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COVER STORY

Carnosine
Nature's pluripotent life extension agent

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A substance that protects and extends the functional life of the body's key building blocks—cells, proteins, DNA, lipids—can be fairly called an agent of longevity. When that agent is safe, naturally present in the body and in food, and has demonstrated prolongation of life span in animals and cultured human cells, it is fundamental to any life extension program. Mounting research suggests that carnosine has just such anti-aging potential.

Carnosine is a multifunctional dipeptide made up of a chemical combination of the amino acids beta-alanine and L-histidine. Long-lived cells such as nerve cells (neurons) and muscle cells (myocytes) contain high levels of carnosine. Muscle levels of carnosine correlate with the maximum life spans of animal species (Hipkiss AR et al., 1995).

Laboratory research on cellular senescence (the end of the life cycle of dividing cells) suggests that these facts may not be coincidences. Carnosine has the remarkable ability to rejuvenate cells approaching senescence, restoring normal appearance and extending cellular life span.

How does carnosine rejuvenate cells? We do not yet know the full answer, but carnosine's properties may point up key mechanisms of tissue and cell aging, as well as the anti-aging measures that counteract them.

Carnosine addresses the biochemical paradox of life: the elements that make and give life—oxygen, glucose, lipids, protein, trace metals—also destroy life in ways that are inhibited by carnosine. Carnosine protects against their destructive sides through potent antioxidant, anti-glycating, aldehyde quenching and metal chelating actions (Quinn PJ et al., 1992; Hipkiss AR, Preston JE et al., 1998). A prime beneficiary is the body's biggest target—its proteins.

The body is made up largely of proteins. Unfortunately, proteins tend to undergo destructive changes as we age, due largely to oxidation and interactions with sugars or aldehydes. These interrelated protein modifications include oxidation, carbonylation, cross-linking, glycation and advanced glycation endproduct (AGE) formation. They figure prominently not only in the processes of aging but also in its familiar signs such as skin aging, cataracts and neurodegeneration. Studies show that carnosine is effective against all these forms of protein modification.

As an antioxidant, carnosine potently quenches that most destructive of free radicals, the hydroxyl radical, as well as superoxide, singlet oxygen and the peroxy radical. Surprisingly, carnosine was the only antioxidant to significantly protect chromosomes from oxidative damage due to 90% oxygen exposure.

Carnosine's ability to rejuvenate connective tissue cells may explain its beneficial effects on wound healing. In addition, skin aging is bound up with protein modification. Damaged proteins accumulate and cross-link in the skin, causing wrinkles and loss of elasticity. In the lens of the eye, protein cross-linking is part of cataract formation. Carnosine eye drops have been shown to delay vision senescence in humans, being effective in 100% of cases of primary senile cataract and 80% of cases of mature senile cataract (Wang AM et al., 2000).

Carnosine levels decline with age. Muscle levels decline 63% from age 10 to age 70, which may account for the normal age-related decline in muscle mass and function (Stuerenberg HJ et al., 1999). Since carnosine acts as a pH buffer, it can keep on protecting muscle cell membranes from oxidation under the acidic conditions of muscular exertion. Carnosine enables the heart muscle to

contract more efficiently through enhancement of calcium response in heart myocytes (Zaloga GP et al., 1997).

The high levels of carnosine in the brain may serve as natural protection against excitotoxicity, copper and zinc toxicity, protein cross-linking and glycation, and especially oxidation of cell membranes. Animal studies show broad protective effects in simulated stroke.

New research shows that copper and zinc dramatically stimulate senile plaque formation in Alzheimer's disease. Chelators of these metals dissolve plaques in the laboratory. Carnosine can also inhibit the cross-linking of amyloid-beta that leads to plaque formation. A signature of Alzheimer's disease is impairment of brain microvasculature. Carnosine protected the cells that line brain blood vessels (endothelial cells) from damage by amyloid-beta (senile plaque material) as well as by products of lipid oxidation and alcohol metabolism in laboratory experiments.

