ISSN 0006-2979, Biochemistry (Moscow), 2012, Vol. 77, No. 4, pp. 313-326. © Pleiades Publishing, Ltd., 2012.
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REVIEW

Carnosine: New Concept for the Function of an Old Molecule*

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Received September 26, 2011
Revision received November 17, 2011

Abstract—In this review, the development of understanding of the biological functions of carnosine is briefly discussed. Carnosine was first described as a component of meat in 1900 by V. S. Gulevitch. Changes in the concepts of the role of carnosine in metabolism are followed starting from the early suggestion that it is the end product of protein degradation to the modern ideas based on demonstrating its specific involvement in intracellular signaling regulation in excitable tissue cells. The discovery of the ability of carnosine to regulate expression of early response genes broadens the concept about carnosine as a cellular peptide regulator. The first attempts for application of carnosine in sport and medical practice are described.

DOI: 10.1134/S0006297912040013

Key words: carnosine, pH buffer, reactive oxygen species, intracellular signaling, neurodegenerative diseases

This story starts in the end of the XIX century. The brilliant student Vladimir Gulevitch, who had excellently graduated from Moscow State University in 1890, began his own research work: he had to compare features of two compounds – the well-known *choline* and a new, only just described substance, the so-called *neurine*. The accumulation of neurine was then believed to be a cause of nervous diseases. After some years of persistent work, Gulevitch presented his doctoral dissertation "About Choline and Neurine. Materials for Chemical Study of the Brain" [1]. This work established that these two substances were identical and that the accumulation in the brain tissues of neurine (choline) was associated with disorders in metabolism of this compound and really accompanied some brain pathologies. The research work by Gulevitch was awarded a gold medal, and this allowed him to work on probation in Europe for two years in a laboratory of his own choice [2].

In the scientific Europe of this period the main interest was focused in chemistry, and the leading country in chemistry was Germany, and the most famous school was the school of Justus Liebig (1803-1873). Liebig was widely known by new principles of organization of a research laboratory, by the discovery of "the minimum law" (this law stated that a shortage of any substance among those given to a plant limited the assimilation by this plant of other compounds even in the case of their excess), and, finally, by a strong group of students of whom nearly half became later the Nobel Prize Laureates. Among Liebig's students the Russian chemist was N. N. Zinin who synthesized aniline.

Gulevitch presented his papers for the work in the laboratory of Liebig's student Albrecht Kossel (Nobel Prize winner of 1910) in Marburg. In this laboratory Gulevitch assimilated quantitative methods of biochemical analysis using as a substrate the so-called "Liebig meat extract" which contained different, still undescribed compounds. On his coming back to Russia, Gulevitch became an extraordinary professor of Kharkov University and continued investigating the Liebig meat extract. And even the first experiments (performed jointly with his student Sergei Amiradgibi) revealed an interesting thing: the content of organic nitrogen in the meat was significantly higher than in specimens of protein nitrogen. Gulevitch concluded that the muscle tissue should hold organic nitrogen-containing non-protein compounds and began to analyze them. These compounds included the two

* On July 10-12, 2011 in the town of Ghent (Belgium) the II International Congress "Carnosine in Exercise and Disease" was held. At the Congress studies on the biological role of carnosine in animal and human tissues were analyzed allowing researchers to suppose a possibility of using this compound in sport medicine and in the treatment of some diseases accompanied by oxidative stress. This review presents material of the introductory lecture presented by the author at the opening of the Congress.

Deceased.

known substances (choline and methylguanidine) and also unknown ones. And two of these unknown substances were present in rather high amounts, and Gulevitch called them carnosine and carnitine (from the Latin *caro*, *carnis* – meat). The amount of carnosine was greater (>1 g per 100 g wet weight in veal) [3]. Therefore, Gulevitch concentrated his studies on this compound. This was the starting of the "Russian page" in World biochemistry – studies on biological activity of carnosine.

RUSSIAN PAGE IN BIOCHEMISTRY

In 1911, the structure of carnosine was established – it was found to be the dipeptide β-alanyl-L-histidine [4]. Later this structure of carnosine was confirmed by its synthesis [5]. Gulevitch thought that carnosine could be generated in tissues as a result of protein decomposition [6]. Nevertheless, he recommended that his students to pay attention to its possible biological role. The first report (1926) describing a physiological effect of carnosine belonged to three of Gulevitch's colleagues: Razenkov, Derwies, and Severin [7]. An intravenous injection of carnosine to dogs caused a transient decrease in blood pressure of the animals and their falling asleep.

However, this finding was then so strongly ahead of the existent knowledge concerning physiological processes that it failed to attract its due attention. Moreover, experiments in this line were considered unreasonable because the described effect was thought to be caused not by the compound itself but rather by admixtures [8]. It was not surprising because this observation was reported 50 years before the discovery of vasodilatation mechanisms and the role of nitrogen oxide in this process [9] and about 70 years before the description of the involvement of carnosine in the regulation of NO metabolism [10-13]. Thus, only at the turn of the XX and XXI centuries carnosine was found to control nitrogen oxide production and thus to influence the blood circulation and blood pressure. If these two lines of studies had intersected, we could be able to use carnosine as a natural regulator blood supply to tissues many years ago.

