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The Roles of Carnosine in Aging of Skeletal Muscle and in Neuromuscular Diseases

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Abstract—Skeletal muscles undergo specific alterations that are related to the aging process. The incidence of several neuromuscular diseases (e.g., amyotrophic lateral sclerosis (ALS), myasthenia gravis, polymyositis, drug-induced myopathies, late-onset mitochondrial myopathy) is age-related. The increased sensitivity to disease of aging muscle represents an additional age-related negative influence in the presence of existing risk factors (such as a genetic predisposition). The potential significance of carnosine lies on one hand in its possible influence on specific physiological changes in muscle associated with the aging process, and on the other in its effect on oxidative stress and the antioxidative system in specific neuromuscular diseases such as ALS or polymyositis.

Key words: carnosine, neuromuscular disease, aging, antioxidant system, denervation, amyotrophic lateral sclerosis, myopathies

AGE-ASSOCIATED CHANGES IN THE SKELETAL MUSCULATURE AND AGE-ASSOCIATED NEUROMUSCULAR DISEASES

The potential significance of carnosine lies on one hand in its possible influence on specific physiological changes in muscle associated with the aging process, and on the other in its effect on oxidative stress and the antioxidative system in specific neuromuscular diseases such as amyotrophic lateral sclerosis (ALS) or polymyositis. Skeletal muscles undergo specific alterations that are related to the aging process. Protein synthesis in the skeletal muscles of old rats is decreased [1, 2]. In humans, a 20% reduction in peripheral muscle strength is found at age 70 as compared to age 20, with a corresponding decrease in muscle mass. Atrophy of the small muscles of the hand is encountered in 50% of the elderly. The deep tendon reflexes are reduced to the point of absent ankle jerks in 45-50% of the elderly [3, 4]. A number of potential catabolic factors have been investigated, in particular the relationship between neuromuscular disease and muscle aging, but up to now the relative contribution of each individual factor in this complex system has not be determined [5-11]. The incidence of several neuromuscular diseases is age-related. The increased sensitivity to disease of aging muscle represents an additional age-related negative influence in the presence of existing risk factors (such as a genetic predisposition). In the case of ALS, the median age at onset of the illness is about 60 years [12], and the incidence increases with each decade of age [13]. The physiological age-associated impairment of the motor neurons and motor units may contribute significantly to the age-dependent incidence of ALS [14, 15]. Neurogenic amyotrophy and the reduced efficiency of reinnervation could explain why increasing age is an independent prognostic risk factor for decreased survival time in ALS [15]. In the familial form of ALS, which is associated with a mutation in superoxide dismutase-1 (SOD-1), permanently raised oxidative stress could lead to accumulated toxicity [16]. Myasthenia gravis has two age-related peaks of incidence. Women are affected more often than men in the second and third decade, which reflects the generally higher autoimmune reactivity of young women. Men are generally affected more often in the sixth and seventh decade, correlating with an increased incidence of thymomas [17]. Drug-induced myopathies are more common in older patients because medications with myotoxic effects are more often prescribed for older patients. However, the drugs themselves are seldom myotoxic: they usually require the addition of a further risk factor, which as a rule is age-associated (such as renal insufficiency as a risk factor for

Abbreviations: ALS) amyotrophic lateral sclerosis; SOD) superoxide dismutase; TBARS) thiobarbituric acid-reactive substances.

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myopathy induced by colchicine or clofibrat) [18]. A decline in creatine clearance with increasing age explains this increasing toxicity. In addition, it is plausible that age-associated changes in muscle protein synthesis amplify the effects of drugs that damage muscle proteins. Late-onset mitochondrial myopathy is characterized by progressive proximal weakening and corresponding histopathological characteristics [19]. This muscle insufficiency could reflect the accumulation of mitochondrial dysfunction with increasing age, either by way of age-associated disturbance to the respiratory chain or by slowly accumulating oxidative stress [20]. Other myopathies such as inflammatory myopathies are among the most common inherited myopathies in the elderly, while dermatomyositis and to a lesser extent also polymyositis are associated with tumors which have a higher incidence in elderly patients [21].