Now that many are cutting down on meat—the main dietary source of carnosine—supplementation becomes especially important. Carnosine is safe, with no toxicity even at dosages above 500 mg per kilogram of body weight in animal studies (Quinn PJ et al., 1992). It is most fortunate that carnosine is safe at high dosages because the body would neutralize lesser amounts of carnosine. The enzyme carnosinase (Quinn PJ et al., 1992) must be saturated with more carnosine than it is able to neutralize in order to make free carnosine available to the rest of the body.

There are thought to be many mechanisms responsible for aging. Consequently, an agent must work along many basic pathways of the aging process in order to control it. Scientists have described carnosine as “pluripotent”—active in a multitude of ways, in many tissues and organs (Hipkiss AR, Preston JE et al., 1998). Carnosine's pluripotent life extension potential places it on a par with CoQ10 as a cornerstone of longevity nutrition.

Biological rejuvenation

It is well known that cells have only a limited capacity to continue to divide through the course of life. For example, human fetal fibroblasts (connective tissue cells) divide no more than about 60 to 80 times in laboratory cultures. By young adulthood, fibroblasts have 30 to 40 divisions left, while in old age no more than 10 to 20 remain.

The limited capacity of the cell to perpetuate itself through division is called the Hayflick Limit, after the scientist who discovered it nearly four decades ago (Hayflick L et al., 1961; Hayflick L, 1965). In concert with telomeres, which count off the rounds of cell division, the Hayflick Limit caps life span at the cellular level. With each division a cell becomes less likely to divide again, until finally it stops dividing altogether and becomes senescent.

As cultured cells approach the Hayflick Limit they divide less frequently and take on strikingly irregular forms. They no longer line up in parallel arrays, assume a granular appearance, and deviate from their normal size and shape (McFarland GA et al., 1994). This distorted appearance, called the senescent phenotype, normally ushers in a twilight state called cellular senescence that until recently was thought to be irreversible (see the article “Carnosine and Cellular Senescence” in this issue).

Extending cell life span

In a remarkable series of experiments, scientists at an Australian research institute have shown that carnosine rejuvenates cells as they approach senescence (McFarland GA, 1999; McFarland GA, 1994). The scientists cultured human fibroblasts (connective tissue cells) from the lung and the foreskin. Fibroblasts that went through many rounds of division, known as late-passage cells, displayed a disorganized, irregular appearance before ceasing to divide. Fibroblasts cultured with carnosine lived longer, retaining youthful appearance and growth patterns.

What is most exciting is the ability of carnosine to reverse the signs of aging in cells approaching senescence. When the scientists transferred late-passage fibroblasts to a culture medium containing carnosine, they exhibited a rejuvenated appearance and often an enhanced capacity to divide. They again grew in the characteristic whorled growth patterns of young fibroblasts, and resumed a uniform appearance. But when they transferred the fibroblasts back to a medium lacking carnosine, the signs of senescence quickly reappeared.

The scientists switched late-passage fibroblasts back and forth several times between the culture media. They consistently observed that the carnosine culture medium restored the juvenile cell phenotype within days, whereas the standard culture medium brought back the senescent cell phenotype.

The carnosine medium also increased life span, even for old cells. The number of PDs, or population doublings, provides a



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convenient measure of cell division. When late-passage lung fibroblasts at 55 PDs (population doublings) were transferred to the carnosine medium, they lived to 69 to 70 PDs, compared to 57 to 61 PDs for the fibroblasts that were not transferred. Moreover, the fibroblasts transferred to the carnosine medium attained a life span of 413 days, compared to 126 to 139 days for the control fibroblasts. Carnosine increased chronological life span more dramatically than PDs in the Australian series of experiments.

When cells in the carnosine medium eventually enter into cellular senescence, they nevertheless retain a normal or less senescent morphology. Carnosine's ability to retain or restore the juvenile phenotype suggests that it may help maintain cellular homeostasis.

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Two Japanese studies demonstrate carnosine's ability to stabilize and protect cultured fibroblasts. The first study shows that carnosine stimulates a factor called vimentin that promotes robustness in cultured fibroblasts (Ikeda D et al., 1999). Vimentin is a structural protein that imparts strength and stability to fibroblasts and endothelial cells.

