

Carnosine Overview

Carnosine (B-alanyl-L-histidine) is a naturally-occurring di-peptide (a combination of two amino acids), found in muscle, brain and other animal and human tissues. Carnosine is a powerful antioxidant and an anti-glycation agent. Glycation is a process whereby unstable protein and sugar molecules build up in the brain causing a browning of brain matter. It also appears to enhance wound healing; reduce lactic acid accumulation; promote muscle recovery; and enhances muscle contraction. Carnosine is found in high concentrations in skeletal muscles and thus the focus of research has been on athletic performance. It appears to be a powerful antioxidant.

Research has implicated carnosine in a variety of physiological processes. Its most widely researched function is as a "broad-spectrum" antioxidant, able to interact with several free radical species including singlet oxygen, hydrogen peroxide, and both peroxy and hydroxyl radicals. Carnosine is also able to inhibit radical-induced cellular damage caused by iron, copper and zinc. It appears to help activate the enzymes responsible for generating muscle contractions (myofibrillar-ATPase) and act as an intramuscular buffering agent to prevent accumulation of lactic acid. Among athletes, muscle carnosine levels are highest in those with high anaerobic demands (rowers and track sprinters), but levels are also elevated in endurance athletes (marathoners) when compared to untrained subjects. There are a number of potential therapeutic actions of carnosine, including antihypertensive effects; immunomodulation; wound healing and anti-tumor/chemopreventive effects

Current research shows that in animal and test-tube experiments, carnosine inhibits oxidation of LDL-cholesterol and reduces development of breast cancer (in rats). High doses of carnosine may also possess some immune-stimulating activity as shown by animal experiments in which survival time in x-ray irradiated mice was increased by about 50% following carnosine intake (50-200mg/kg.day – a very large dose). It also appears to promote wound healing as shown by animal experiments in which 6-20mg/kg/day for 2 weeks reduced the size and depth of gastric ulcers and accelerated regeneration of the damaged tissue.

Calculating the pool of muscle dipeptides (mainly carnosine) accounts for about 10%-40% of the pH-buffering capacity of muscle tissue. Therefore, during intense exercise, carnosine may play an important role in preventing the reduction in pH caused by lactic acid accumulation – and thereby improving exercise performance. Animal studies in racehorses have shown that muscle carnosine concentrations are higher in muscles with a high percentage of fast-twitch glycolytic fibers and lower in muscles with predominantly slower twitch oxidative fiber types. In addition to its potential effects on anaerobic metabolism (lactic acid), carnosine may enhance oxidative (aerobic) metabolism by increasing the efficiency of mitochondria to produce cellular energy.

Dietary Sources: The average daily intake of carnosine from foods is probably in the range of 50-250mg (based on a diet containing at least one serving, 3-4 ounces, of beef, pork or chicken).

Dosage: Oral doses of 1-3 grams per day have been used with success in managing immune system function in cancer patients.

Side Effects: Rodent experiments have suggested that carnosine is extremely safe – no adverse toxic effects are noted even at doses up to 500mg/kg body weight (about 35 grams for an average-sized man).

(Source: www.supplementwatch.com)

Research Overview

Research on carnosine shows the following effects:

1. Protects superoxide dismutase against oxidation
2. Prevents the accumulation of age-related free radicals
3. May protect against the oxidative stress associated with Alzheimer's
4. Protects neuronal and endothelial cells from damage
5. Has anti-glycating properties
6. Improves memory in Alzheimer's
7. Improves cognition in Alzheimer's
8. Protect against malondialdehyde toxicity
9. Provide protection to cells and molecules from free radical damage
10. Delays aging in human cells
11. Protects against toxic aldehydes and thus offers protection from diabetes complications, inflammatory ailments, and alcohol-related liver disease
12. Affects protein metabolism
13. Affects cellular homeostasis

14. Prevents development of senility features
15. Reduces lipid peroxide production
16. Aids in wound healing
17. Enhances the immune system

Carnosine Abstracts (47)

1. Bull Exp Biol Med. 2003 Feb;135(2):130-2. Protective effect of carnosine on Cu,Zn-superoxide dismutase during impaired oxidative metabolism in the brain in vivo. Stvolinskii SL, Fedorova TN, Yuneva MO, Boldyrev AA. Institute of Neurology, Russian Academy of Medical Sciences, Moscow. sls@bio.inevro.msk.ru

Natural hydrophilic antioxidant carnosine protects cerebral cytosolic Cu,Zn-superoxide dismutase (SOD) under conditions of oxidative stress in various in vivo models: short-term hypobaric hypoxia in rats and accumulation of age-related changes in senescence-accelerated mice (SAMP). Administration of carnosine preventing Cu,Zn-SOD inactivation reduced mortality in rats and prolonged average life span in SAMP-mice.

2. Bull Exp Biol Med. 2002 Jun;133(6):559-61. Effect of carnosine on *Drosophila melanogaster* lifespan. Yuneva AO, Kramarenko GG, Vetreshchak TV, Gallant S, Boldyrev AA. M. V. Lomonosov Moscow State University, Moscow.

A positive dose-dependent effect of carnosine (beta-alanyl-L-histidine) on the lifespan of male *Drosophila melanogaster* flies was shown. The mean lifespan of male flies receiving 200 mg/liter carnosine approached that of females. At the same time carnosine had no effect on the lifespan of female flies. This positive effect of carnosine probably reflects its protective action against age-related accumulation of free radicals and did not depend on carnosine metabolism in the body. Addition of 200 mg/liter histidine and beta-alanine (separately or in combination) had no effect on the mean lifespan of flies.

3. Biogerontology. 2001;2(1):19-34. AGES in brain ageing: AGE-inhibitors as neuroprotective and anti-dementia drugs? Dukic-Stefanovic S, Schinzel R, Riederer P, Munch G. Physiological Chemistry I, Biocenter, University of Wurzburg, Germany.

In Alzheimer's disease, age-related cellular changes such as compromised energy production and increased radical formation are worsened by the presence of AGEs as additional, AD specific stress factors. Intracellular AGEs (most likely derived from methylglyoxal) crosslink cytoskeletal proteins and render them insoluble. These aggregates inhibit cellular functions including transport processes and contribute to neuronal dysfunction and death. Extracellular AGEs, which accumulate in ageing tissue (but most prominently on long-lived protein deposits like the senile plaques) exert chronic oxidative stress on neurons. In addition, they activate glial cells to produce free radicals (superoxide and NO) and neurotoxic cytokines such as TNF-alpha. Drugs, which inhibit the formation of AGEs by specific chemical mechanisms (AGE-inhibitors), including aminoguanidine, carnosine, tenilsetam, OPB-9195 and pyridoxamine, attenuate the development of (AGE-mediated) diabetic complications. Assuming that 'carbonyl stress' contributes significantly to the progression of Alzheimer's disease, AGE-inhibitors might also become interesting novel therapeutic drugs for treatment of AD.

4. Proc Natl Acad Sci U S A. 1996 May 14;93(10):4765-9. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. Forster MJ, Dubey A, Dawson KM, Stutts WA, Lal H, Sohal RS. Department of Pharmacology, University of North Texas Health Science Center, Fort Worth, 76107, USA.

The hypothesis that age-associated impairment of cognitive and motor functions is due to oxidative molecular damage was tested in the mouse. In a blind study, senescent mice (aged 22 months) were subjected to a battery of behavioral tests for motor and cognitive functions and subsequently assayed for oxidative molecular damage as assessed by protein carbonyl concentration in different regions of the brain. The degree of age-related impairment in each mouse was determined by comparison to a reference group of young mice (aged 4 months) tested concurrently on the behavioral battery. The age-related loss of ability to perform a spatial swim maze task was found to be positively correlated with oxidative molecular damage in the cerebral cortex, whereas age-related loss of motor coordination was correlated with oxidative molecular damage within the cerebellum. These results support the view that oxidative stress is a causal factor in brain senescence. Furthermore, the findings suggest that age-related declines of cognitive and motor performance progress independently, and involve oxidative molecular damage within different regions of the brain.

5. J Histochem Cytochem. 1998 Jun;46(6):731-5. Cytochemical demonstration of oxidative damage in Alzheimer disease by immunochemical enhancement of the carbonyl reaction with 2,4-dinitrophenylhydrazine. Smith MA, Sayre LM, Anderson VE, Harris PL, Beal MF, Kowall N, Perry G. Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106, USA.

