C).

ischemia and the following reperfusion, a cascade of events such as increased calcium release, the overexpression of COX-2 and iNOS both of which are important free-radical generators and trigger neuronal cell death in selected brain areas including the hippocampal cornu ammonis 1 (CA1) (1,22,52,181). Curcumin exerted a neuroprotective effect in rats who underwent ischemia/reperfusion injury and this effect has been related to the direct scavenger effect of curcumin as well as to a curcumin-induced interference with the apoptotic machinery, increase in antioxidant molecules (GSH) and enzymes (catalase, superoxide dismutase) (181-183).

4.2. Carnitines and Acetyl-L-carnitine

L-carnitine (LC) (Figure 4A) is a natural compound and its biological role is to facilitate the transport of fatty acids to mitochondria. Dietary LC derives from the intake of red meats, but the endogenous synthesis of LC from the amino acid precursors lysine and methionine has been also documented (184). The dietary intake of LC in humans ranges from 1 to 15 μmol/kg body weight/day, whereas the rate of biosynthesis is about 1-2 μmol/kg body weight/day (185). After oral ingestion, dietary LC is well absorbed by simple or carrier-mediated diffusion and its bioavailability is 54-86 %; conversely, the bioavailability of exogenous LC is much lower, in the range 5-18 % (184,185). This paradoxical effect can be explained considering that the absorption of LC decreases as the intake of LC increases, this to maintain the concentration of LC constant (184,185). The normal plasma concentration of LC in healthy adults with a mixed diet is 40-50 μM (184,186). When administered at doses 30-100 mg/kg p.o. in humans, L-carnitine peak plasma concentrations were 27-91 μM after 3 hours, and returned to the baseline within 24 h (185,187). L-carnitine undergoes acetylation in rodents and human intestine thus forming esterified compounds such as acetyl-L-carnitine (ALC), which is endowed with biological activity *per se* (184,185). Interestingly, ALC diffuses across membranes much better than LC and its efflux in the systemic circulation has been calculated to be 4-times greater than that of LC (185,188). Data from AD patients have shown that after supplementation with pharmacological doses of

ALC (2 g/day) for 55 days, its plasma concentrations increased from 7.2 to 10.3 μM (185). In the plasma, neither LC nor ALC are bound to proteins (184). The volume of distribution of LC differs considering the dietary or exogenous source being approximately 3000 liters and 20-50 liters, respectively (184). This great difference in the volume of distribution between dietary and supplemental LC depends on the different degree of absorption, slow accumulation in tissues such as the muscle and rate of kidney elimination (see below), and therefore these numbers should be considered purely indicative (184). It is interesting to underline that ALC is able to cross blood-brain-barrier; as shown by Parnetti *et al.* AD patients treated with ALC i.v. or p.o. for 10-60 days have an increased concentration of ALC in the cerebrospinal fluid up to 3.55 nmol/ml (189). In human subjects treated with LC i.v. its elimination half-life ranged from 3 to 12 hours (184). However due to the long-lasting release of LC by the muscle, the total time of turnover from the body has been estimated to be 66 days (185). L-carnitine is metabolised by the intestine to γ-butyrobetaine and trimethylamine, the former excreted by the feces, the latter in urine (184,185). Accordingly, the renal clearance of LC which is about 1-3 ml/min suggesting an extensive rate of tubular reabsorption, significantly increases at values close to the creatinine clearance with the increase in LC plasma concentrations indicating that tubular reabsorption approaches full saturation (184). This last finding is very important and contributes to explain how exogenous LC is almost completely excreted during the first 12 hours after administration whereas dietary LC is reabsorbed (184). Due to its elimination mainly through the kidney, LC should be administered very carefully to patients affected by renal impairment (190).