The second Japanese study showed that carnosine preserves the integrity of rat fibroblasts in a nutritionally deficient culture medium (Kantha SS et al., 1996). Fibroblasts grown in this culture medium lost their characteristic form after one week, while those grown in the carnosine supplemented culture retained their healthy appearance. After four weeks those fibroblasts grown in the carnosine medium retained cellular integrity, while the others were no longer viable.

The study also examined levels of 8-hydroxydeoxyguanosine (8-OH dG), a marker of oxidative damage to DNA, in fibroblast cultures with and without carnosine. They found that carnosine significantly reduced 8-hydroxydeoxyguanosine levels in fibroblasts after four weeks of continuous culture. DNA oxidation is thought to contribute importantly not only to cellular senescence, but also to carcinogenesis, and indeed 8-hydroxydeoxyguanosine has been proposed as a marker for cancer risk (Kasai H, 1997).

Carnosine's revitalizing effects on cultured fibroblasts may explain why it improves post-surgical wound healing. Another Japanese study showed that carnosine enhances granulation, a healing process in which proliferating fibroblasts and blood vessels temporarily fill a tissue defect (Nagai K et al., 1986). A Brazilian study showed that granulation tissue developed and matured faster, with a higher level of collagen biosynthesis, in carnosine treated rats (Vizioli MR et al., 1983). The Japanese study also presented evidence that carnosine restores the body's regenerative potential suppressed by common drugs.

Extending organism life span

Do carnosine's rejuvenating effects on cells extend to the entire organism? Similar anti-senescence effects have now been demonstrated in mice. A new Russian study tested the effect of carnosine on life span and indicators of senescence in senescence-accelerated mice (Yuneva MO et al., 1999; Boldyrev AA et al., 1999). Half the mice were given carnosine in their drinking water starting at two months of age. Carnosine extended the life span of the treated mice by 20% on average, compared to the mice not fed carnosine.

Carnosine did not alter the 15 month maximum life span of the senescence-accelerated mice strain, but it did significantly raise the number of mice surviving to old age. The mice given carnosine were about twice as likely to reach the "ripe old age" of 12 months as untreated mice. It also improved indicators of senescence measured at the "old age" of ten months.

Carnosine distinctly improved the appearance of the aged mice, whose coat fullness and color remained much closer to that of young animals. Significantly more carnosine-treated mice had glossy coats (44% vs. 5%), while significantly fewer had skin ulcers (14% vs. 36%). However, carnosine did not affect the loss or texture of hair. Carnosine significantly reduced the rates of spinal lordokyphosis (spinal curvature) and periophthalmic lesions, but did not affect corneal opacities.

The sharpest contrast between the treated and untreated mice was seen in their behavior. Only 9% of the untreated mice displayed normal behavioral reactivity, compared to 58% of the carnosine treated mice.

The researchers also measured biochemical indicators associated with brain aging. Brain membranes of the carnosine treated mice had significantly lower levels of MDA (malondialdehyde), a highly toxic product of membrane lipid oxidation. MAO-B (monoamine oxidase B) activity was 44% lower in the carnosine-treated mice, indicating maintenance of dopamine metabolism.



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Glutamate binding to its cellular receptors nearly doubled in the carnosine treated group. Since glutamate is the main excitatory neurotransmitter, this may explain the more normal behavioral reactivity of the carnosine-fed mice.

This study showed that carnosine significantly improved most measures of appearance, physiological health, behavior, and brain biochemistry—as well as extended life span—in senescence-accelerated mice. The researchers therefore conclude that “carnosine-treated animals can be characterized as more resistant to the development of features of aging” (Boldyrev AA et al., 1999).

Protein carbonylation

The reason why older people—and animals—look different than younger ones has to do with changes in the proteins of the body. Proteins are the substances most responsible for the daily functioning of living organisms, which gives protein deterioration its dramatic impact on the body's function and appearance. Many lines of research over the last decade converge on protein modification as a major pathway for aging and degenerative disease. These modifications result from oxidation (as by free radicals) and interrelated processes such as protein-sugar reactions (glycation).

Modified proteins accumulate as we age, while carnosine levels are declining. Once a protein is modified it has lost its ability to function normally, and when a significant portion of the body's protein has reached this point, the body becomes more prone to degenerative diseases.