Formation of carbonyls derived from lipids, proteins, carbohydrates, and nucleic acids is common during oxidative stress. For example, metal-catalyzed, "site-specific" oxidation of several amino acid side-chains produces aldehydes or ketones, and peroxidation of lipids generates reactive aldehydes such as malondialdehyde and hydroxynonenal. Here, using in situ 2,4-dinitrophenylhydrazine labeling linked to an antibody system, we describe a highly sensitive and specific cytochemical technique to specifically localize biomacromolecule-bound carbonyl reactivity. When this technique was applied to tissues from cases of Alzheimer disease, in which oxidative events including lipoperoxidative, glycoxidative, and other oxidative protein modifications have been reported, we detected free carbonyls not only in the disease-related intraneuronal lesions but also in other neurons. In marked contrast, free carbonyls were not found in neurons or glia in age-matched control cases. Importantly, this assay was highly specific for detecting disease-related oxidative damage because the site of oxidative damage can be assessed in the midst of

concurrent age-related increases in free carbonyls in vascular basement membrane that would contaminate biochemical samples subjected to bulk analysis. These findings demonstrate that oxidative imbalance and stress are key elements in the pathogenesis of Alzheimer disease.

6. Carnosine prevents activation of free-radical lipid oxidation during stress. Gulyaeva NV, Dupin AM, Levshina IP. *Bull Exp Biol Med.* 1989; 107(2):148-152. No abstract available.

Neurosci Lett. 1998 Feb 13;242(2):105-8. Toxic effects of beta-amyloid(25-35) on immortalised rat brain endothelial cell: protection by carnosine, homocarnosine and beta-alanine. Preston JE, Hipkiss AR, Himsworth DT, Romero IA, Abbott JN. Institute of Gerontology, King's College London, UK. j.preston@kcl.ac.uk

The effect of a truncated form of the neurotoxin beta-amyloid peptide (A beta25-35) on rat brain vascular endothelial cells (RBE4 cells) was studied in cell culture. Toxic effects of the peptide were seen at 200 microg/ml A beta using a mitochondrial dehydrogenase activity (MTT) reduction assay, lactate dehydrogenase release and glucose consumption. Cell damage could be prevented completely at 200 microg/ml A beta and partially at 300 microg/ml A beta, by the dipeptide carnosine. Carnosine is a naturally occurring dipeptide found at high levels in brain tissue and innervated muscle of mammals including humans. Agents which share properties similar to carnosine, such as beta-alanine, homocarnosine, the anti-glycating agent aminoguanidine, and the antioxidant superoxide dismutase (SOD), also partially rescued cells, although not as effectively as carnosine. We postulate that the mechanism of carnosine protection lies in its anti-glycating and antioxidant activities, both of which are implicated in neuronal and endothelial cell damage during Alzheimer's disease. Carnosine may therefore be a useful therapeutic agent.

7. *FEBS Lett.* 1995 Aug 28;371(1):81-5. Non-enzymatic glycosylation of the dipeptide L-carnosine, a potential anti-protein-cross-linking agent. Hipkiss AR, Michaelis J, Syrris P. Division of Biomolecular Engineering, CSIRO, North Ryde, NSW, Australia.

The dipeptide carnosine (beta-alanyl-L-histidine) was readily glycosylated non-enzymatically upon incubation with the sugars glucose, galactose, deoxyribose and the triose dihydroxyacetone. Carnosine inhibited glycation of acetyl-Lys-His-amide by dihydroxyacetone and it protected alpha-crystallin, superoxide dismutase and catalase against glycation and cross-linking mediated by ribose, deoxyribose, dihydroxyacetone, dihydroxyacetone phosphate and fructose. Unlike certain glycated amino acids, glycated carnosine was non-mutagenic. The potential biological and therapeutic significance of these observations are discussed.

8. *Biochim Biophys Acta.* 1997 Feb 27;1360(1):17-29. Influence of advanced glycation end-products and AGE-inhibitors on nucleation-dependent polymerization of beta-amyloid peptide. Munch G, Mayer S, Michaelis J, Hipkiss AR, Riederer P, Muller R, Neumann A, Schinzel R, Cunningham AM. Theodor-Boveri-Institute (Biocenter), Wurzburg, Germany. muench@biozentrum.uni-wuerzburg.de

Nucleation-dependent polymerization of beta-amyloid peptide, the major component of plaques in patients with Alzheimer's disease, is significantly accelerated by crosslinking through Advanced Glycation End-products (AGEs) in vitro. During the polymerization process, both nucleus formation and aggregate growth are accelerated by AGE-mediated crosslinking. Formation of the AGE-crosslinked amyloid peptide aggregates could be attenuated by the AGE-inhibitors Tenilsetam, aminoguanidine and carnosine. These experimental data, and clinical studies, reporting a marked improvement in cognition and memory in Alzheimer's disease patients after Tenilsetam treatment, suggest that AGEs might play an important role in the etiology or progression of the disease. Thus AGE-inhibitors may generally

9. *Biochem Biophys Res Commun.* 1998 Jul 9;248(1):28-32. Carnosine protects proteins against methylglyoxal-mediated modifications. Hipkiss AR, Chana H. Molecular Biology and Biophysics Group, King's College London, United Kingdom. alan.hipkiss@kcl.ac.uk

Methylglyoxal (MG) (pyruvaldehyde) is an endogenous metabolite which is present in increased concentrations in diabetics and implicated in formation of advanced glycosylation end-products (AGEs) and secondary diabetic complications. Carnosine (beta-alanyl-L-histidine) is normally present in long-lived tissues at concentrations up to 20 mM in humans. Previous studies showed that carnosine can protect proteins against aldehyde-containing cross-linking agents such as aldose and ketose hexose and triose sugars, and malon-dialdehyde, the lipid peroxidation product. Here we examine whether carnosine can protect protein exposed to MG. Our results show that carnosine readily reacts with MG thereby inhibiting MG-mediated protein modification as revealed electrophoretically. We also investigated whether carnosine could intervene when proteins were exposed to an MG-induced AGE (i.e. lysine incubated with MG). Our results show that carnosine can inhibit protein modification induced by a lysine-MG-AGE; this suggests a second intervention site for carnosine and emphasizes its potential as a possible non-toxic modulator of diabetic complications.

10. *Free Radic Biol Med.* 2000 May 15;28(10):1564-70. Carnosine reacts with a glycated protein. Brownson C, Hipkiss AR. Division of Biomolecular Science, GKT School of Biomedical Sciences, King's College London, Guy's Campus, London Bridge, London, UK.

Oxidation and glycation induce formation of carbonyl (CO) groups in proteins, a characteristic of cellular aging. The dipeptide carnosine (beta-alanyl-L-histidine) is often found in long-lived mammalian tissues at relatively high concentrations (up to 20 mM). Previous studies show that carnosine reacts with low-molecular-weight aldehydes and ketones. We examine here the ability of carnosine to react with ovalbumin CO groups generated by treatment of the protein with methylglyoxal (MG). Incubation of MG-treated protein with carnosine accelerated a slow decline in CO groups as measured by dinitrophenylhydrazine reactivity. Incubation of [¹⁴C]-carnosine with MG-treated ovalbumin resulted in a radiolabeled precipitate on addition of trichloroacetic acid (TCA); this was not observed with control, untreated protein. The presence of lysine or N-(alpha)-acetylglycyl-lysine methyl ester caused a decrease in the TCA-precipitable radiolabel. Carnosine also inhibited cross-linking of the MG-treated ovalbumin to lysine and normal, untreated alpha-crystallin. We conclude that carnosine can react with protein CO groups (termed "carnosinylation") and thereby modulate their deleterious interaction with other polypeptides. It is proposed that, should similar reactions occur intracellularly, then carnosine's known "anti-aging" actions might, at least partially, be explained by the dipeptide facilitating the inactivation/removal of deleterious proteins bearing carbonyl groups.

11. *Neurosci Lett.* 1997 Dec 5;238(3):135-8. Protective effects of carnosine against malondialdehyde-induced toxicity towards cultured rat brain endothelial cells. Hipkiss AR, Preston JE, Himswoth DT, Worthington VC, Abbot NJ. Molecular Biology and Biophysics Group, King's College London, Strand, UK.