S. E. Severin continued methodical studies on carnosine in biological objects. It was shown to be present in large amounts in skeletal muscles of various animals [14] and to be intensively decomposed in kidneys [15] but not in muscles [16, 17]. Attempts to reveal synthesis of carnosine in kidneys and liver gave different results: Severin's colleague N. P. Meshkova failed to detect a noticeable accumulation of carnosine in these tissues [18], whereas Parshin et al. [19] in some publications indicated a possibility of such accumulation. The scientific discussion between two laboratories led to the statement that tissues capable of synthesizing carnosine should be unable to provide for its degradation. This opinion seems to be still reasonable.

The structure of carnosine is specified by the presence of a β-amino acid, and this is responsible for its resistance to the majority of known peptidases. An enzyme capable of cleaving carnosine and termed carnosinase has been found in the liver, blood serum, and kidneys [20-22]. Now two isoforms of carnosinase are described, the serum (EC 3.4.13.20) and tissue (cytosolic) (EC 3.4.13.18) ones, which are different in molecular properties and substrate specificity [22]. This has been recently demonstrated in elegant experiments of Aldini et al. [23] who determined hydrolysis of L- and D-isomers of carnosine by kidneys, liver, and blood serum. Both isomers were hydrolyzed in the liver at the same rate, whereas serum and kidney carnosinase could be cleave at a normal rate only L-carnosine and not D-carnosine.

Data about the existence of carnosine-related compounds began to appear even from the late 1920s. In muscles of goose (*Anser anser*) a carnosine derivative methylated by N^1 of the imidazol ring was detected and termed *anserine* [24, 25]. An N^3-methylated carnosine derivative initially termed *balenine* and then *aphidine* was found in dolphins and whales [26]. Now more than ten carnosine derivatives are known (Table 1). They are all found in excitable tissues – skeletal and cardiac muscles, nervous tissue; and a certain correlation is observed between the content of carnosine or its derivatives and the functional activity of the tissue [27, 28].

In 1954, Harms and Winnik [30] reported the existence of a specific enzyme capable of synthesizing carnosine and anserine. Razina studied localization of this enzyme in different tissues [31]. Later carnosine synthase (EC 6.3.2.11) was isolated and partially purified from chicken cardiac muscle [32] and human muscles [33]. Very recently these studies resulted in preparing the purified enzyme [34] and its characterization [35].

Interesting data have been obtained recently showing that carnosine synthase can also produce β-alanyllysine, β-alanylornithine, and β-alanylarginine. In fact, these dipeptides can be detected in muscle but in very small amounts. They are considered to be "faulty metabolites" that are hydrolyzed by β-alanyl-L-lysine peptidase of muscles, which prevents their accumulation in muscle tissue [35].

Among studies on the physiological role of carnosine, the demonstration of its ability to strengthen muscle activity seems to be especially interesting. In these experiments performed on nerve–muscle preparations of the frog sartorius muscle, 10 mM carnosine (the typical concentration for this tissue) was introduced in the medium at the stage of fatigue caused by the rhythmic stimulation of the nerve [36]. This resulted in recovery of contractility and its maintenance for rather a prolonged time. In the literature this effect was termed "Severin's phenomenon". Application of anserine influenced similarly. This phenomenon was analyzed by Severin at the III International Biochemical Congress in Brussels [37].

Table 1. The family of carnosine and related compounds [29]

Trivial name	Rational name	Tissue localization	Year of discovery
Carnosine	β-alanyl-L-histidine	skeletal muscles and brain of vertebrates, skin of snakes and frogs	1900
Anserine	β-alanyl-N^1-methylhistidine	skeletal muscles, heart, and brain of vertebrates	1929
Ophidine	β-alanyl-N^3-methylhistidine	muscles of snakes, dolphins, and whales	1939
Homocarnosine	γ-aminobutyrylhistidine	brain of humans and animals	1962
Neurosine	N-acetylhistidine	central nervous system and eye tissues	1964
Homoanserine	γ-aminobutyryl-N^1-methylhistidine	brain and cardiac muscle	1969
Carcinine	β-alanylhistamine	central nervous system and cardiac muscle	1975
N-Acetylcarnosine	N-acetyl-β-alanyl-L-histidine	brain and heart of vertebrates	1975
N-Acetylhomocarnosine	N-acetyl-γ-aminobutyrylhistidine	brain tissues	1975
N-Acetylmethylhistidine	N-acetyl-N^1-methylhistidine	cardiac muscle and brain	1988
N-Acetylanserine	N-acetyl-β-alanyl-N^1-acetylhistidine	cardiac muscle	1988

Studies on the action of carnosine on muscle activity were performed by many of Severin's students: E. A. Mishukova, N. K. Nagradova, I. M. Bocharnikova, E. V. Petushkova, and A. A. Boldyrev. More intensive muscle activity was found to be accompanied by accumulation of larger amounts of lactic acid in both the muscle and the surrounding solution, although by the end of the experiment in the more intensively working muscle the lactate amount frequently was lower than in the parallel muscle that worked less intensively. The total amount of accumulated lactate was compared with the work performed by the muscle, and it occurred that in the presence of carnosine the lactate accumulation was 25-30% lower per unit of work, and this seemed to correspond to the lower energy expended per unit of work [38]. Moreover, ATP contents by the end of the experiment were decreased approximately to the same level in both muscles. And it was supposed that at least partial increase in the efficiency of muscle contractions caused by carnosine could be associated with its influence on viscosity and elasticity of the muscle fiber. This speculation is indirectly confirmed by the increased rates of both contraction and relaxation of the muscle in the presence of carnosine [38a].