The telltale sign of destructive protein modification is the protein carbonyl group. Accumulation of proteins with carbonyl groups is a molecular indicator of cell aging. Protein carbonyl levels increase markedly in the last third of the life span, rising almost exponentially with age in a wide variety of animal species and tissues. In humans, about a third of proteins become carbonylated later in life. At that level, these aberrant proteins are considered likely to have deleterious effects on most aspects of cellular function (Stadtman ER et al., 2000).

Many pathways of protein modification produce carbonyl groups, including oxidation of amino acid side chains, glycation and reactions with aldehydes and lipid peroxidation products (Berlett BS et al., 1997; Stadtman ER et al., 2000, 1992). The multiplicity of mechanisms behind protein modification places this problem beyond the scope of simple antioxidants. A pluripotent agent is needed whose biochemical profile matches this array of mechanisms. Carnosine emerges as the most promising broad spectrum shield against protein modification.

Carnosine addresses the major pathways through which proteins become carbonylated through its antioxidant and anti-glycation actions, its ability to quench reactive aldehydes and chelate metals, and its effectiveness against lipid peroxidation. Carnosine's properties fit the mechanisms of protein carbonylation so well as to invite the speculation that evolution “designed” carnosine to protect proteins from carbonylation and other deleterious modifications.

An excellent example of carnosine's broad-spectrum defense against protein modification is provided by MDA (malondialdehyde). This noxious product of lipid peroxidation causes protein carbonylation, cross-linking, glycation and AGE formation (Burcham PC et al., 1997).

Carnosine inhibits MDA from carbonylating albumin (the main serum protein) and crystallin (eye lens protein) in a concentration-dependent manner. MDA glycates albumin leading to cross-linking and production of advanced glycation end products (AGEs), however these changes too were prevented by carnosine. Table 1 summarizes some of the many laboratory studies demonstrating that carnosine protects proteins against diverse protein damaging agents.

Study	Test Substance(s)	Protein Damaging Agent	Inhibited or Reversed Carbonylation?	Inhibited Cross-Linking or AGE Formation?
Hipkiss Preston, et al., 1998	Serum albumin (the major plasma protein)	MDA (lipid oxidation product)	X	X
	Serum albumin (the major plasma protein)	Hypochlorite ions (inflammatory response product)	X	X
	DNA & histone (DNA protein)	Formaldehyde or acetaldehyde	NA	X
Hipkiss Preston, et al., 1997	Crystallin (eye lens protein)	MDA (lipid oxidation product)	X	X

Hipkiss & Chana, 1998; Hipkiss & Brownson, 2000	Ovalbumin (albumin from egg white)	Methylglyoxal (promotes AGE formation)	X	X
Munch, Mayer, et al., 1997	Amyloid beta (forms senile plaques when cross-linked)	Fructose	NA	X
Hipkiss, Michaelis, Syrris, 1995	SOD (key intercellular antioxidant)	Dihydroxyacetone	NA	X
	Catalase (enzyme that catalyzes breakdown of hydrogen peroxide)	Fructose	NA	X
Hipkiss, Michaelis, syrris, et al., 1995	Anti-thrombin III (anticoagulant blood protein), serum albumin, or crystallin	Fructose	NA	X

Table 1. Protective effects of carnosine on protein carbonylation, cross-linking and AGE formation. NA = Not Applicable (not measured in study)

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Glycation and AGE formation

One of the processes that carbonylates proteins, glycation, is itself recognized as a major cause of aging and degenerative disease. Glycation occurs when proteins react with sugars. Then, through a series of reactions including oxidation, advanced glycation end products (aptly called AGEs) form.

AGEs accelerate aging processes and promote degenerative disease. This is not surprising when one considers that AGE formation in the body is the chemical equivalent of the browning of food in the oven—and equally irreversible. When proteins accumulate AGEs they do in fact turn brown. The “slow oven” of AGE formation turns proteins fluorescent, and cross-links them to a point where the body cannot break them down. As AGEs build up, tissues lose tone and resiliency and organ systems degenerate. For example, AGEs are now recognized as an important factor in atherosclerosis (Bierhaus A et al., 1998), cataracts, Alzheimer's disease (Munch G et al., 1998), and loss of skin elasticity (see “Skin Aging” in the article “Carnosine and Cellular Senescence” in this issue).