Malondialdehyde (MDA) is a deleterious end-product of lipid peroxidation. The naturally-occurring dipeptide carnosine (beta-alanyl-L-histidine) is found in brain and innervated tissues at concentrations up to 20 mM. Recent studies have shown that carnosine can protect proteins against cross-linking mediated by aldehyde-containing sugars and glycolytic intermediates. Here we have investigated whether carnosine is protective against malondialdehyde-induced protein damage and cellular toxicity. The results show that carnosine can (1) protect cultured rat brain endothelial cells against MDA-induced toxicity and (2) inhibit MDA-induced protein modification (formation of cross-links and carbonyl groups).

12. *Cell Mol Neurobiol.* 1997 Apr;17(2):259-71. Biochemical and physiological evidence that carnosine is an endogenous neuroprotector against free radicals. Boldyrev AA, Stvolinsky SL, Tyulina OV, Koshelev VB, Hori N, Carpenter DO. M. V. Lomonosov Moscow State University, Moscow, Russia.

1. Carnosine, anserine, and homocarnosine are endogenous dipeptides concentrated in brain and muscle whose biological functions remain in doubt. 2. We have tested the hypothesis that these compounds function as endogenous protective substances against molecular and cellular damage from free radicals, using two isolated enzyme systems and two models of ischemic brain injury. Carnosine and homocarnosine are both effective in activating brain Na, K-ATPase measured under optimal conditions and in reducing the loss of its activity caused by incubation with hydrogen peroxide. 3. In contrast, all three endogenous dipeptides cause a reduction in the activity of brain tyrosine hydroxylase, an enzyme activated by free radicals. In hippocampal brain slices subjected to ischemia, carnosine increased the time to loss of excitability. 4. In in vivo experiments on rats under experimental hypobaric hypoxia, carnosine increased the time to loss of ability to stand and breath and decreased the time to recovery. 5. These actions are explicable by effects of carnosine and related compounds which neutralize free radicals, particularly hydroxyl radicals. In all experiments the effective concentration of carnosine was comparable to or lower than those found in brain. These observations provide further support for the conclusion that protection against free radical damage is a major role of carnosine, anserine, and homocarnosine.

13. *Ann N Y Acad Sci.* 1998 Nov 20;854:37-53. Pluripotent protective effects of carnosine, a naturally occurring dipeptide. Hipkiss AR, Preston JE, Himswoth DT, Worthington VC, Keown M, Michaelis J, Lawrence J, Mateen A, Allende L, Eagles PA, Abbott NJ. Molecular Biology and Biophysics Group, King's College London, Strand, United Kingdom. alan.hipkiss@kcl.ac.uk

Carnosine is a naturally occurring dipeptide (beta-alanyl-L-histidine) found in brain, innervated tissues, and the lens at concentrations up to 20 mM in humans. In 1994 it was shown that carnosine could delay senescence of cultured human fibroblasts. Evidence will be presented to suggest that carnosine, in addition to antioxidant and oxygen free-radical scavenging activities, also reacts with deleterious aldehydes to protect susceptible macromolecules. Our studies show that, in vitro, carnosine inhibits nonenzymic glycosylation and cross-linking of proteins induced by reactive aldehydes (aldose and ketose sugars, certain triose glycolytic intermediates and malondialdehyde (MDA), a lipid peroxidation product). Additionally we show that carnosine inhibits formation of MDA-induced protein-associated advanced glycosylation end products (AGEs) and formation of DNA-protein cross-links induced by acetaldehyde and formaldehyde. At the cellular level 20 mM carnosine protected cultured human fibroblasts and lymphocytes, CHO cells, and cultured rat brain endothelial cells against the toxic effects of formaldehyde, acetaldehyde and MDA, and AGEs formed by a lysine/deoxyribose mixture. Interestingly, carnosine protected cultured rat brain endothelial cells against amyloid peptide toxicity. We propose that carnosine (which is remarkably nontoxic) or related structures should be explored for possible intervention in pathologies that involve deleterious aldehydes, for example, secondary diabetic complications, inflammatory phenomena, alcoholic liver disease, and possibly Alzheimer's disease.

14. *Exp Cell Res.* 1994 Jun;212(2):167-75. Retardation of the senescence of cultured human diploid fibroblasts by carnosine. McFarland GA, Holliday R.

We have examined the effects of the naturally occurring dipeptide carnosine (beta-alanyl-L-histidine) on the growth, morphology, and lifespan of cultured human diploid fibroblasts. With human foreskin cells, HFF-1, and fetal lung cells, MRC-5, we have shown that carnosine at high concentrations (20-50 mM) in standard medium retards senescence and rejuvenates senescent cultures. These late-passage cultures preserve a nonsenescent morphology in the presence of carnosine, in comparison to the senescent morphology first described by Hayflick and Moorhead. Transfer of these late-passage cells in medium containing carnosine to unsupplemented normal medium results in the appearance of the senescent phenotype. The serial subculture of cells in the presence of carnosine does not prevent the Hayflick limit to growth, although the lifespan in population doublings as well as chronological age is often increased. This effect is obscured by the normal variability of human fibroblast lifespans, which we have confirmed. Transfer of cells approaching senescence in normal medium to medium supplemented with carnosine rejuvenates the cells but the extension in lifespan is variable. Neither D-carnosine, (beta-alanyl-D-histidine), homocarnosine, anserine, nor beta-alanine had the same effects as carnosine on human fibroblasts. Carnosine is an antioxidant, but it is more likely that it preserves cellular integrity by its effects on protein metabolism.

15. Exp Gerontol. 1999 Jan;34(1):35-45. Further evidence for the rejuvenating effects of the dipeptide L-carnosine on cultured human diploid fibroblasts. McFarland GA, Holliday R. CSIRO Division of Molecular Science, Sydney Laboratory, North Ryde, Australia.

We have confirmed and extended previous results on the beneficial effects of L-carnosine on growth, morphology, and longevity of cultured human fibroblasts, strains MRC-5 and HFF-1. We have shown that late-passage HFF-1 cells retain a juvenile appearance in medium containing 50 mM carnosine, and revert to a senescent phenotype when carnosine is removed. Switching cells between medium with and without carnosine also switches their phenotype from senescent to juvenile, and the reverse. The exact calculation of fibroblast lifespans in population doublings (PDs) depends on the proportion of inoculated cells that attach to their substrate and the final yield of cells in each subculture. We have shown that carnosine does not affect cell attachment, but does increase longevity in PDs. However, the plating efficiency of MRC-5 cells seeded at low density is strongly increased in young and senescent cells by carnosine, as shown by the growth of individual colonies. We have also demonstrated that very late-passage MRC-5 cells (with weekly change of medium without subculture) remain attached to their substrate much longer in medium containing carnosine in comparison to control cultures, and also retain a much more normal phenotype. Carnosine is a naturally occurring dipeptide present at high concentration in a range of human tissues. We suggest it has an important role in cellular homeostasis and maintenance. Biosci Rep. 1999 Dec;19(6):581-7.

16. Carnosine, the protective, anti-aging peptide.

Boldyrev AA, Gallant SC, Sukhich GT.

Center for Molecular Medicine, Department of Biochemistry, Biological Faculty, MV Lomonosov, Moscow State University, Vorobjovy Gory, Russia. aab@1.biocenter.bio.msu.ru

Carnosine attenuates the development of senile features when used as a supplement to a standard diet of senescence accelerated mice (SAM). Its effect is apparent on physical and behavioral parameters and on average life span. Carnosine has a similar effect on mice of the control strain, but this is less pronounced due to the non-accelerated character of their senescence processes.