Severin paid special attention to the necessity of adjusting pH of solutions to the same value to exclude the pH-dependent component in the carnosine effect – he wanted to emphasize that the observed phenomenon was specific not for all but only for excitable tissues. However, the ability of carnosine molecule to bind protons, which V. P. Skulachev called an activity of mobile biological buffer, also contributes to its effect: the pK of carnosine is 6.8 (pK of anserine is 7.04), i.e. it is in the more acidic region than the physiological pH value of 7.4. In other words, during intensive muscle work carnosine and anserine effectively prevent acidification of the intracellular medium [39].

Later the ability of carnosine to regulate osmotic pressure was described. Abe [40] analyzed changes in the contents of this dipeptide in muscles of diadromous fishes on their passing from fresh into sea water and back, and he found that the carnosine level in the muscles changed in 10-24 h, increasing in salt and decreasing in fresh water; these changes were up to 30% of the initial level of carnosine. He concluded that carnosine, in addition to its ability to function as a pH buffer, could also regulate osmotic pressure in muscle tissue.

Nevertheless, the data available by this time indicated that a correlation should exist between carnosine and anserine and functions of excitable tissues, and this was analyzed by Severin in his plenary lecture at the X International Biochemical Congress in New York [41]. By this period stable interest in carnosine also appeared in the international biochemistry community.

DIRECT AND INDIRECT ANTIOXIDANT ACTIONS

Some new findings attracted the attention to the biological activity of carnosine in the end of the XX century.

First, Severin's phenomenon was reproduced in mammals *in vivo*. Rats were injected with carnosine (150 mg/g body weight) and forced to run in a wheel until exhaustion. The carnosine-injected animals ran 25-30% longer than the control animals, and the lactate content (measured by *in vivo* NMR) in muscles actively involved in the exercise was significantly lower in the carnosine-treated rats that in the control ones [42]. Moreover, the carnosine content in the muscles working until exhaustion was significantly lower, although its diminution was not accompanied by an increase in the levels of histidine or β-alanine [43].

Second, attention was attracted to the significantly weaker induction of lipid peroxidation in muscle homogenates than in liver, despite a significantly higher content of vitamin E in the liver. This finding was considered to be due to the presence of carnosine in muscle, and it was supposed that carnosine could reduce α-tocopherol and thus increase the antioxidant ability of the tissue [44]. Later carnosine was shown to be unable to recover the stores of oxidized tocopherol as differentiated from ascorbic acid [45], but it could prevent the accumulation of lipid hydroperoxides [46].

Considering the ability of carnosine to chelate heavy metal ions (copper, iron, cobalt, cadmium), it would reasonable to think that the protection by carnosine against lipid peroxidation was due to a decrease in active concentrations of variable valence metals. The binding constant of iron ions by carnosine calculated from values of the chemiluminescent response of phospholipid liposomes on their oxidation was $2.3 \cdot 10^{-3}$ M^{-1} [47]. However, in some cases the effect of carnosine was observed also at the lower concentrations (0.1-0.5 mM) [43, 48]. Thus, it was concluded that the effect of carnosine was not limited only to its ability to chelate iron ions.

Now it is established that in excitable tissues of animals carnosine is a natural hydrophilic antioxidant of direct action [49, 50], and this was an independent conclusion of different investigators [51-53]. Carnosine and anserine prevented with a similar efficiency the oxidative damage to DNA in human lymphocytes in the presence of Fe^{2+}, Cu^{2+}, or H_2O_2 (as determined by the so-called "single cell gel electrophoresis", Comet assay) [54]. A direct antiradical activity of carnosine was demonstrated in experiments with cell cultures: the death of differentiating PC12 cells incubated with NMDA also could be prevented by carnosine [55]. Moreover, these authors found that the protective effect of carnosine was weakened by both α-fluoromethylhistidine (a selective and irreversible inhibitor of histidine decarboxylase) and thioperamide (a selective antagonist of H1 and H3 histamine receptors). Therefore, they concluded that the protective effect of carnosine should be directed to NMDA receptors and also to some histamine receptors.

Based on these findings, carnosine was supposed to protect the organism under conditions of general oxidative stress. In fact, addition of carnosine to the diet of mice (50 mg per kg body weight) increased their resistance to γ-irradiation [56]. Radiation induces multiple damages of tissue macromolecules, and the genetic apparatus of intensively dividing cells is the most sensitive to such breaks. Therefore, radiation damages first of all the immune system cells, and this decreases the organism's viability. Figure 1 shows that radiation of mice with a half-lethal dose of 5 Gy after a short delay results in a massive death of the animals, whereas in the carnosine-treated animals the latent period before the appearance of deaths becomes longer and the lethality by the 30th day of the experiment is decreased twofold. The protective effect of carnosine manifested itself not only on its administration before the irradiation but also 1 h after it. And colonies of stem cells were actively produced in spleens of the carnosine-treated mice that indicated an enhanced power of the immune system in its struggle with the consequences of irradiation [57].