AGEs exert their harmful effects on two levels. Most obviously, they physically impair proteins, DNA and lipids, altering their chemical properties. They also act as cellular signals, triggering a cascade of destructive events when they attach to their cellular binding sites (see sidebar titled “AGEs and RAGE”). One consequence is a 50-fold increase in free radical generation. Since oxidative stress is often described as a “fixative” of AGE formation, a vicious cycle can ensue of oxidative stress and AGE accumulation.

Carnosine is by far the safest and most effective natural anti-glycating agent. Studies in a wide variety of experimental models demonstrate that carnosine inhibits protein glycation and AGE formation (see Table 1 at the end of Page 2).

Through its structural resemblance to the sites that glycyating agents attack in proteins, carnosine is thought to act as a “sacrificial sink.” When carnosine becomes glycyated, it spares proteins from the same fate. Glycyated carnosine is not mutagenic, in contrast to amino acids such as lysine which becomes mutagenic when glycyated, according to the well-known Ames test (Hipkiss AR, Michaelis J, Syrris P, et al., 1995).

Carnosine not only inhibits the formation of AGEs, it can also protect normal proteins from the toxic effects of AGEs that have already formed. An elegant experiment carried out at King's College, University of London, made this point (Brownson C et al., 2000; Hipkiss AR et al., 2000). The scientists employed a glycyating agent called methylglyoxal (MG) that reacts with lysine and arginine residues in body proteins.

The scientists used MG to glycyate ovalbumin (egg white protein). This produced a brown colored solution typical of the “browning” effect of glycyation. They then incubated the glycyated albumin with a normal protein, a-crystallin, from the lens of the eye. The glycyated albumin formed cross-links with the crystallin, but this was inhibited by carnosine.

The study demonstrated that carnosine can stop protein damage from spreading to healthy proteins. It also found evidence that carnosine reacts with and removes the carbonyl groups in glycyated proteins. This study reinforces the body of research demonstrating carnosine's unique three-stage protection against accumulation of aberrant proteins: carnosine protects against protein carbonylation, inhibits damaged proteins from damaging healthy proteins, and helps the proteolytic system dispose of damaged and unneeded proteins.

AGEs & RAGE

The main binding site for AGEs is appropriately called RAGE (receptor for AGEs). The binding of AGEs to RAGE induces cellular activation and intracellular oxidative stress, which lead to generation of assorted cytokines, growth factors and transcription factors such as nuclear factor kappa beta (Schmidt AM et al., 1999).

AGE binding to RAGE tends to be self-amplifying, since the more AGEs bind to RAGE, the more RAGE binding sites develop. This creates a “positive feedback loop” leading to spreading waves of cellular activation and tissue damage (Schmidt AM et al., 1999).

The implications of the discovery of RAGE become revolutionary when one considers that amyloid-beta, the senile plaque material in Alzheimer's disease, also binds to RAGE with similar effects (Yan SD et al., 1996). Scientists do not yet know how AGEs and amyloid-beta might interact in stimulating the RAGE response in Alzheimer's disease.

DNA is organized into chromosomes, each of which contains a double helical DNA structure carrying the genes. Oxidative stress causes breaks and other aberrations in the chromosome that accumulate with age. A fascinating experiment shows the paradoxical effects of antioxidants on oxidative damage to chromosomes (Gille JJ et al., 1991). This study used hyperoxia, exposure to nearly pure oxygen (90%), as a physiologically natural oxidative stressor. Hyperoxia is thought to generate free radicals at the same intracellular sites where they normally form over time.

The scientists tested the ability of several antioxidants—including vitamin C, n-acetylcysteine (NAC), vitamin E, carnosine and a form of glutathione—to protect the chromosomes in Chinese hamster ovary cells from oxidative damage. Some of the antioxidants tested acted instead as pro-oxidants: they increased chromosomal damage, aggravating the effects of hyperoxia. It is a well known phenomenon that single antioxidants can sometimes exert a pro-oxidant effect in the body, which is the reason people take multiple antioxidants. In this study, only one antioxidant, carnosine, significantly reduced chromosomal damage. Cells cultured without any antioxidant exhibited 133 chromosomal aberrations per 100 cells. Carnosine reduced this level of damage by two-thirds, to only 44 chromosomal aberrations per 100 cells. Carnosine preserved 68% of cells fully intact, as compared to 46% of the control cells.