Acetyl-L-carnitine has been proposed to have beneficial effects in preventing the loss of brain function which typically occurs during aging and neurodegenerative disorders. The main mechanism of action of ALC is the improvement of mitochondrial respiration which allows the neuron to produce ATP necessary to maintain the normal membrane potential (191). However, ALC has been shown to be neuroprotective through a variety of other effects such as the increase in PKC activity (191). Interestingly ALC counteracted the loss of NMDA receptors in neuronal membrane and increased the production of neurotrophins, two effects strictly related to synaptic plasticity (191). Recent studies have shown that ALC reduces Aβ toxicity in primary cortical neuronal cultures by increasing both HO-1 and Hsp70 expression (192). Studies in rats have shown that chronic ALC treatment increases life-span, improves cognitive behaviour in aged animals and guarantees long-term memory performance (191). Furthermore, chronic ALC treatment has been shown to prevent age-related changes in mitochondrial respiration and decrease oxidative stress biomarkers through the up-regulation of HO-1, Hsp70 and superoxide dismutase-2 in senescent rat (193). Taken together, these pre-clinical studies suggested that ALC treatment could be beneficial for the treatment of age-related diseases and the potential use in humans has been encouraged. Patients affected by AD and treated with ALC at doses ranging from 1 to 2 g/day for 6-12 months, have

shown an improved performance on several cognitive tests such as word recognition, name learning and word list recall with respect to placebo-treated patients, but none of these effects was significant (191,194). In two clinical studies, ALC 3 g/day for 1 year significantly reduced cognitive decline only in early-onset AD patients (195,196), but this evidence was not confirmed in a later *ad hoc* designed study (197). Consistently, we have demonstrated that acetyl-L-carnitine 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. The results from this study show for the first time that acetyl-L-carnitine induces heme oxygenase-1 and Hsp60 heat shock proteins, and that this effect may involve the transcription factor Nrf2, implying the conceivable possibility that acetyl-L-carnitine, by promoting acetylation of DNA-binding proteins, can induce post-translational modifications of critical target proteins endowed with DNA competence and transactivating activity (198). Very importantly, this new envisioned role of ALC as a molecule endowed with the capability of potentiating the cellular stress response pathways appear to be a promising alternative therapeutic approach for those pathophysiological conditions where stimulation of the HO pathway is warranted (198).

4.3. Carnosine

Carnosine is a natural dipeptide (β-alanyl-L-histidine), (Figures 4B,C), present in long-lived mammalian tissues (199) and has numerous roles as proton buffer, metal chelator, antioxidant, antiglycating, immunostimulant, antitumoral and wound-healing agent (200,201). Since β-alanine is non-proteinogenic amino acid, it is obvious that carnosine is not product of protein catabolism: Instead it is synthesized enzymatically by carnosine synthetase, an enzyme present in brain and muscle that shows broad substrate specificity (202).The hydrolysis of carnosine is catalyzed by two enzymes recently cloned and characterized (203). Both enzymes belong to the M20 metalloprotease family. The enzyme named CN1 exhibits narrow specificity and the characteristics of the enzyme previously designated X-His dipeptidase or carnosinase (204). The enzyme named CN2 displays broad substrate specificity and is ubiquitously expressed like the enzyme previously designated cytosol non-specific dipeptidase (205). In the brain, carnosine has been found in glial cells and in some type of neurons (206); uptake of carnosine has been found to be mediated by a high affinity, energy-dependent dipeptide transport system, identified as the peptide transporter PepT2 (207). Carnosine has been shown to delay ageing in cultured human fibroblasts (208 268), male Drosophila (209) and senescence-accelerated mice (210). Therapeutic potential has also been invoked in cataractogenesis (211) and diabetes (212–214). The occurrence of carnosine and its analogue homocarnosine (γ-aminobutyryl-histidine) in brain, and homocarnosine in CSF, and their age-related alterations (215,216) suggested a role for these peptides with respect to suppression of onset or progression of AD and other neurodegenerative diseases (217,218). In brain cells, carnosine has been shown to be neuroprotective