Nagai revealed that in old animals carnosine could support the immune system weakened because of age-related changes in the animals' organism [58]. Moreover, the effect of carnosine was clearly age-specific: in mice of different age its effect on the immune system was opposite. In old animals carnosine injection increased the quantity of colony-forming cells in the spleen, whereas in young animals the quantity of colonies in the spleen was actually decreased. Thus, carnosine was concluded to act in the organism as an immunity modifier [59].

Injection of carnosine (100 mg/kg body weight) into rats under cold stress conditions lowered the level of lipid hydroperoxides and end products of lipid peroxidation (malonic dialdehyde, MDA) in tissues and also increased their resistance to cold stress [60]. The protective function of carnosine preventively injected into rats (150 mg/kg body weight) was also shown on the model of experimental brain stroke (ischemia/reperfusion of the brain). In the group with the experimental ischemia 54% of animals died, whereas the pretreatment with carnosine decreased the lethality threefold. Moreover, in the carnosine-pretreated animals the training for food searching in the T-shaped labyrinth was associated with a decreased number of errors, and the learning level achieved was retained also after the episode of ischemia [61]. These data were confirmed later by description of the neuroprotective effect of carnosine under conditions of focal ischemia in mice [62].

Note that *in vivo* determinations of microcirculation revealed a time-dependence of disorders in the blood supply to the animals' brain. The blood flow rate in the damaged region was 25% lowered already 15 min after the ischemia episode, after 30 min it was 32%, and this decreased level remained at the 24th hour after the ischemia. However, in the carnosine-treated animals the decrease in the flow rate during the initial period was only 13-15% and was only 5% below the normal level at the 24th hour of the experiment [63].

The ability of carnosine to act as an indirect antioxidant was initially shown in model experiments with superoxide dismutase (SOD), an enzyme regulating the level of superoxide oxygen anion triggering free radical processes. SOD is highly sensitive to hydroxide radical, which causes protein fragmentation and inhibits its enzymatic activity [64]. Therefore, it was not surprising that the introduction of carnosine into an *in vitro* system generating reactive oxygen species (ROS) protected the SOD activity [64, 65]. But a similar effect of carnosine observed in the *in vivo* system and even manifested in significantly lower concentrations indicated that its action should be mediated through some additional mechanisms [65].

The discrepancy between the acting concentrations of carnosine in the *in vitro* and *in vivo* experiments was repeatedly considered by researchers. Thus, effective carnosine concentrations under conditions of experimental brain ischemia in animals were at least the order of magnitude lower than in model experiments *in vitro* [29, 43, 66]. Therefore, carnosine was admitted to be capable also of manifesting an indirect antioxidant effect due to increasing the efficiency of endogenous protection against oxidative stress. This was in good agreement with Skulachev's viewpoint [39] about the protective effect of carnosine as an intracellular mobile proton buffer.

In fact, on acidification of the medium the superoxide anion can interact with protons, which convert superoxide into the peroxyl radical having higher oxidative activity. The presence in the medium of carnosine, which is a strong buffer (pK 6.8), will prevent accumulation of protons and the conversion of superoxide anion into the more active oxidizer [67].

For assessment of the protective effect of carnosine, a model of prenatal hyperhomocysteinemia was recently used to create stable oxidative stress in all tissues of the body [68, 69]. In this model an increase in the homocysteine (HC) level in blood plasma of pregnant rats was created due to peroral load with *methionine* (1 g/kg body weight daily) starting from the second trimester of pregnancy. At the normal blood plasma content of total HC in rodents (7-12 μM) the alimentary load with methionine increased its concentration three-fourfold [69]. This resulted in the birth of progeny that developed under conditions of hyperhomocysteinemia. The number of newborns and their weight were sharply decreased along with nearly twofold increase in the HC content in their blood: from 5.4 to 9.8 μM.

Homocysteine is very toxic for cells of the nervous and immune systems: an increased level of HC in the blood flow is associated with disorders in blood–brain barrier stability and the accumulation of HC in brain tissues. As a structural analog of glutamate, HC activates both ionotropic and metabotropic glutamate receptors. The most dangerous is the HC-induced activation of ionotropic glutamate receptors activated by N-methyl-D-aspartate (NMDA) [70]. These receptors responsible

