

REVIEW

Carnosine: physiological properties and therapeutic potential

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Introduction

Carnosine and related dipeptides are naturally-occurring histidine-containing compounds. They are found in several tissues, particularly in skeletal muscle. Since carnosine was discovered in Russia in 1900, there have been many theories about its biological functions, but none has been proved beyond reasonable doubt [1, 2]. However, this compound is reported to possess antioxidant, buffering, immune-enhancing and neurotransmitter actions. The aim of this review is to discuss carnosine's properties, functions and therapeutic potential with special reference to its antioxidant activity.

Synthesis and metabolism

Carnosine was first isolated from Liebig's meat extract [2] and was subsequently identified as β -alanyl-histidine (Figure 1) [3]. Since then, various aminoacyl amino acids—such as its methylated derivative, anserine (β -alanyl-methylhistidine) [4], homocarnosine (γ -aminobutyryl-histidine) [5] and others—have been isolated from excitable tissues [6]. All these peptides are structurally similar and are synthesized by carnosine synthetase, an enzyme with broad substrate specificity [7].

Carnosine is an endogenously synthesized dipeptide which is present in brain, cardiac muscle, kidney, stomach, olfactory bulbs and in large amounts in skeletal muscle [2, 8]. Figure 2 shows its metabolic pathway and indicates several biological roles of carnosine and its products [8]. Carnosine methylation gives rise to anserine or ophidine; its hydrolysis leads to histidine and β -alanine. During decarboxylation of histidine, histamine is formed, whose interaction with β -alanine results in carcine, a compound with

unknown functions. β -alanine, besides being an indispensable component of coenzyme A, is a product of pyrimidine base degradation and may have a role in stimulating collagen synthesis in tissues [9].

Tissue carnosine concentrations are influenced by diet. Dietary histidine deficiency reduces skeletal muscle carnosine concentration in rats, while high dietary histidine supplementation increases it [10]. Supplementation with high concentrations of dietary carnosine also increases skeletal carnosine concentrations [10]. However, dietary carnosine supplementation does not affect carnosine concentrations in heart, liver or skeletal muscle. Supplementation with both carnosine and α -tocopherol results in greater carnosine concentrations in liver and α -tocopherol concentrations in liver and heart than supplementation with α -tocopherol alone. This suggests an *in vivo* inter-relationship between carnosine and α -tocopherol [11].

Methods used for direct measurements of carnosine and related compounds in human and animal tissues include high-performance liquid chromatography, immunoassay and nuclear magnetic resonance spectroscopy. Combined solid-phase extraction and reversed-phase gradient high-performance liquid chromatography methods provide a sensitive, reproducible and

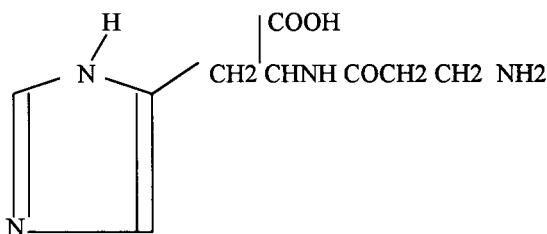


Figure 1. Chemical structure of carnosine.

selective quantification of carnosine and related compounds [12].

Antioxidant activity

Carnosine is a water-soluble natural metabolite of animal tissues. It has antioxidant properties due to its biological function of scavenging active oxygen species. Carnosine is a scavenger of hydroxyl and superoxide radicals and a strong quencher of singlet molecular oxygen [13–16]. The antioxidant activity of carnosine has also been demonstrated in oxidation model systems containing linoleic acid emulsions [13], skeletal muscle microsomes and sarcoplasmic reticulum [17]. Because of its water solubility, carnosine provides cells with an antioxidant system that functions in the cytosolic environment, where water-soluble oxidation mediators (such as transition metals and oxygen radicals) are often present in high concentrations.