NEUROMUSCULAR DISEASE, OXIDATIVE STRESS, AND ANTIOXIDANT SYSTEM

In various neuromuscular diseases, oxidative free radical effects on neuromuscular structures are suspected as etiological factors. Damage to cellular structures by free radicals appears when these free radicals are present at elevated concentrations or when endogenous antioxidative protection systems are damaged. However, these postulated mechanisms have not been studied in sufficient detail, and in particular in human muscle little data is available [22]. Free radicals also have physiological roles, notably by way of the lipid peroxidation of the cell membrane by polyunsaturated fatty acids [23-25]. The handling of free radicals by the organism is therefore delicately balanced. Skeletal muscle is in principle especially endangered because of its strong oxidative metabolism, but the corresponding antioxidative protection mechanisms are present. These are contributed by non-enzymatic antioxidants such as reduced glutathione and vitamin C and E, as well as enzymatic systems like SOD, catalase, glutathione peroxidase, and glutathione reductase. The antioxidative protection system of a muscle is in a dynamic equilibrium: a lack of one factor can to a certain extent be compensated by an increase in other factors [26]. The enzyme activities of SOD and catalase are markedly elevated in the skeletal muscle of aging rats [27-29]. On the other hand, glutathione peroxidase activity falls with increasing age and increases after 10 weeks treadmill training. All antioxidative enzymes, such as SOD, glutathione peroxidase, catalase, and glutathione reductase increase significantly after an intensive and acute training program [29, 30]. From this it can be concluded that both aging processes and training can lead to increased oxidative stress in skeletal muscle [29, 31, 32]. A few studies have been conducted on the significance of free radicals in neuromuscular diseases and on the motoneuronal damage that may be caused by free radicals in the familial form of ALS [16].

THE SIGNIFICANCE OF CARNOSINE FOR SKELETAL MUSCLE AGING AND NEUROMUSCULAR DISEASES

Carnosine is present in large amounts in skeletal muscle and is actively synthesized by muscle cells in culture [34]. Isolated fatigued skeletal muscle resumes contraction in response to the addition of carnosine to the surrounding medium[1], while carnosine has a calcium-sensitizing action in chemically skinned striated muscle [33]. The stimulatory effect of carnosine on the physiological parameters of muscle contraction is quite marked.

Carnosine-related peptides are also actively synthesized by muscle cells in culture [34]. Carnosine exhibits excellent buffering capacity at physiological pH values: concentrations of anserine and carnosine are such that they can provide up to 40% of the pH-buffering capacity of skeletal muscle [35]. Higher concentrations of carnosine are found in white muscle fibers, where high levels of anaerobic metabolism are common [36]. Carnosine can maintain intracellular pH at a physiological level, although this may play only a small role in human muscle [37]. The biological activities of carnosine may be primarily due to one property, its membrane-protective effect. Boldyrev et al. first reported that carnosine has membrane-protecting properties [38][2]: it was demonstrated that carnosine and anserine could decrease membrane lipid oxidation rates as determined by measuring TBARS [38]. Carnosine is capable of inhibiting lipid oxidation catalyzed by a number of substances and mechanisms [39-42]. The antioxidant activity of carnosine and anserine has been demonstrated in a variety of systems [41]. The inhibition of lipid oxidation by carnosine and anserine is concentration dependent, with significant antioxidant activity occurring at concentrations comparable to those in skeletal muscle tissues [41]. The hydrophilic nature of carnosine and anserine provides protection in the cytosolic environment where many lipid oxidation catalysts and free radicals are found. Carnosine and anserine have been found to exhibit up to 60% protection against lipid oxidation at a concentration of 10 mM [41]. The antioxidant mechanism of carnosine and anserine has been postulated to be due to metal chelation or free radical scavenging.