17. Effect of carnosine on age-induced changes in senescence-accelerated mice Yuneva M.O.; Bulygina E.R.; Gallant S.C.; Kramarenko G.G.; Stvolinsky S.L.; Semyonova M.L.; Boldyrev A.A. Prof. A.A. Boldyrev, Department of Biochemistry, School of Biology, Moscow State University, Vorobjovy Gory, 119899 Moscow Russian Federation Author Email: aab@1.biocenter.bio.msu.ru Journal of Anti-Aging Medicine (J. ANTI-AGING MED.) (United States) 1999 , 2/4 (337-342)

The effect of carnosine on the life span and several brain biochemical characteristics in senescence-accelerated mice-prone 1 (SAMP1) was investigated. A 50% survival rate of animals treated with carnosine increased by 20% as compared to controls. Moreover, the number of animals that lived to an old age significantly increased. The effect of carnosine on life span was accompanied by a decrease in the level of 2'-tiobarbituric acid reactive substances (TBARS), monoamine oxidase b (MAO b), and Na/K-ATPase activity. There was also an increase in glutamate binding to N-methyl-D-aspartate receptors. These observations are consistent with the conclusion that carnosine increases life span and quality of life by diminishing production of lipid peroxides and reducing the influence of reactive oxygen species (ROS) on membrane proteins.

18. Salganik R.I.; Dikalova A.; Dikalov S.; La D.; Bulygina E.; Stvolinsky S.; Boldyrev A. Dr. R.I. Salganik, 2217B, McGavran-Greenberg Hall, School of Public Health, University of North Carolina, Chapel Hill, NC 27599 United States Author Email: rsalganik@unc.edu Journal of Anti-Aging Medicine (J. ANTI-AGING MED.) (United States) 2001 , 4/1 (49-54)

Impairment of long-term memory is characteristic of aging and some neurodegenerative diseases associated with the increased generation of reactive oxygen species (ROS). An inbred OXYS rat strain was developed from Wistar rats with an inherited

overproduction of ROS, manifesting impairment of long-term memory and oxidative damage of cell structures and functions. A highly inbred OYXR strain harboring oxidative patterns close to normal Wistar rats served as a control. Alterations of brain neurochemical functions in OXYS rats and the possibility of protecting them with different antioxidants were studied. Assaying the oxidative DNA lesion, 8-hydroxydeoxyguanine (8-OHdG), and lipid peroxidation-induced etheno-DNA adducts in rat liver DNA indicated a high oxidative stress in OXYS rats. We found that the Na/K-ATPase activity, N-methyl-D-aspartate (NMDA) receptors, and the integrity of sulfhydryl (SH) groups, parameters associated with memory-related neurochemical mechanisms, were altered in OXYS rat brains compared to that of control OXYR rats. Protection of neurochemical functions was investigated by long-term treatment of OXYS rats with different antioxidants, namely, 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene; BHT), 2,6-dimethyl-3-hydroxypyridine (emoxipine), and beta-alanyl-L-histidine (carnosine). We determined that BHT protected rat brains from the oxidative alteration of Na/K-ATPase but did not protect NMDA receptors and SH groups. Emoxipine protected rat brain from oxidative impairment of SH group, but did not protect NMDA receptors and Na/K-ATPase. Carnosine protected from oxidative impairment rat brain NMDA receptors, Na/K-ATPase, and protein SH groups.

19. *Biochemistry (Mosc)*. 2000 Jul;65(7):807-16. Interactions between carnosine and zinc and copper: implications for neuromodulation and neuroprotection. Trombley PQ, Horning MS, Blakemore LJ. Biomedical Research Facility, Department of Biological Science, Florida State University, Tallahassee, Florida 32306-4340, USA. trombley@neuro.fsu.edu.

This review examines interactions in the mammalian central nervous system (CNS) between carnosine and the endogenous transition metals zinc and copper. Although the relationship between these substances may be applicable to other brain regions, the focus is on the olfactory system where these substances may have special significance. Carnosine is not only highly concentrated in the olfactory system, but it is also contained in neurons (in contrast to glia cells in most of the brain) and has many features of a neurotransmitter. Whereas the function of carnosine in the CNS is not well understood, we review evidence that suggests that it may act as both a neuromodulator and a neuroprotective agent. Although zinc and/or copper are found in many neuronal pathways in the brain, the concentrations of zinc and copper in the olfactory bulb (the target of afferent input from sensory neurons in the nose) are among the highest in the CNS. Included in the multitude of physiological roles that zinc and copper play in the CNS is modulation of neuronal excitability. However, zinc and copper also have been implicated in a variety of neurologic conditions including Alzheimer's disease, Parkinson's disease, stroke, and seizures. Here we review the modulatory effects that carnosine can have on zinc and copper's abilities to influence neuronal excitability and to exert neurotoxic effects in the olfactory system. Other aspects of carnosine in the CNS are reviewed elsewhere in this issue.

20. *Brain Res*. 2000 Jan 3;852(1):56-61. Endogenous mechanisms of neuroprotection: role of zinc, copper, and carnosine. Horning MS, Blakemore LJ, Trombley PQ. Biomedical Research Facility, Department of Biological Science, Florida State University, Tallahassee 32306-4340, USA. horning@neuro.fsu.edu

Zinc and copper are endogenous transition metals that can be synaptically released during neuronal activity. Synaptically released zinc and copper probably function to modulate neuronal excitability under normal conditions. However, zinc and copper also can be neurotoxic, and it has been proposed that they may contribute to the neuropathology associated with a variety of conditions, such as Alzheimer's disease, stroke, and seizures. Recently, we demonstrated that carnosine, a dipeptide expressed in glial cells throughout the brain as well as in neuronal pathways of the visual and olfactory systems, can modulate the effects of zinc and copper on neuronal excitability. This result led us to hypothesize that carnosine may modulate the neurotoxic effects of zinc and copper as well. Our results demonstrate that carnosine can rescue neurons from zinc- and copper-mediated neurotoxicity and suggest that one function of carnosine may be as an endogenous neuroprotective agent.

21. *Neuroscience*. 1999;94(2):571-7. Carnosine protects against excitotoxic cell death independently of effects on reactive oxygen species. Boldyrev A, Song R, Lawrence D, Carpenter DO. International Center for Biotechnology and Center for Molecular Medicine, MV Lomonosov Moscow State University, Department of Biochemistry, School of Biology, Russia.

The role of carnosine, N-acetylcarnosine and homocarnosine as scavengers of reactive oxygen species and protectors against neuronal cell death secondary to excitotoxic concentrations of kainate and N-methyl-D-aspartate was studied using acutely dissociated cerebellar granule cell neurons and flow cytometry. We find that carnosine, N-acetylcarnosine and homocarnosine at physiological concentrations are all potent in suppressing fluorescence of 2',7'-dichlorofluorescein, which reacts with intracellularly generated reactive oxygen species. However, only carnosine in the same concentration range was effective in preventing apoptotic neuronal cell death, studied using a combination of the DNA binding dye, propidium iodide, and a fluorescent derivative of the phosphatidylserine-binding dye, Annexin-V. Our results indicate that carnosine and related compounds are effective scavengers of reactive oxygen species generated by activation of ionotropic glutamate receptors, but that this action does not prevent excitotoxic cell death. Some other process which is sensitive to carnosine but not the related compounds is a critical factor in cell death. These observations indicate that at least in this system reactive oxygen species generation is not a major contributor to excitotoxic neuronal cell death.

22. *Cell Mol Neurobiol*. 1999 Feb;19(1):45-56. Carnosine: an endogenous neuroprotector in the ischemic brain. Stvolinsky SL, Kukley ML, Dobrota D, Matejovicova M, Tkac I, Boldyrev AA. Institute of Neurology, Russian Academy of Medical Sciences, Moscow, Russia.

1. The biological effects of carnosine, a natural hydrophilic neuropeptide, on the reactive oxygen species (ROS) pathological generation are reviewed. 2. We describe direct antioxidant action observed in the in vitro experiments. 3. Carnosine was found to effect metabolism indirectly. These effects are reflected in ROS turnover regulation and lipid peroxidation (LPO) processes. 4. During brain ischemia carnosine acts as a neuroprotector, contributing to better cerebral blood flow restoration, electroencephalography (EEG) normalization, decreased lactate accumulation, and enzymatic protection against ROS. 5. The data presented demonstrate that carnosine is a specific regulator of essential metabolic pathways in neurons supporting brain homeostasis under unfavorable conditions.

23. Surgery. 1986 Nov;100(5):815-21. Action of carnosine and beta-alanine on wound healing. Nagai K, Suda T, Kawasaki K, Mathuura S.