Many studies have demonstrated, both at the tissue and organelle level, that carnosine and related peptides may prevent peroxidation of model membrane systems. These have led to the suggestion that they represent water-soluble counterparts to lipid-soluble antioxidants (such as α -tocopherol) in protecting cell membranes from oxidative damage [1]. An increase in tissue α -tocopherol concentrations, for example, decreases susceptibility to lipid oxidation reactions in liver, kidney and skeletal muscle [18]. Dietary supplementation with carnosine, α -tocopherol or both is effective in decreasing the formation of thiobarbituric

acid-reactive substances in rat skeletal muscle homogenate, the combination of α -tocopherol and carnosine being more effective than dietary carnosine alone. These data suggest that dietary supplementation with carnosine and α -tocopherol may modulate tissue carnosine and α -tocopherol concentrations and the formation of thiobarbituric acid-reactive substances in rat skeletal muscle homogenates [11].

Carnosine may inhibit lipid oxidation by a combination of free-radical scavenging and metal chelation. A study in which two animal models of ischaemic brain injury were used found that carnosine increased the time to loss of excitability and decreased the time to recovery. This action was thought to be due to carnosine's protective properties against free-radical damage [19]. The ability of carnosine to suppress significantly the development of ischaemic reperfusion contracture and to support the restoration of contractile force during reperfusion has been shown in an isolated rat heart muscle model. At the same time there is a decrease of myoglobin and nucleoside release from myocytes, indicating a membrane-protecting effect of carnosine [20].

Buffering activities

At physiological pH, both carnosine and anserine exhibit remarkable buffering activity, a function which may explain some of their biological roles [21]. At weakly alkaline pH, carnosine is easily able to suppress lipid peroxidation. Its buffering action becomes important during muscle activity, where

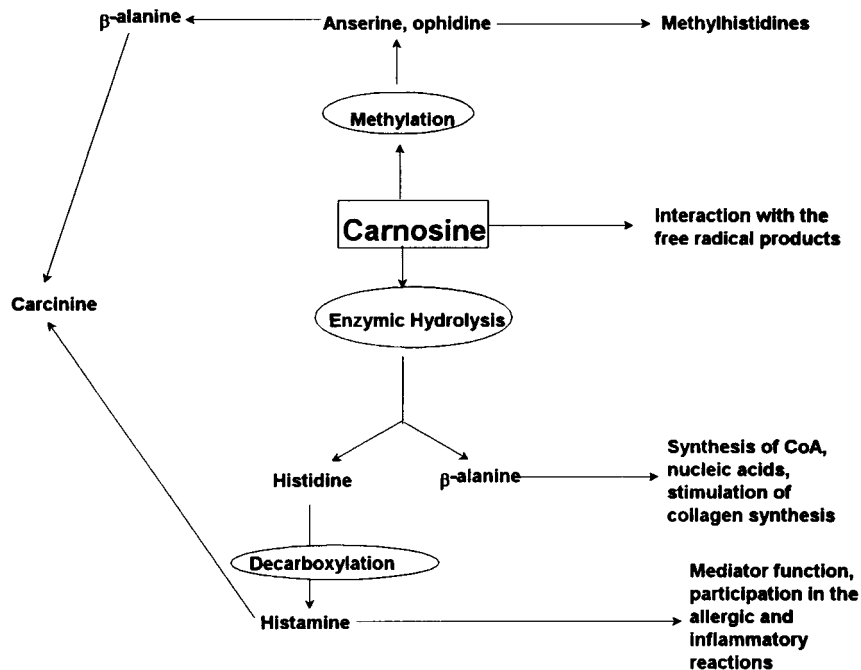


Figure 2. Carnosine metabolism in tissues.

acidification of the intracellular environment takes place. This allows carnosine to maintain its suppression of peroxidation. In addition, carnosine also exhibits heavy metal ion binding properties, which inhibit some enzymatic reactions [8]. However, in a case-controlled study muscle buffer capacity and carnosine concentration of biopsy samples from the vastus lateralis were assessed before and after 16 weeks of isometric endurance training. Neither muscle buffer capacity nor carnosine concentration changed after training [22].