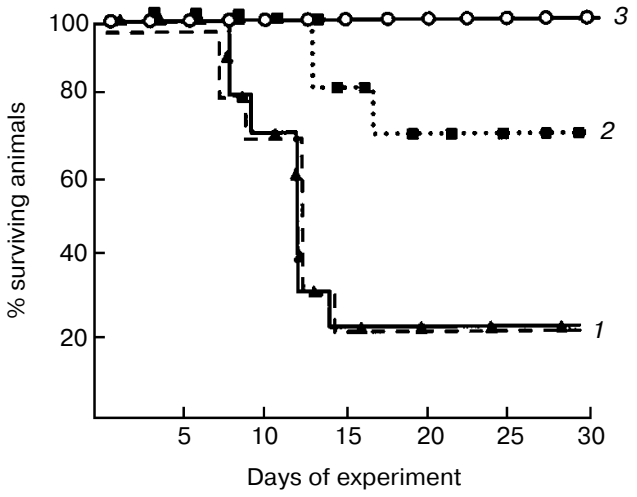

Fig. 1. Effect of carnosine on survival of mice after γ-irradiation (5 Gy). *1*) Mice irradiated without taking carnosine; *2*) irradiated mice given carnosine (50 mg/kg) daily beginning from the day of irradiation; *3*) intact mice. Each group included 10 adult animals.

for long-term potentiation, image recognition, and memory formation were earlier thought to be specific components of the nervous tissue. NMDA receptors were recently found in the immune system [71], where they are responsible for molecular processes underlying cell immunity: recognizing foreign proteins and formation of the immune response through synthesis and secretion of cytokines [72, 73].

The increased level of HC in blood circulation leads to a constant activation of NMDA receptors in cells of the nervous and immune systems and to dysfunction in the activities of these important systems. This phenomenon, called excitotoxicity in the case of nervous tissue [74], is responsible for the cell death caused either by apoptosis or by necrosis depending on the duration and intensity of the activation [75]. After the discovery of NMDA receptors in immune system cells, such as lymphocytes [76, 77], neutrophils [78], and also in erythrocytes and cardiomyocytes [79, 80], it becomes clear that hyperhomocysteinemia can be toxic not only for nervous cells but also for the cardiac muscle and immune system cells. Moreover, under conditions of long-term circulation of HC in blood this compound can also display long-term toxic effects, in particular, it can cause destruction of the cytoskeleton. Thus, on long-term exposure (as occurs in the organism under conditions of hyperhomocysteinemia) HC can lower the level of phosphorylation of vimentin and cause reorganization of actin molecules in the cytoskeleton [81]. This effect of HC can cause the death of nerve cells in neurodegenerative diseases. It seems that similar mechanisms of HC toxicity can manifest themselves on interaction not only with the nervous system cells. Note that hyperhomocysteinemia is associ-

ated with multiple lesions of the endothelial cell wall in the vascular system, which are forerunners of atherosclerosis, repeated brain strokes, and infarctions. This also explains the disorders in the development of progeny caused by hyperhomocysteinemia.

Cognitive abilities of animals also suffer under conditions of hyperhomocysteinemia. Using the Morris water maze test for evaluation of learning and memorizing reveals that these animals find the platform in a water pool more slowly and are weaker in memorizing the information. They show slower mean rate of swimming and, respectively, longer time of searching for the platform [69].

A daily addition of carnosine (150 mg/kg body weight) to the diet of pregnant rats displayed various protective effects on their litters: the number and weight of the newborns became normal and information memorizing was similar to that of intact animals (Fig. 2). The protective effect of carnosine can be most easily explained by its ability to limit the accumulation of free radicals in the cells expressing NMDA receptors, which are shown to be activated by homocysteine. However, this mechanism can be not the only one – the injection of carnosine is accompanied by a transient increase in its stationary level in blood and tissues, it can undergo metabolic conversions, in particular, be degraded by carnosinase with production of histidine. Carnosine decarboxylation can produce histamine, which is an activator of histamine receptors especially important for small muscle cells lining the vessels. Some findings supporting this mechanism have been considered above [55], and additional data will be discussed in the final part of the review.

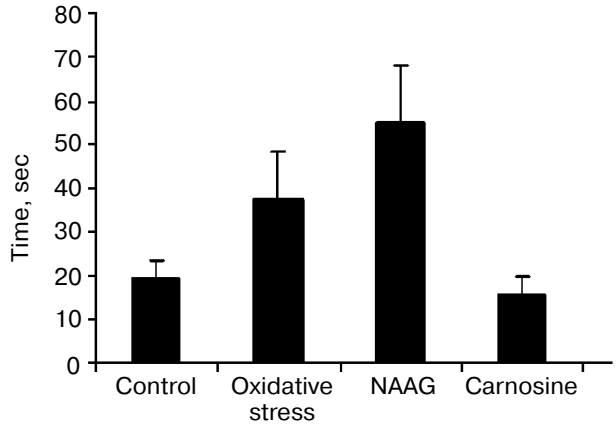

Fig. 2. Assessment of the ability for learning in the Morris test (time for searching for platform in a swimming pool, seconds). Animal groups (n = 12): intact (Control); animals subjected to a prenatal hypoxic stress (Oxidative stress); animals subjected to oxidative stress and given either N-acetylaspartyl glutamate (NAAG), or carnosine (Carnosine). Ordinate axis, time of platform searching. NAAG was injected twice a day in the dose of 1.5 mg/kg body weight 1 day before and 1 day after the hypoxic stress; carnosine (150 mg/kg) was injected daily starting from the day of exposure to hypoxic stress.

Thus, effects of carnosine injected into the organism can be more versatile. But it is important that its protective effect is realized notwithstanding the retention of the high stationary blood level of HC. In other words, the protective effect of carnosine is due not to decreasing the HC level in the tissues but due to protection against its toxic action [69].