In rats treat-given hydrocortisone to suppress healing, tensile strength of the skin at the site of an incision wound was significantly higher in rats locally treated with carnosine than in untreated animals. Similar effects on the tensile strength of the skin were observed by the administration of beta-alanine and histidine, but not of beta-alanine alone. Exogenous carnosine was degraded in the body by carnosinase and histidine decarboxylase to yield histamine. Since beta-alanine, the other degradation product of carnosine, was found to stimulate the biosynthesis of nucleic acids and collagen, histamine derived from carnosine is considered to have enhanced the process of wound healing by stimulating effusion at the initial stage of inflammation. Thus, the enhancement by carnosine of wound healing may be ascribed to stimulation of early effusion by histamine and of collagen biosynthesis by beta-alanine. The wound-healing effects of carnosine were further demonstrated by the observation that carnosine significantly increased granulation suppressed by cortisone, mitomycin C, 5-fluorouracil, and bleomycin.

24. Nippon Seirigaku Zasshi. 1986;48(11):735-40. [Immuno-enhancing actions of carnosine and homocarnosine] [Article in Japanese] Nagai K, Suda T.

Immuno-enhancing actions of carnosine, beta-alanine, homocarnosine, and gamma-aminobutyric acid were studied in ddY mice by evaluating plaque-forming cell reaction against sheep red blood cells. Animals were administered the test agents in prior to, or simultaneously with, various treatments that are known to reduce immune function such as administration of the anti-tumor agents, mitomycin C and 5-fluorouracil, immunosuppressant cyclophosphamide, antiinflammatory agent hydrocortisone, or cancer implantation and gamma-irradiation. Experiments were performed also in aged mice with reduced immune function. The administration of these drugs showed non-specific immuno-enhancing effects under all conditions examined and on all cell groups that may have been affected by these immunosuppressive stimulus.

25. Nippon Seirigaku Zasshi. 1986;48(11):741-7. [Antineoplastic effects of carnosine and beta-alanine--physiological considerations of its antineoplastic effects] [Article in Japanese] Nagai K, Suda T.

Antineoplastic effects of carnosine (CAR) and beta-alanine (ALA), were examined in vivo using ddY mice implanted with the solid tumor Sarcoma-180. The sarcoma was treated with trypsin, 10⁵ cells were implanted subcutaneously in the back of the animals, and CAR and ALA were administered subcutaneously 2 cm from the implantation site starting on the next day. The animals treated with ALA alone showed prolongation of survival to a T/C value of 132%; the growth of the tumor was inhibited and mortality reduced in those treated with CAR alone. Regression of the tumor was observed in the animals treated with either drug. The effects of these agents were enhanced when administered in combination with the non-specific active immuno-enhancing agent OK-432. More than half the animals treated with CAR and OK-432 survived the observation period (T/C greater than 218%), and survival was prolonged in those treated with ALA and OK-432 to a T/C value of 132%. The agents also showed potent antineoplastic effects on Sarcoma-180 when the tumor had been attenuated in vivo with mitomycin C (MMC).

26. Nippon Seirigaku Zasshi. 1986;48(6):572-9. [Immunoregulative effects of homocarnosine and gamma-aminobutyric acid] [Article in Japanese] Nagai K, Suda T.

The effects of homocarnosine and GABA on antibody production (PFC reaction) and cellular immunity (delayed hypersensitivity reaction, DHR) were examined in vivo. In mice treated with these agents, PFC reaction to 2 X 10⁷ SRBC was enhanced but that to 1 X 10⁹ SRBC was suppressed; moreover, immunoreaction was reduced in immature mice (2-2.5 weeks old) but was increased in aged mice (30 weeks old or above). These agents had optimal doses on the PFC reaction in mice given 1 X 10⁸ SRBC and DHR, and induced recovery of immunofunction suppressed by the administration of MMC.

27. Nippon Seirigaku Zasshi. 1986;48(6):564-71. [Immunoregulative effects of carnosine and beta-alanine] [Article in Japanese] Nagai K, Suda T.

Physiological factors involved in immunity and tissue repair with regulate homeostasis, a physiological function of the connective tissue, are as yet unidentified. We earlier detected the granulation-promoting action of carnosine, and reported on the acceleration of tissue repair in experimental as well as clinical studies. In that study, immunoregulatory effects of carnosine and beta-alanine were examined by the plaque-forming cell (PFC) count and delayed hypersensitivity reaction (DHR). The PFC value increased in mice pretreated with these agents. In these mice, PFC reaction to 2 X 10⁷ SRBC was enhanced but that to 1 X 10⁹ SRBC was

suppressed. The agents also suppressed excess immunoreaction in immature mice but increased weakened immunoreaction in aged animals. Furthermore, the agents had the optimal doses for the enhancement of both PFC reaction to 1×10^8 SRBC and DHR to 1% picryl chloride. They also induced recovery of immunofunction suppressed by the administration of MMC. Carnosine and beta-alanine exerts immunoregulatory effects by activating both T and B cells. Our observations indicated that the agents not only promote tissue repair but also help maintain homeostasis and accelerate spontaneous healing.

28. Effects of carnosine on the development of rat sponge-induced granulation tissue. II. Histoautoradiographic observations on collagen biosynthesis. Vizioli MR, Blumen G, Almeida OP, et al. *Cell Mol Biol*. 1983; 29(1):1-9. No abstract available.

29. *Cell Struct Funct*. 1999 Apr;24(2):79-87. Carnosine stimulates vimentin expression in cultured rat fibroblasts. Ikeda D, Wada S, Yoneda C, Abe H, Watabe S. Laboratory of Aquatic Molecular Biology and Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Japan.

Two-dimensional electrophoretic gel profiles were compared between rat 3Y1 fibroblasts cultured in the presence and absence of 30 mM L-carnosine (beta-alanyl-L-histidine) for one week without any replenishment of medium. While a number of cellular proteins changed their expression levels by the addition of carnosine, we identified one of the most prominently varied proteins as vimentin. Immunoblot analysis with anti-vimentin antibody demonstrated that the vimentin levels increased about 2-fold after one-week culture in the presence of carnosine. We also confirmed that the increase of vimentin expression was dependent on the concentration of carnosine added to the medium. Moreover, when cultured cells were stained with anti-vimentin antibody and observed by light microscopy, most cells grown in the presence of carnosine were found to have markedly developed vimentin filaments. The increase of vimentin expression was also observed by adding with carnosine related dipeptides, N-acetylcarnosine and anserine.

30. *Cell Mol Life Sci*. 2000 May;57(5):747-53. A possible new role for the anti-ageing peptide carnosine. Hipkiss AR, Brownson C. Biomolecular Sciences Division, GKT School of Biomedical Sciences, King's College London, UK. alan.hipkiss@kcl.ac.uk

The naturally occurring dipeptide carnosine (beta-alanyl-L-histidine) is found in surprisingly large amounts in long-lived tissues and can delay ageing in cultured human fibroblasts. Carnosine has been regarded largely as an anti-oxidant and free radical scavenger. More recently, an anti-glycating potential has been discovered whereby carnosine can react with low-molecular-weight compounds that bear carbonyl groups (aldehydes and ketones). Carbonyl groups, arising mostly from the attack of reactive oxygen species and low-molecular-weight aldehydes and ketones, accumulate on proteins during ageing. Here we propose, with supporting evidence, that carnosine can react with protein carbonyl groups to produce protein-carbonyl-carnosine adducts ('carnosinylated' proteins). The various possible cellular fates of the carnosinylated proteins are discussed. These proposals may help explain anti-ageing actions of carnosine and its presence in non-mitotic cells of long-lived mammals.

31. *Biogerontology*. 2000;1(3):217-23. Carnosine reacts with protein carbonyl groups: another possible role for the anti-ageing peptide? Hipkiss AR, Brownson C. Biomolecular Sciences Division, GKT School of Biomedical Sciences, King's College London, Guy's Campus London Bridge, London EC1 1UL, UK. alan.hipkiss@kcl.ac.uk

Carnosine (beta-alanyl-L-histidine) can delay senescence and provoke cellular rejuvenation in cultured human fibroblasts. The mechanisms by which such a simple molecule induces these effects is not known despite carnosine's well documented anti-oxidant and oxygen free-radical scavenging activities. Carbonyl groups are generated on proteins post-synthetically by the action of reactive oxygen species and glycating agents and their accumulation is a major biochemical manifestation of ageing. We suggest that, in addition to the prophylactic actions of carnosine, it may also directly participate in the inactivation/disposal of aged proteins possibly by direct reaction with the carbonyl groups on proteins. The possible fates of these 'carnosinylated' proteins including the formation of inert lipofuscin, proteolysis via the proteasome system and exocytosis following interaction with receptors are also discussed. The proposal may point to a hitherto unrecognised mechanism by which cells/organisms normally defend themselves against protein carbonyls.