Membrane protection properties

Studies with sarcoplasmic reticulum membrane fragments demonstrate that calcium-pump uncoupling and inhibition of Ca-ATPase by lipid peroxidation can be inhibited or reduced by the addition of either carnosine or anserine [8]. When added to the reaction mixtures, both compounds led to a decrease in malondialdehyde concentrations (implying a reduction in lipid peroxidation) in a dose-dependent fashion. Different models of lipid peroxidation produced similar results. The hydrophilic nature of part of the carnosine molecule allows it virtually to adhere to breaks (caused by oxidation) in the membrane lipid bilayer and effectively 'quench' peroxidation products formed near the damage.

Other properties of carnosine

Carnosine levels fall in muscle tissues after starvation [23], infection [24], trauma [25] and shock [26]. Infection and trauma may be associated with cellular calcium dysregulation and myocardial depression [27]. Carnosine administration improves cardiac contractility, increases myocyte free intracellular calcium levels, releases calcium from the sarcoplasmic reticulum and increases the calcium sensitivity of the contractile proteins [27]. Therefore carnosine may have a role in the regulation of intracellular calcium and contractility in cardiac cells.

The ability of carnosine to modulate human neutrophil function with respect to interleukin-1 β production and apoptosis has been tested [28]. Carnosine increases interleukin-1 β production and suppresses apoptosis, suggesting that it may have the capacity to modulate the immune response in human neutrophils.

Normal diploid human cells can grow in high concentrations of carnosine. Moreover, carnosine treatment can extend the life-span of these cells and prevent the appearance of the usual signs of senescence [29]. In the absence of pyruvate, however, physiological concentrations of carnosine are cytotoxic to neoplastic and tumour cells [30].

Potential therapeutic applications

During ageing, proteins become oxidized and cross-linked. These modifications are inducible by deleterious aldehydes such as glucose, fructose and the peroxidation product malondialdehyde. Methylglyoxal may also promote similar protein modifications, especially associated with secondary complications in diabetes mellitus. Carnosine readily reacts with many deleterious aldehydes, presumably because it has a target amino group with proximal imidazole and carboxyl groups. Carnosine can inhibit protein modification induced by lysine-methylglyoxal-advanced glycosylation end-products; this emphasizes carnosine's potential as a possible non-toxic modulator of diabetic complications [31].

In a prospective randomized study of the effect of different enteral diets on wound healing, dietary carnosine improved wound healing when administered as part of a complete enteral formula. This may be of clinical relevance since few enteral formulas currently contain carnosine [32].

Through its anti-glycating and antioxidant activities, both of which are implicated in neuronal and endothelial cell damage in Alzheimer's disease, carnosine may therefore be a useful therapeutic agent [33].

In Russia, stable eye drops of 5% carnosine have been developed and permitted by the Ministry of Health for medical use. In clinical trials on 109 patients, carnosine eye drops exerted a good therapeutic effect in corneal erosion, trophic keratitis and bullous keratopathy [34].

In rats, intraperitoneal administration of carnosine inhibited experimentally-induced gastric erosion of the stomach and duodenum and also improved healing of mucosal defects [35].

Carnosine may have protective functions additional to its antioxidant 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, *in vitro*, inhibits protein glycation (formation of cross-links, carbonyl groups and advanced glycosylation end-products) and DNA-protein cross-linking [36].

Key points

- Carnosine is a water-soluble endogenously synthesized dipeptide which is found in brain, cardiac muscle, kidney, stomach, olfactory bulbs and in large amounts in skeletal muscle.
 - Carnosine may have potentially useful physiological and therapeutic functions, which include antioxidant and free-radical scavenging properties. It is also a likely lipofuscin (age pigment) precursor and possible modulator of diabetic complications, atherosclerosis and Alzheimer's disease.
 - Many of carnosine's biological functions are still uncertain, and further focused research is required.
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