All these effects of carnosine can be considered as manifestations of its indirect antioxidant action promoting the enhancement of the endogenous system of antioxidant protection.

COMPARISON OF PROPERTIES OF CARNOSINE AND RELATED COMPOUNDS

The family of Carnosine Related Compounds (CRC) includes substances with similar structure (Table 1). Studies on the composition of extracts from muscles in ontogenesis of birds have revealed that before hatching, protein synthesis sharply increases along with a decrease in the levels of all amino acids, including histidine. Carnosine also appears in the muscles during this period, although its level is rather high even in embryos. The carnosine level continues to increase after the hatching and then becomes stable. Then at a certain stage of the postnatal development (in birds it is the flight-starting period) the carnosine level begins to decrease along with an increase in the level of anserine [82].

The stepwise conversion of histidine into carnosine and then into anserine along with the improvement of muscle functioning in ontogenesis was shown for ducks, rooks, and rabbits [82-84], and this stimulated more careful analysis of contents of carnosine and relative compounds in muscles of animals different in their place in evolution. Although such comparing is far from perfect, it is obvious that "the evolution tree" (with a few exceptions) shows the replacement of histidine by carnosine and then by anserine (for whales and snakes by ophidine). Note that animals adapted to physiological hypoxia (aqueous mammals capable of a long-term retention of respiration) have in the muscles especially high concentrations of carnosine or ophidine (Table 2). And tissues of invertebrates contain neither carnosine nor anserine.

It was reasonably to suggest that the described conversions of carnosine in excitable tissues should occur under the influence of enzymes, despite the absence of sufficient experimental proof. However, enzymatic generation of anserine is shown [84], as well as the production of homocarnosine under the influence of carnosine synthase in the presence of large amount of γ-aminobutyric acid. In the literature there are no direct data on enzymatic conversions of carnosine into carcinine or on generation of acetylated derivatives of carnosine, anserine, and homocysteine. Nevertheless, just the existence of the family of carnosine-related compounds can be biologically reasonable.

Table 2. Contents of carnosine and relative compounds (mg% per tissue wet weight) in tissues of invertebrates and vertebrates [27, 56, 85]

Animals	β-Alanine	Histidine	Carnosine	Anserine	Ophidine
Invertebrates (*Invertebrata*)					
Actinia (*Actiniaria* sp.)	–	10	–	–	–
Crab (*Brachyura* sp.)	–	7-15	–	–	–
Oyster (*Ostrea gigas*)	150	7	–	–	–
Calmar (*Teuthida* sp.)	5	65	–	–	–
Octopus (*Octopus octopus*)	345	2	–	–	–
Cyclostomes (Cyclostomata)					
Lampreys (*Petromyzonidae* sp.)	63	25	80	128	–
Fishes (*Pisces*)					
Spurdog (*Squalus acanthias*)	105	–	–	–	–
Sturgeon (*Acipenser* sp.)	140	–	252	–	–
Salmon trout (*Oncorhynchus* sp.)	–	traces	–	400	–
Herring (*Clupea* sp.)	–	160	–	–	–
Bonito (*Gymnosarda* sp.)	–	1620	–	–	–
Chum (*Oncorhynchus* sp.)	–	15	–	1020	–
Cod (*Gadus morrhua*)	220	–	–	60	–
Croaker (*Argyrosomus* sp.)	–	66	–	527	–
Amphibia					
Frog (*Rana* sp.)					
musculus rectus abdominis	–	–	220	–	–
cardiac muscle	–	–	36	–	–
Reptiles (Reptilia)					
Sea snake (*Aipysurus duboisii*)	–	–	–	–	560
King cobra (*Ophiophagus hannah*)	2	2	–	–	120
Birds (Aves)					
Chicken (*Gallus gallus*)					
pectoral muscle	–	1	278	983	–
Pigeon (*Columba livia*)					
pectoral muscle	–	1	20	110	–
Rook (*Corvus frugilegus*)					
pectoral muscle	–	–	–	348	–
muscles of extremities	–	–	–	43	–
Mammals (Mammalia)					
Cow (*Bovinae* sp.)					
thigh muscles	–	–	150	25	–
cardiac muscle	–	–	19	–	–
Rabbit (*Oryctolagus cuniculus*)					
musculus longissimus of the back	–	1	72	437	–
muscles of leg	–	6	70	400	–
Cat (*Felis catus*)					
musculus gastrocnemius	7	4	150	200	–
Blue whale (*Balaenoptera musculus*)					
musculus longissimus of the back	–	10	90	–	1080
Dolphin (*Delphinus* sp.)	–	–	220	–	480
Human (*Homo sapience*)	–	–	150-220	–	–

Experiments of two types can be useful for elucidation of this problem. The first type is associated with quantitative comparison of the antioxidant activity of these compounds, and such experiments have been performed on a model of oxidation of blood plasma lipoproteins induced by bivalent iron ions [86]. This comparison has shown that carnosine derivatives are different in preventing the oxidation of lipoproteins. Carnosine is more efficient than homocarnosine, and anserine is more efficient than carnosine; acetylation by the free β-amino group decreases the protective efficiency of the molecule. A significant difference in the efficiency leads to the suggestion that the metabolism of these compounds in the organism could mediate the efficiency of the antioxidant protection. In the absence of requirements for antioxidants, acetylation of carnosine will be sufficient for decreasing its ability to react with radicals. On the contrary, methylation of carnosine will increase the antioxidant efficiency of the molecule. This hypothesis allowed us to predict the presence in tissue enzymes responsible for carnosine metabolism [87].