CARNOSINE AND GLYCATION

32. *Mech Ageing Dev* 2001 Sep 15;122(13):1431-45 Carnosine, the anti-ageing, anti-oxidant dipeptide, may react with protein carbonyl groups. Hipkiss AR, Brownson C, Carrier MJ. Division of Biomolecular Sciences, GKT School of Biomedical Sciences, King's College London, Guy's Campus, London Bridge, London SE1 1UL, UK. alan.hipkiss@kcl.ac.uk

Carnosine (beta-alanyl-L-histidine) is a physiological dipeptide which can delay ageing and rejuvenate senescent cultured human fibroblasts. Carnosine's anti-oxidant, free radical- and metal ion-scavenging activities cannot adequately explain these effects. Previous studies showed that carnosine reacts with small carbonyl compounds (aldehydes and ketones) and protects macromolecules against their cross-linking actions. Ageing is associated with accumulation of carbonyl groups on proteins. We consider here whether carnosine reacts with protein carbonyl groups. Our evidence indicates that carnosine can react non-enzymically with protein carbonyl groups, a process termed 'carnosinylation'. We propose that similar reactions could occur in cultured fibroblasts and in vivo. A preliminary experiment suggesting that carnosine is effective in vivo is presented; it suppressed

diabetes-associated increase in blood pressure in fructose-fed rats, an observation consistent with carnosine's anti-glycating actions. We speculate that: (i) carnosine's apparent anti-ageing actions result, partly, from its ability to react with carbonyl groups on glycated/oxidised proteins and other molecules; (ii) this reaction, termed 'carnosinylation,' inhibits cross-linking of glycoxidised proteins to normal macromolecules; and (iii) carnosinylation could affect the fate of glycoxidised polypeptides.

33. *Biochemistry (Mosc)* 2000 Jul;65(7):771-8 Carnosine and protein carbonyl groups: a possible relationship. Hipkiss AR. Division of Biomolecular Sciences, GKT School of Biomedical Sciences, King's College London, London SE1 1UL, UK. alan.hipkiss@kcl.ac.uk.

Carnosine has been shown to react with low-molecular-weight aldehydes and ketones and has been proposed as a naturally occurring anti-glycating agent. It is suggested here that carnosine can also react with ("carnosinylate") proteins bearing carbonyl groups, and evidence supporting this idea is presented. Accumulation of protein carbonyl groups is associated with cellular ageing resulting from the effects of reactive oxygen species, reducing sugars, and other reactive aldehydes and ketones. Carnosine has been shown to delay senescence and promote formation of a more juvenile phenotype in cultured human fibroblasts. It is speculated that carnosine may intracellularly suppress the deleterious effects of protein carbonyls by reacting with them to form protein-carbonyl-carnosine adducts, i.e., "carnosinylated" proteins. Various fates of the carnosinylated proteins are discussed including formation of inert lipofuscin and proteolysis via proteasome and RAGE activities. It is proposed that the anti-ageing and rejuvenating effects of carnosine are more readily explainable by its ability to react with protein carbonyls than its well-documented antioxidant activity.

34. *Neurosci Lett* 1998 Feb 13;242(2):105-8 Toxic effects of beta-amyloid(25-35) on immortalised rat brain endothelial cell: protection by carnosine, homocarnosine and beta-alanine. Preston JE, Hipkiss AR, Himsworth DT, Romero IA, Abbott JN. Institute of Gerontology, King's College London, UK. j.preston@kcl.ac.uk

The effect of a truncated form of the neurotoxin beta-amyloid peptide (A beta25-35) on rat brain vascular endothelial cells (RBE4 cells) was studied in cell culture. Toxic effects of the peptide were seen at 200 microg/ml A beta using a mitochondrial dehydrogenase activity (MTT) reduction assay, lactate dehydrogenase release and glucose consumption. Cell damage could be prevented completely at 200 microg/ml A beta and partially at 300 microg/ml A beta, by the dipeptide carnosine. Carnosine is a naturally occurring dipeptide found at high levels in brain tissue and innervated muscle of mammals including humans. Agents which share properties similar to carnosine, such as beta-alanine, homocarnosine, the anti-glycating agent aminoguanidine, and the antioxidant superoxide dismutase (SOD), also partially rescued cells, although not as effectively as carnosine. We postulate that the mechanism of carnosine protection lies in its anti-glycating and antioxidant activities, both of which are implicated in neuronal and endothelial cell damage during Alzheimer's disease. Carnosine may therefore be a useful therapeutic agent.

35. *Biochemistry (Mosc)* 1997 Oct;62(10):1119-23 Change in the functional properties of actin by its glycation in vitro. Kuleva NV, Kovalenko ZS. Department of Biochemistry, School of Biology and Soil Sciences, St. Petersburg State University, Universitetskaya Naberezhnaya 7/9, Vasil'evskii Ostrov, St. Petersburg, Russia.

The influence of glycation (non-enzymatic glycosylation) on structural and functional properties of actin of rabbit skeletal muscle and the effects of the natural anti-glycating dipeptide carnosine were studied. Glucose (0.5 M), fructose (0.5 M), and glyceraldehyde (0.05 M) were used as glycating agents. Marked changes in the structural and functional properties were observed in the presence of glyceraldehyde when high-molecular-weight components appear. This was followed by a decrease in the ability of actin to activate myosin ATPase, to polymerize, and to inhibit DNase I. In the presence of 0.05 M carnosine, the quantity of high-molecular-weight products decreased and myosin ATPase activation was retained. Since muscle tissue contains millimolar quantities of carnosine, glycation of actin associated with changes in its properties is evidently more likely to occur in non-muscle cells.

CARNOSINE AND DEGENERATIVE

36. *Biochim Biophys Acta*. 2000 Dec 15;1524(2-3):162-70. Enhanced oxidative damage by the familial amyotrophic lateral sclerosis-associated Cu,Zn-superoxide dismutase mutants. Kang JH, Eum WS. Department of Genetic Engineering, Division of Natural Sciences, Chongju University, 360-764, Chongju, South Korea. jhkang@chongju.ac.kr

Some cases of familial amyotrophic lateral sclerosis (FALS), a degenerative disorder of motor neurons, is associated with mutation in the Cu,Zn-superoxide dismutase (SOD) gene SOD1. The purified FALS mutant and wild-type Cu,Zn-SODs expressed in *Escherichia coli* cells have identical dismutation activity whereas the hydroxyl radical formation of FALS mutants was enhanced relative to that of the wild-type enzyme. These higher free radical-generating activities of mutants facilitated the release of copper ions from their own molecules. The reaction of the mutants with hydrogen peroxide enhanced DNA strand breaks and lipid peroxidation. The results suggested that the enhanced oxidative damage of macromolecules is mediated in the Cu,Zn-SOD mutants and hydrogen peroxide system via the generation of hydroxyl radicals by a combination of the higher free radical-generating activities of mutants and a Fenton-like reaction of copper ions released from oxidatively damaged Cu,Zn-SODs. Carnosine has been proposed to act as antioxidant in vivo. We investigated whether carnosine could protect the oxidative damage

induced by FALS mutants. Carnosine effectively inhibited the DNA cleavage and lipid peroxidation. These results suggest that the higher free radical-generating function of FALS mutants can lead to increased oxidative damage of macromolecules which further implicates free radical-mediated motor neuronal injury in the pathogenesis of FALS and carnosine may be explored as potential therapeutic agents for FALS patients.