Neurodegeneration

The brain's rich supply of oxygen, glucose, membrane lipids and metals may explain why it is also richly endowed with carnosine. Carnosine suppresses oxidative stress, protein-sugar interactions leading to AGE formation (see above), lipid peroxidation, and copper and zinc toxicity. Moreover, carnosine's ability to forestall cellular senescence may help sustain the long lives of neurons, which do not divide to form new cells. We will survey carnosine's neuroprotective actions, with special attention to Alzheimer's disease.

Brain aging and degeneration are marked by protein carbonylation. A highly sensitive and specific assay was recently developed for protein carbonyls. Applied to human brain tissue, this assay reveals that the carbonyl content of neurons is several times as high in Alzheimer's disease patients as in control subjects similar in age (Smith MA et al., 1998).

Advances in cell culturing techniques permit scientists for the first time to maintain neurons in culture for extended periods. Scientists at the University of Kentucky used these techniques to study "aging in a dish" (Aksenova MV et al., 1999). They found that cultured neurons from the hippocampus of the rat fetus begin to rise in protein carbonyl content about a week before noticeable changes in neuronal viability appear. At a point when only 10% to 20% of neurons are no longer viable, protein carbonyl levels have already doubled. They observed swollen, unhealthy cell bodies in many of the cells with high carbonyl levels.

The Kentucky study also reinforced earlier findings correlating protein oxidation with declining activity of the energy-transfer enzyme creatine kinase, which is very sensitive to oxidation. This leads to diminished energy metabolism in the brain, a hallmark of Alzheimer's disease.

Animal studies demonstrate that brain protein carbonylation is associated with cognitive and behavioral dysfunctions. A study in senescent mice found that protein carbonyl levels in the brain cortex correlate with the degree of cognitive impairment, while levels in the cerebellum correlate with motor deficits (Forster MJ et al., 1996). An earlier study in aged gerbils showed increased protein carbonyl levels are associated with spatial memory loss (Carney JM et al., 1991, 1994).

Membrane lipid peroxidation

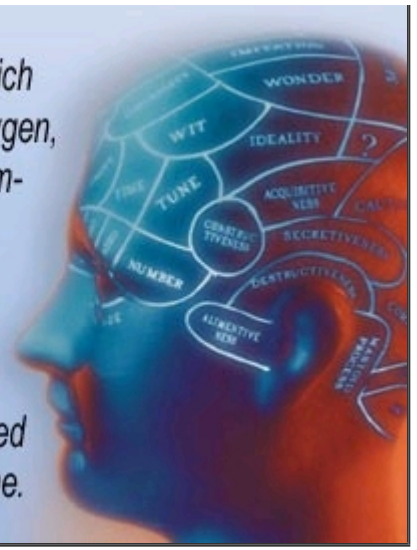
A major source of oxidative damage and cellular dysfunction in the brain is the oxidation of polyunsaturated lipids in the membranes of brain cells and their extensions such as axons. This chain reaction spreads oxidative damage and generates highly neurotoxic byproducts such as HNE and other aldehydes which are quenched by carnosine.

In Alzheimer's disease, lipid peroxidation products are thought to interfere with critical membrane proteins involved in cellular signaling and in transporting ions, glucose and glutamate. Their impairment leads to membrane depolarization, metabolic deficit, excitotoxicity and increased vulnerability to oxidative assault (Mark RJ et al., 1997; Butterfield DA, 1999).

As we have seen, carnosine feeding suppressed lipid peroxidation in the brains of old senescence-accelerated mice. Another mouse study tested the effects of carnosine on mice stressed with electric shocks for two hours (Gulyaeva NV et al., 1989). Carnosine protected brain cells from damage by lipid peroxidation products while increasing the "flowability" of cell membranes.