On the other hand, carnosine derivatives are more resistant to the action of carnosinase [88]. Enzymatic hydrolysis of carnosine, anserine, acetylcarnosine, and homocarnosine is characterized by the same value of K_m but by different maximal rates (and for all derivatives the rate is lower than for carnosine). This means that metabolic conversions of carnosine allow it to escape from the control of carnosinase and thus save the reserve of antioxidant molecules from exhaustion.

NEW PROPERTIES OF THE "OLD MOLECULE"

New features of this molecule known for the long time were described in the end of the XX and the beginning of the XXI century. Thus, carnosine was shown to prevent modifications of proteins by aldehydes (methylglyoxal, malonic dialdehyde) [89]. Carnosine was shown to bind molecular products of lipid oxidative conversions, in particular, 4-hydroxy-trans-2-nonenal [23]. Due to this ability, carnosine can protect macromolecules of the cell against hydroxynonenal, which forms functionally inactive adducts with proteins and nucleic acids. This ability is likely to represent a new mechanism for protein protection against products accumulated during the development of oxidative stress [67].

Interesting observations were reported in 2003 by Kondrashova and coauthors [90]: they quantitatively characterized a spontaneous self-organization of the mitochondrial network in rat liver homogenates and found that carnosine prevented the mitochondrial network dissociation during storage of the isolated mitochondria. They think that because of the experimental conditions (low carnosine concentration of 50 μM and natural antioxidant protection of liver cells) this effect of carnosine cannot be explained by the antioxidant properties of the compound. This effect mimics the earlier observations by Meshkova that carnosine increased the coupling of oxidative phosphorylation in mitochondria, and that this effect was the stronger the "older" was the mitochondria preparation (cited after [28]).

At present, the concept of the regulatory role of ROS in the neuronal activity seems to be generally adopted [91], because the dependence of the genome state on the redox status of the cell is well known [92, 93]. In fact, free radicals, which influence the redox status of the cells, act as secondary messengers regulating the expression of various genes. This concept stimulated studies for elucidating a possibility of carnosine influence on the expression of redox-sensitive genes in cell cultures. Thus, in a culture of rat astrocytes lipopolysaccharides or interferon-γ caused hyperproduction of NO radical, and under these conditions there were an accumulation of oxidized proteins and an increased expression of genes associated with the regulation of oxidative stress, *HSP32* and *HSP70*. An addition into the medium of 300 μM carnosine 1 h before inducing the stress decreased the accumulation of oxidized proteins and prevented the expression of Hsp32 and Hsp70 [94].

Carnosine was recently shown to regulate the production of interleukin 8 by influencing translation mechanisms [95]. It was also found that carnosine induced expression of the antimetastatic gene NM23-H1 and inhibited expression of matrix metalloprotease, thus limiting hepatocarcinoma growth [96]. And a short time later carnosine was reported to prevent the development of glioblastoma in mice due to lowering the expression of the genome regulation factors HIF1a and NFκB [97].

It was important to elucidate how activation of glutamate receptors of the NMDA-class could contribute to the long-term potentiation processes involved in learning and memory. Activation of NMDA receptors is known to cause the growth of intracellular ROS, which are a factor of phosphorylation of the signaling kinase cascade with a kinase family for activation of which phosphorylation is required (Fig. 3). Information from the cell membrane to the nucleus is transduced due to sequential involvement of these kinases among which ERK1/2, p38, and JNK are crucial because they influence the inclusion of specific genes (the so-called early response genes) determining the type of cell reaction to external stimuli [98].

An important feature of NMDA receptors is their ability to change levels of ROS and other factors determining the genome state and thus to influence the expression of the early response genes, which can either adapt the cell to environmental changes or to cause cell death. And just due to easiness of receptor switching from normal functioning to inducing cell death they are characterized as excitotoxic.

It seems that the switching of NMDA receptor functioning mode depends on the nature of the activating lig-

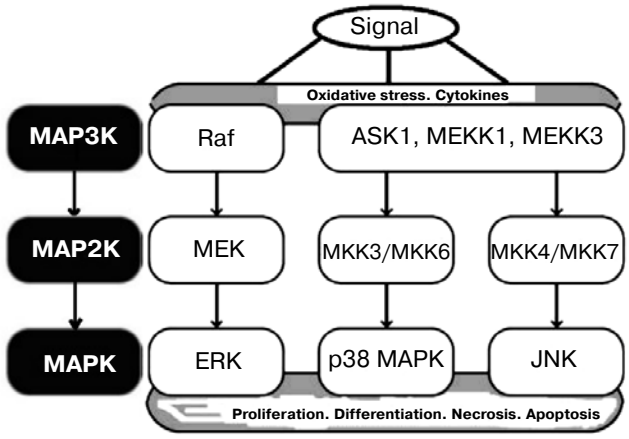

Fig. 3. Scheme of MAPK signaling cascades (after [97] with modifications). Signals from the environment onto mitogen-activated proteinases (MAPK) are transduced through sequential phosphorylation of the cascade members – kinases responsible for phosphorylation of MAPK (MAP2K) and kinases responsible for phosphorylation of MAP2K (MAP3K). The terminal link of the MAP-kinase cascade is represented by ERK1/2, p38, and JNK; the level of their phosphorylation (activation) is often used for assessment of the ability of the cell to express transcription factors responsible for the cell adaptive response.