GLYCATION AND CARNOSINE SEARCH

37. Life Sci. 2003 Apr 25;72(23):2603-16. The polyamines spermine and spermidine protect proteins from structural and functional damage by AGE precursors: a new role for old molecules? Gugliucci A, Menini T. Biochemistry Laboratory, Division of Basic Medical Sciences, Touro University, College of Osteopathic Medicine, 1310 Johnson Lane, Mare Island, Vallejo, CA 94592, USA. agugliuc@touro.edu

Due to the importance of glycation in the genesis of diabetic complications, an intense search for synthetic new antiglycation agents is ongoing. However, a somewhat neglected avenue is the search for endogenous compounds that may inhibit the process and be a source of prodrugs. Based on their ubiquity, their polycationic nature, their essential role in growth, their relatively high concentrations in tissues, and their high concentrations in sperm, we hypothesized that polyamines inhibit glycation and that might be one of their so far elusive functions. In this study we demonstrate a potent antiglycation effect of physiological concentrations of the polyamines spermine and spermidine. We employed two approaches: in the first, we monitored structural changes on histones and ubiquitin in which polyamines inhibit glycation-induced dimer and polymer formation. In the second we monitored functional impairment of catalytic activity of antithrombin III and plasminogen. Protection is afforded against glycation by hexoses, trioses and dicarbonyls AGE precursors and is comparable to those of aminoguanidine and carnosine.

38. Biochem Biophys Res Commun. 2003 Jan 3;300(1):75-80. Carnosine promotes the heat denaturation of glycated protein. Yeargans GS, Seidler NW. Department of Biochemistry, University of Health Sciences, 1750 Independence Avenue, Kansas City, MO 64106-1453, USA.

Glycation alters protein structure and decreases biological activity. Glycated proteins, which accumulate in affected tissue, are reliable markers of disease. Carnosine, which prevents glycation, may also play a role in the disposal of glycated protein. Carnosinylation tags glycated proteins for cell removal. Since thermostability determines cell turnover of proteins, the present study examined carnosine's effect on thermal denaturation of glycated protein using cytosolic aspartate aminotransferase (cAAT). Glycated cAAT (500 microM glyceraldehyde for 72h at 37 degrees C) increased the T(0.5) (temperature at which 50% denaturation occurs) and the Gibbs free energy barrier (DeltaG) for denaturation. The enthalpy of denaturation (DeltaH) for glycated cAAT was also higher than that for unmodified cAAT, suggesting that glycation changes the water accessible surface. Carnosine enhanced the thermal unfolding of glycated cAAT as evidenced by a decreased T(0.5) and a lowered Gibbs free energy barrier. Additionally, carnosine decreased the enthalpy of denaturation, suggesting that carnosine may promote hydration during heat denaturation of glycated protein.

39. Life Sci. 2002 Mar 1;70(15):1789-99. Effects of thermal denaturation on protein glycation. Seidler NW, Yeargans GS. Department of Biochemistry, University of Health Sciences, Kansas City, MO 64106, USA. nseidler@uhs.edu

Protein denaturation occurs at sites of inflammation. We hypothesized that denatured protein may provide a more susceptible target for glycation, which is a known mediator of inflammation. We examined the effects of thermal denaturation on the susceptibility of protein glycation using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and aspartate aminotransferase (AAT) as our target proteins. GAPDH and AAT are ubiquitous proteins that exhibited very different thermal stabilities. Glycating agents, methylglyoxal (MG) and glyceraldehyde (Glyc), caused an increase in the formation of advanced glycation endproducts (AGEs) in native and denatured GAPDH and AAT. The effects of the glycating agents were more pronounced with the denatured proteins. In addition to nitroblue tetrazolium (NBT)- reactivity, our measured endpoints were absorbance ($\lambda = 365$ nm) and fluorescence ($\lambda_{ex} = 370$ nm; $\lambda_{em} = 470$ nm) properties that are typically associated with protein glycation. We also looked at carnosine's ability to prevent glycation of native and denatured protein. Carnosine, an endogenous histidine dipeptide, exhibits anti-inflammatory activity presumably due to its anti-oxidant and anti-glycation properties. Carnosine prevented Glyc-induced AGE formation in both native and denatured AAT suggesting that carnosine's anti-inflammatory activity may be due in part to carnosine's ability to prevent glycation of denatured protein.

40. Ann N Y Acad Sci. 2002 Apr;959:285-94. Reaction of carnosine with aged proteins: another protective process? Hipkiss AR, Brownson C, Bertani MF, Ruiz E, Ferro A. GKT School of Biomedical Sciences, King's College London, Guy's Campus, London Bridge, London SE1 1UL, United Kingdom. alan.hipkiss@kcl.ac.uk

Cellular aging is often associated with an increase in protein carbonyl groups arising from oxidation- and glycation-related phenomena and suppressed proteasome activity. These "aged" polypeptides may either be degraded by 20S proteasomes or cross-link to form structures intractable to proteolysis and inhibitory to proteasome activity. Carnosine (beta-alanyl-L-histidine) is present at surprisingly high levels (up to 20 mM) in muscle and nervous tissues in many animals, especially long-lived species. Carnosine can delay senescence in cultured human fibroblasts and reverse the senescent phenotype, restoring a more juvenile

appearance. As better antioxidants/free-radical scavengers than carnosine do not demonstrate these antisenescence effects, additional properties of carnosine must contribute to its antisenescence activity. Having shown that carnosine can react with protein carbonyls, thereby generating "carnosinylated" polypeptides using model systems, we propose that similar adducts are generated in senescent cells exposed to carnosine. Polypeptide-carnosine adducts have been recently detected in beef products that are relatively rich in carnosine, and carnosine's reaction with carbonyl functions generated during amino acid deamidation has also been described. Growth of cultured human fibroblasts with carnosine stimulated proteolysis of long-labeled proteins as the cells approached their "Hayflick limit," consistent with the idea that carnosine ameliorates the senescence-associated proteolytic decline. We also find that carnosine suppresses induction of heme-oxygenase-1 activity following exposure of human endothelial cells to a glycated protein. The antisenescence activity of the spin-trap agent alpha-phenyl-N-t-butyl nitron (PBN) towards cultured human fibroblasts resides in N-t-butyl-hydroxylamine, its hydrolysis product. As hydroxylamines are reactive towards aldehydes and ketones, the antisenescence activity of N-t-butyl-hydroxylamine and other hydroxylamines may be mediated, at least in part, by reactivity towards macromolecular carbonyls, analogous to that proposed for carnosine.

41. Biosci Biotechnol Biochem. 2002 Jan;66(1):36-43. Effect of carnosine and related compounds on the inactivation of human Cu,Zn-superoxide dismutase by modification of fructose and glycolaldehyde. Ukeda H, Hasegawa Y, Harada Y, Sawamura M. Department of Bioresources Science, Faculty of Agriculture, Kochi University, Nankoku, Japan. hukeda@cc.kochi-u.ac.jp

Glycolaldehyde, an intermediate of the Maillard reaction, and fructose, which is mainly derived from the polyol pathway, rapidly inactivate human Cu,Zn-superoxide dismutase (SOD) at the physiological concentration. We employed this inactivation with these carbonyl compounds as a model glycation reaction to investigate whether carnosine and its related compounds could protect the enzyme from inactivation. Of eight derivatives examined, histidine, Gly-His, carnosine and Ala-His inhibited the inactivation of the enzyme by fructose ($p < 0.001$), and Gly-His, Ala-His, anserine, carnosine, and homocarnosine exhibited a marked protective effect against the inactivation by glycolaldehyde ($p < 0.001$). The carnosine-related compounds that showed this highly protective effect against the inactivation by glycolaldehyde had high reactivity with glycolaldehyde and high scavenging activity toward the hydroxyl radical as common properties. On the other hand, the carnosine-related compounds that had a protective effect against the inactivation by fructose showed significant hydroxyl radical-scavenging ability. These results indicate that carnosine and such related compounds as Gly-His and Ala-His are effective anti-glycating agents for human Cu,Zn-SOD and that the effectiveness is based not only on high reactivity with carbonyl compounds but also on hydroxyl radical scavenging activity.

42. J Biol Chem. 2001 Dec 28;276(52):48967-72. Epub 2001 Oct 24. Chelating activity of advanced glycation end-product inhibitors. Price DL, Rhett PM, Thorpe SR, Baynes JW. Department of Chemistry and Biochemistry, University of South Carolina, Columbia, South Carolina 29208, USA.