The study found that mice pretreated with carnosine displayed brain and blood levels of lipid peroxidation products more than 85% lower than in the untreated mice, and more than 70% lower even than in control mice who did not receive shocks. Brain SOD (superoxide dismutase) antioxidant activity was six times higher in the carnosine treated mice. Essential membrane phospholipid levels dropped by 9% in the untreated mice, but carnosine treatment actually raised them by 26%.

The Brain's rich supply of oxygen, glucose, membrane lipids and metals may explain why it is also richly endowed with carnosine.



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Excitotoxicity and stroke

A pathology common to many neurological disorders is excitatory toxicity, or excitotoxicity. It is caused by an excess of, or excessive sensitivity to, glutamate—the main excitatory neurotransmitter. Excitotoxicity triggers a cascade of events including membrane polarization, ending in cell death. Oxidative stress and excitotoxicity are thought to reinforce each other in a vicious cycle.

It is probable that excitotoxic complications determine the long-term effects of stroke. In Alzheimer's disease, laboratory experiments show that amyloid-beta sensitizes cultured neurons to excitotoxic death (Doble A, 1999).

Carnosine and glutamate are found together in presynaptic terminals in the brain. Experimental evidence shows that carnosine protects cells against excitotoxic death, supporting the notion that carnosine serves the same purpose in the brain. An interesting Russian study showed that rat cerebellar cells incubated in carnosine were resistant to excitotoxic cell death from the glutamate analogs NMDA and kainite (Boldyrev A et al., 1999).

Copper and zinc

Copper and zinc are neurological double-edged swords. While the body cannot live without them, new research from Florida State University confirms that they can also be neurotoxic (Horning MS et al., 2000). Abnormal copper-zinc metabolism is implicated in Alzheimer's disease, stroke, seizures and many other diseases with neurological components.

Copper and zinc are thought to modulate synaptic transmission, but are rapid neurotoxins at the concentrations reached when they are released from synaptic terminals. The brain must buffer these metals so that they can perform their functions without neurotoxicity. The new research on copper and zinc toxicity shows that carnosine provides that buffering action.

When scientists exposed rat neurons to physiological concentrations of copper or zinc, the neurons died. However carnosine, at a modest physiological concentration, protected the neurons from the toxic effects of these metals (Horning MS et al., 2000).

A spate of recent research papers point up the central role of copper and zinc in the development of Alzheimer's disease. Levels of these metals are elevated in the Alzheimer's disease brain, even more so in the amyloid-beta plaques ("senile plaques") which are the central feature of the disease (see the sidebar "Copper and Zinc in Alzheimer's disease").

A groundbreaking study discovered that chelators of copper and zinc solubilize (dissolve) aggregates of amyloid-beta in post-mortem human tissue samples from the brains of Alzheimer's disease patients (Cherny RA et al., 1999). The researchers conclude, "agents that specifically chelate copper and zinc ions but preserve Mg(II) and Ca(II) may be of therapeutic value in Alzheimer's disease."

Carnosine fits this profile, offering in addition pH buffering and hydroxyl radical scavenging actions. Not only does carnosine chelate copper and zinc, but the presence of copper and zinc ions enhances carnosine's potency as a scavenger of the superoxide radical (Gulyaeva NV, 1987). This is especially significant since amyloid-beta damages brain endothelial (blood vessel wall) cells quickly and at low concentrations by generating oxidative stress, particularly in the form of superoxide radicals (Thomas T et al., 1996). Microvascular damage is the harbinger of Alzheimer's disease, preceding its

Copper and zinc in Alzheimer's disease

Copper and zinc contribute to amyloid-beta formation and toxicity through a host of mechanisms. When amyloid-beta aggregates, as it does in plaque formation, it becomes more neurotoxic. Laboratory experiments show that tiny amounts of zinc and especially copper cause amyloid-beta to aggregate.

The mildly acidic environment characteristic of Alzheimer's disease dramatically increases aggregation of amyloid-beta by copper ions (Atwood CS et al., 1998). Inflammation, thought to aggravate and possibly cause Alzheimer's disease, also creates an acidic environment. Moreover, the acidosis, inflammation and disturbed energy metabolism associated with the disease are thought to increase copper and zinc levels, thus setting the stage for accelerated formation of amyloid-beta plaques (Atwood CS et al., 1998).

other pathological features.