because of its ability to counteract both oxidative and nitrosative stress related to several pathological conditions including ischemia (219-221), methamphetamine neurotoxicity (222) and neurodegenerative disorders (223). Importantly, carnosine may indirectly influence neuronal excitability by modulating the effect of zinc and copper (224,225) (Figure 4C). Furthermore, carnosine has been shown prevent β-amyloid aggregation and toxicity (226) and this effect can be due to the known ability of this peptide to inhibit protein misfolding and avoid the formation of advanced-glycation end products (201). More recently, it has been reported that the peptide is able to protect against Aβ-induced neurotoxicity in differentiated rat PC12 cells by regulation of glutamate release and glutamate release and NMDA receptor trafficking (227,228). Interestingly, carnosine plasma levels have been found lower in AD patients than in age-matched controls (229). Furthermore, carnosine has been shown to counteract peroxynitrite-dependent protein alterations such as tyrosine nitration (230) and to inhibit the NO-dependent activation of guanylate cyclase (231). Recent evidence demonstrated that carnosine prevents the up-regulation of iNOS and the induction of both HO-1 and Hsp-70 following strong nitrosative conditions (232); in addition, a correlation has been found between cell protection and NO free-radical scavenging activity of carnosine that showed direct NO-trapping ability in cell-free experiments (233). Taken together, these findings allow us to propose the metabolic pathway leading to carnosine formation as a potential target for the prevention and/or treatment of neurodegenerative disorders. In addition, new carnosine, homocarnosine and anserine derivatives have been synthesized and characterized, showing chelating and antioxidant properties similar to those of the parent dipeptides (234-237). In addition, these new compounds survive to attack by carnosinases (238) and should be explored for their ability to suppress age-related neurodegeneration where increased zinc and copper could play a role (239-241).

5. 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. Maintaining or recovering the activity of vitagenes can decrease the occurrence of age-related diseases with resulting prolongation of a healthy life span (1,20,21).

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 neurodegeneration. As one potentially successful approach, potentiation of endogenous secondary antioxidants 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 bilirubin (22,23,38,39). Thus, HO-1 can amplify intracellular cytoprotective mechanisms against a variety of insults.

Reducing energy intake by controlled caloric restriction or intermittent fasting increases lifespan and protects various tissues against disease, in part, by hormesis mechanisms that increase cellular stress resistance. Examples of such preconditioning mechanisms have been defined hormesis (242), particularly 'neurohormesis' if related only to CNS (243). Here we priviledge the term of vitagene, rather than the more general biological term of hormesis, to indicate the integrated network of protective mechanisms under control of redox-sensitive genes and related signaling pathways that result in increased expression of specific genes, such as those responding to antioxidant compounds. Although only as a speculative attempt, the term vitagene has appeared one time in the past literature, where was used in a different sense, equivalent to "longevity assurance genes", with a role in determining the evolutionary natural lifespan (244). However, the first evidence-based notion identifying vitagenes with stress responsive genes such as HO-1, Hsps, TrxR and sirtuins have been provided by our group (1,2,20,21,51,192,193,245-248).

Stimulation of various maintenance and repair pathways through exogenous interventions (mild stress or compounds targeting the heat shock signal pathway), might have biological significance as a novel approach to delay the onset of various age-associated disorders (192,193,198), opening intriguing perspectives 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 and resistance to neurodegenerative insult. Notably, by maintaining or recovering the activity of vitagenes could be possible to delay the aging process and decrease the occurrence of age-related diseases with resulting prolongation of a healthy life span (1,20,21). Furthermore, future development of novel chemically-modified antioxidant drugs (e.g., mitochondrial targeted compounds) should result in effectively slowing disease progression. Yet, the association with new drug-delivery-systems may be desirable and useful for the therapeutic use of antioxidants in human neurodegenerative diseases. Presented here is strong evidence that a functional interplay between stress response genes is important for cell stress tolerance, highlighting compelling reason for a renewed effort to understand the central role of this most extraordinary defense system in biology and medicine.

6. ACKNOWLEDGMENTS

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Abbreviations: BV, biliverdin; BVR, biliverdin reductase; BR, bilirubin;. c-FLIP: cellular FLICE-like inhibitory protein; IAP-1: inhibitor of apoptosis protein 1; IAP-2: inhibitor of apoptosis protein 2; XIAP: X-linked inhibitor of apoptosis protein; 5-LOX: 5-lipooxygenase; COX-2: cyclooxygenase-2; IL-6 : interleukin-6; MMP-9: matrix metalloproteinase-9; Epo: erythropoietin; VEGF: Vascular Endothelial Growth Factor;ROS: Reactive oxygen species; iNOS: inducible nitric oxide synthase;TNF: tumor necrosis factor; HIF-1: Hypoxia Inducible Factor-1; NFkB: nuclear factor kB; Hsp70: heat shock protein 70; TRXr: thioredoxin reductase; HO-1: heme oxygenase-1

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