The advanced glycation end-product (AGE) hypothesis proposes that accelerated chemical modification of proteins by glucose during hyperglycemia contributes to the pathogenesis of diabetic complications. The two most commonly measured AGEs, N (epsilon)-(carboxymethyl)lysine and pentosidine, are glycoxidation products, formed from glucose by sequential glycation and autoxidation reactions. Although several compounds have been developed as AGE inhibitors and are being tested in animal models of diabetes and in clinical trials, the mechanism of action of these inhibitors is poorly understood. In general, they are thought to function as nucleophilic traps for reactive carbonyl intermediates in the formation of AGEs; however alternative mechanisms of actions, such as chelation, have not been rigorously examined. To distinguish between the carbonyl trapping and antioxidant activity of AGE inhibitors, we have measured the chelating activity of the inhibitors by determining the concentration required for 50% inhibition of the rate of copper-catalyzed autoxidation of ascorbic acid in phosphate buffer. All AGE inhibitors studied were chelators of copper, as measured by inhibition of metal-catalyzed autoxidation of ascorbate. Apparent binding constants for copper ranged from approximately 2 mM for aminoguanidine and pyridoxamine, to 10-100 microm for carnosine, phenazinediamine, OPB-9195 and tenilsetam. The AGE-breakers, phenacylthiazolium and phenacyldimethylthiazolium bromide, and their hydrolysis products, were among the most potent inhibitors of ascorbate oxidation. We conclude that, at millimolar concentrations of AGE inhibitors used in many in vitro studies, inhibition of AGE formation results primarily from the chelating or antioxidant activity of the AGE inhibitors, rather than their carbonyl trapping activity. Further, at therapeutic concentrations, the chelating activity of AGE inhibitors and AGE-breakers may contribute to their inhibition of AGE formation and protection against development of diabetic complications.

43. Free Radic Biol Med. 2000 May 15;28(10):1564-70. Carnosine reacts with a glycated protein. Brownson C, Hipkiss AR. Division of Biomolecular Science, GKT School of Biomedical Sciences, King's College London, Guy's Campus, London Bridge, London, UK.

Oxidation and glycation induce formation of carbonyl (CO) groups in proteins, a characteristic of cellular aging. The dipeptide carnosine (beta-alanyl-L-histidine) is often found in long-lived mammalian tissues at relatively high concentrations (up to 20 mM). Previous studies show that carnosine reacts with low-molecular-weight aldehydes and ketones. We examine here the ability of carnosine to react with ovalbumin CO groups generated by treatment of the protein with methylglyoxal (MG). Incubation of MG-treated protein with carnosine accelerated a slow decline in CO groups as measured by dinitrophenylhydrazine reactivity. Incubation of [14 C]-carnosine with MG-treated ovalbumin resulted in a radiolabeled precipitate on addition of trichloroacetic acid (TCA); this was not observed with control, untreated protein. The presence of lysine or N-(alpha)-acetylglucyl-lysine methyl ester caused a decrease in the TCA-precipitable radiolabel. Carnosine also inhibited cross-linking of the MG-treated ovalbumin to lysine

and normal, untreated alpha-crystallin. We conclude that carnosine can react with protein CO groups (termed "carnosinylation") and thereby modulate their deleterious interaction with other polypeptides. It is proposed that, should similar reactions occur intracellularly, then carnosine's known "anti-aging" actions might, at least partially, be explained by the dipeptide facilitating the inactivation/removal of deleterious proteins bearing carbonyl groups.

44. J Biochem Mol Toxicol. 2000;14(4):215-20. Carnosine prevents the glycation-induced changes in electrophoretic mobility of aspartate aminotransferase. Seidler NW. University of Health Sciences, Department of Biochemistry, Kansas City, MO 64106-1453, USA. NSEIDLER@fac1.uhs.edu

Carbohydrate-derived aldehydes cause irreversible loss of protein function via glycation. We previously observed that glyceraldehyde 3-phosphate (Glyc3P) abolishes the enzyme activity of cardiac aspartate aminotransferase (cAAT). We also examined the protective effects of carnosine against Glyc3P-induced loss of enzyme activity. The present study looked at carnosine's prevention of Glyc3P-induced change in protein structure. Purified cAAT (2 mg protein/mL) was incubated with various concentrations of carnosine (1-20 mM) in the presence of Glyc3P (500 microM) for 4 days at 37 degrees C. Following incubation, samples were analyzed by SDS-polyacrylamide gel electrophoresis. Carnosine showed prevention of protein modification at carnosine-to-Glyc3P ratios of 10:1 or greater. There was a progressive loss of the unmodified cAAT protein band as Glyc3P concentration was increased. Additionally, the gel position of the Glyc3P-modified cAAT protein varied over time. The apparent molecular weight (MWapp) of the Glyc3P-modified cAAT protein that formed after 1 day at 37 degrees C (500 microM) was greater than its MWapp after 2 days, suggesting that a chemical rearrangement of the initial adduct occurs. These observations support the hypothesis that carnosine is an antiglycation agent and that its mechanism of action involves prevention of protein modification.

45. Tsitologija. 2000;42(1):66-71. [Nonenzymatic glycosylation of and oxidative damage to actin in vitro and in vivo] [Article in Russian] Kuleva NV, Zalesova ZS. St. Petersburg State University.

A study was made the influence exerted by non-enzymatic glycosylation (glycation) and oxidative destruction on structural and functional parameters of actin (free NH₂-groups, advanced glycation end product and bityrosine cross-linking content, DNase inhibition by G-actin and myosin Mg(2+)-ATPase activation by F-actin). The functional properties of actin were shown to change under high molecular weight product formation and oxidative destruction: the extent of DNAase I inhibition decreases (from 70 to 40%) and the extent of myosin Mg(2+)-ATPase decreases (by 40%). Carnosine prevents actin oligomer formation and oxidative destruction which favours preservation of the protein functional properties.

46. Arch Toxicol. 1999 Aug;73(6):307-9. Carnosine prevents glyceraldehyde 3-phosphate-mediated inhibition of aspartate aminotransferase. Swearingin TA, Fitzgerald C, Seidler NW. Department of Biochemistry, University of Health Sciences, 1750 Independence Boulevard, Kansas City, MO 64106-1453, USA.

Post-mitotic tissues, such as the heart, exhibit high concentrations (20 mM) of carnosine (beta-alanyl-L-histidine). Carnosine may have aldehyde scavenging properties. We tested this hypothesis by examining its protective effects against inhibition of enzyme activity by glyceraldehyde 3-phosphate (Glyc3P). Glyc3P is a potentially toxic triose; Glyc3P inhibits the cardiac aspartate aminotransferase (cAAT) by non-enzymatic glycosylation (or glycation) of the protein. cAAT requires pyridoxal 5-phosphate (PyP) for catalysis. We observed that carnosine (20 mM) completely prevents the inhibition of cAAT activity by Glyc3P (5 mM) after brief incubation (30 min at 37 degrees C). After a prolonged incubation (3.25 h) of cAAT with Glyc3P (0.5 mM) at 37 degrees C, the protection by carnosine (20 mM) persisted but PyP availability was affected. In the absence of PyP from the assay medium, cAAT activities (plus Glyc3P) were 95 +/- 18.2 micromol/min per mg protein (mean +/- SD), minus carnosine and 100 +/- 2.4, plus carnosine; control activity was 172 +/- 3.9. When PyP (1.0 microM) was included in the assay medium, cAAT activities (plus Glyc3P) were 93 +/- 14.8, minus carnosine and 151 +/- 16.8, plus carnosine, P < 0.001; control activity was 180 +/- 17.7. These data, which showed carnosine moderating the effects of both Glyc3P and PyP, suggest that carnosine may be an endogenous aldehyde scavenger.

47. Int J Biochem Cell Biol. 1998 Aug;30(8):863-8. Carnosine, a protective, anti-ageing peptide? Hipkiss AR. Molecular Biology and Biophysics Group, King's College London, Strand, UK.

Carnosine (beta-alanyl-L-histidine) has protective functions additional to anti-oxidant and free-radical scavenging roles. It extends cultured human fibroblast life-span, kills transformed cells, protects cells against aldehydes and an amyloid peptide fragment and inhibits, in vitro, protein glycation (formation of cross-links, carbonyl groups and AGEs) and DNA/protein cross-linking. Carnosine is an aldehyde scavenger, a likely lipofuscin (age pigment) precursor and possible modulator of diabetic complications, atherosclerosis and Alzheimer's disease.

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