One theory of Alzheimer's disease development holds that the distorted microvasculature seen in the disease is the primary cause of Alzheimer's, impairing delivery of nutrients to the brain (de la Torre JC, 1997). An experiment on rat brain endothelium shows that carnosine prevents this damage. When endothelium was incubated with amyloid-beta and a physiological concentration of carnosine, damage to endothelial cells was significantly reduced or completely eliminated (Preston JE et al., 1998).

In another experiment carried out by the same British team, carnosine protected brain endothelial cells from damage by MDA (malondialdehyde), a toxic product of lipid peroxidation. Carnosine inhibited protein carbonylation and cross-linking, while protecting cellular and mitochondrial function (Hipkiss AR et al., 1997). A third experiment showed that carnosine also protects these cells against the toxicity of acetaldehyde, which is produced when alcohol is metabolized (Hipkiss AR et al., 1998).

AGEs and amyloid plaque

Carnosine thus works along multiple pathways that prevent the formation of amyloid plaque, inhibit amyloid-beta toxicity and promote amyloid plaque breakdown in laboratory experiments. An examination of how the plaques form reveals an additional pathway.

The first step in plaque formation is thought to be the slow and reversible development of a nucleus, followed by a phase of rapid cross-linking and growth. AGEs (see "Glycation and AGE formation") accelerate both these steps by cross-linking soluble monomers to form insoluble aggregates. In fact, researchers hypothesize that the crucial step in the formation of a stable amyloid nucleus is the cross-linking of amyloid-beta by AGEs (Munch G et al., 1997).

These researchers found that amyloid-beta cross-linking was inhibited by three AGE inhibitors: the pharmaceuticals aminoguanidine and tenilsetam, and carnosine. Tenilsetam has demonstrated clinical benefit in Alzheimer's disease. The researchers incubated amyloid-beta with fructose, which is abundant in the brain and cross-links proteins up to ten times faster than glucose. Soluble amyloid disappeared as aggregates formed, driven by AGE cross-linking. All three AGE inhibitors prevented cross-linking of amyloid-beta, keeping nearly 100% of it in a soluble form.

Given the brain's dependence upon glucose for energy metabolism and the unusually high ratio of fructose to glucose in the brain (about 1:4, compared to 1:20 in plasma), it seems likely that carnosine serves as a natural glycation inhibitor in the brain.

We have seen that carnosine extends life span at the level of the cell and of the organism. It is equally beneficial to dividing cells and non-dividing cells such as neurons. Moreover, like CoQ10, nature appears to have anticipated us in the purposes carnosine naturally serves in the body.

Surpassing the many supplements that address few and limited aging mechanisms, carnosine stands out as the most promising pluripotent life extension discovery since The Life Extension Foundation introduced Coenzyme Q10 to the American public nearly twenty years ago.

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In the presence of copper ions amyloid-beta is thought to generate hydrogen peroxide, which can then react with iron or copper ions to produce highly neurotoxic hydroxyl radicals. In addition, copper forms complexes with amyloid-beta that markedly potentiate amyloid-beta neurotoxicity (Huang X et al., 1999).

The brain must buffer copper and zinc so that they can perform their functions without inducing toxicity. New research show that copper and zinc toxicity in the brain can be buffered by carnosine. (Horning MS et al., 2000)

Stroke Neuroprotection

Two Russian studies show that carnosine protects the brain in simulated strokes (Stvolinsky SL et al., 1999; Boldyrev AA et al., 1997). In the first experiment, rats were exposed to low pressure hypoxia. Rats given carnosine beforehand were able to keep standing and breathing almost twice as long as the others. After the hypoxia, carnosine treated rats were able to stand after 4.3 minutes, as compared to 6.3 minutes for the untreated rats.

The second study simulated stroke through arterial occlusion. Rats treated with carnosine displayed a more normal EEG, less lactate accumulation (a common measure of injury severity), and better cerebral blood flow restoration. The study also demonstrated that carnosine preserves activity of a key enzyme, Na/K-ATPase, which extracts energy stored in ATP to drive the cellular sodium pump. Na/K-ATPase inhibition has been found to correlate with edema in the ischemic (blood-deprived) region.



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