[1] Editor's note: This phenomenon was first described by Severin, S. E., et al. (1953) *Doklady AN SSSR*, **91**, 691-694.

[2] Editor's note: First publications on this item: Severin, S. E., et al. (1984) *Voprosy Med. Khim.*, **30**, 32-36; Boldyrev, A. A., et al. (1987) *Biochem. Int.*, **15**, 1105-1113.

Carnosine is capable of chelating metals, but its chelating activity varies depending on the type of metal ion [39, 43, 44]. It is suggested that carnosine forms a complex with copper in a manner that decreases the reactivity of copper and that carnosine might be capable of scavenging free radicals: carnosine can inactivate hydroxyl radicals [39], while carnosine and anserine have been found to efficiently quench singlet oxygen [43, 44], and a zinc–carnosine chelate compound was found to be capable of scavenging superoxide anion radicals [43, 44]. Copper chelates of carnosine also possess SOD activity against superoxide anion radicals. The pH has little influence on the overall antioxidant activity of carnosine. As antioxidants, the histidine-containing dipeptides could be therapeutically used to protect against lipid oxidation. Research has successfully shown that carnosine could serve as an anti-inflammatory agent [43, 44]. Declining carnosine concentrations in skeletal muscles with increasing age[1] or in neuromuscular diseases could therefore induce a functional deterioration and structural changes by way of a decline in the protective antioxidant effects; these effects could work in synergy with specific atrophic and pathological mechanisms. The determination of tissue free carnosine concentrations in skeletal muscles from patients with neuromuscular diseases and in skeletal and heart muscles from rats of various ages showed that age of the patients emerged as a significant negative predictor of carnosine concentrations [7]. Free carnosine concentrations in rat skeletal muscles also showed a significant negative correlation with the ages of the rats. A decline in free carnosine concentration of 63% takes place between age 10 and age 70 in human subjects, and in rats a continuous decline is observed, amounting to about 37% on average between the third and the 23rd month of life. This is a substantial reduction in both humans and rats. It was concluded that the age-related decline in muscle mass strength and function could be associated with decreased tissue concentrations of the putative membrane-protective antioxidant carnosine. In patients with neuromuscular diseases, this could be an additional factor leading to decompensation of a previously stable situation, involving respiratory deterioration or immobilization. It has been shown that under normal conditions for muscle function, carnosine from blood is not accumulated by muscle cells. In consequence, the phenomenon reported here is probably not related to altered or reduced nutrient supply in aging, but appears to be a muscle-specific phenomenon. In addition, significantly decreased carnosine concentrations in muscle biopsies in ALS were demonstrated [7]. It is therefore plausible that this reduction in carnosine concentration is caused by the progressive denervation which occurs in ALS. Experimental denervation of muscle results in a marked decrease in the level of histidine-containing dipeptides, so that this mechanism could be involved in the age-induced reduction in tissue carnosine. This might indeed have significance for possible therapeutic measures in ALS or other neuromuscular diseases. In particular, when age effects are superimposed on neuromuscular diseases, worsening of the overall condition of the skeletal muscles and immobilization of the patient is possible. In addition, carnosine has muscle-independent effects on the treatment of inflammatory arthritis and on wound healing [45]. It is therefore possible that age-related changes in inflammatory processes and wound healing, especially in polymyositis or dermatomyositis, could also be associated with the age- and disease-related decline in carnosine concentration.

[1] Editor's note: Decrease in histidine dipeptide content in skeletal and cardiac muscles with aging (Johnson, P., and Hammer, J. (1992) *Comp. Biochem. Physiol.*, **103B**, 981-984) or after muscle exercise both *in vitro* (Boldyrev, A. (1993) *Int. J. Biochem.*, **25**, 1101-1107) and *in vivo* (Hong, H., and Johnson, P. (1995) *Biochem. Soc. Trans.*, **23**, 542S) was also noted and discussed by other authors.

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