and and on the time course of the activation. The *in vitro* incubation of neurons with NMDA or glutamate caused a short-term increase in the fraction of the phosphorylated form of the central component of the MAP kinase cascade, ERK1/2, responsible for the signal transduction to the cell genome [99, 100]. However, the incubation of neurons for 30 min with homocysteine resulted in long-term activation of ERK1/2 and finally in the necrotic death of cells. The incubation of cells with HC and carnosine abolished the toxic effect of HC – the level of cell death was the same as in the intact cell suspension (Fig. 4).

For elucidation of the components of the protective effect, it was important to study the effect of carnosine on the activation of signaling kinases. Figure 5 shows that L-carnosine prevents ERK1/2 phosphorylation despite the presence of HC. Note that phosphorylation of this kinase in the presence of HC and D-carnosine and also of a dipeptide with the inverse sequence of amino acids, L-histidyl-β-alanine, has a similar time profile. Moreover, the three dipeptides studied prevent the HC-induced phosphorylation of JNK [100]. It could be supposed that this effect should lead to abolishment of the HC-caused lethality. However, a significant decrease in the lethality of neurons incubated with HC was observed only in the presence of L-carnosine and did not occur in the presence of its structural analogs [101]. Thus, it is concluded that carnosine has several points of interaction in the regulation of neuronal activity, and even compounds with a similar structure do not possess all its functional capabilities (Fig. 5).

Data on the properties of carnosine are very far from completeness, and this is demonstrated by reports about its protective effect under conditions of oxidative stress caused in neurons by a toxic derivative of HC – homocysteic acid (HCA) – that induces both apoptotic and necrotic death of neurons. And the addition of carnosine into the HCA-containing medium protected against both types of cell death (Fig. 6).

Incubation of neurons with HCA leads to accumulation of free radicals which are thought, on one hand, to activate apoptosis and, on the other hand, to cause oxidative damages of cellular components and cell necrosis. And it is reasonable to ask whether the cell protection by

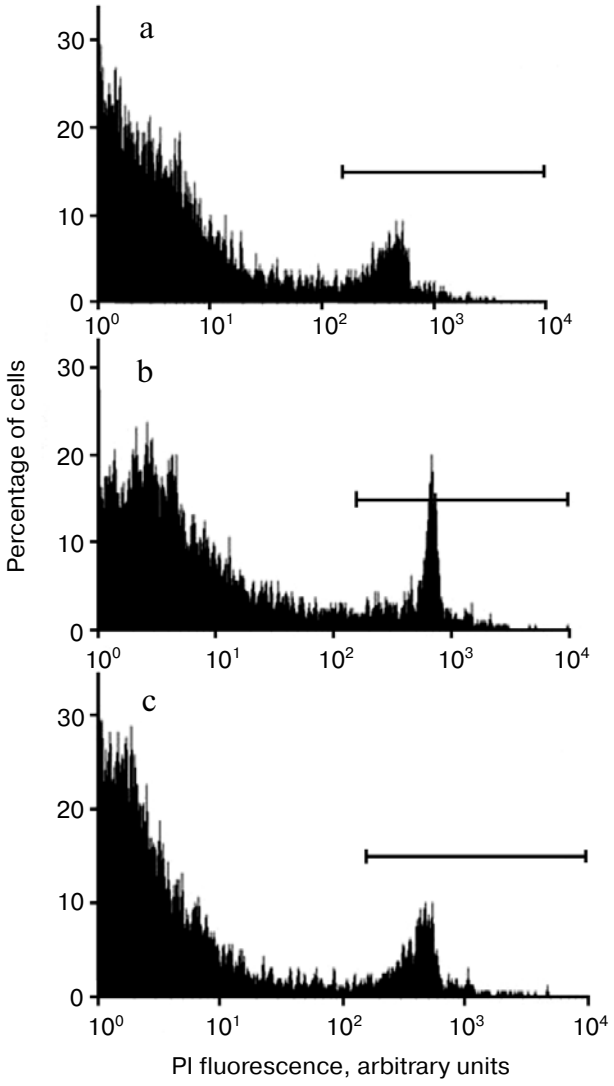

Fig. 4. Carnosine prevents the death of neurons under conditions of oxidative stress caused by the 30-min incubation in the presence of 0.5 mM homocysteine. a) Intact cells; b) upon 30-min incubation with HC; c) upon incubation with HC in the presence of 1 mM carnosine. The horizontal line corresponds to dead cells (stained by propidium iodide (PI)). The quantity of dead cells is 11 ± 2% (a), 17 ± 1% (b), and 9 ± 1% (c).

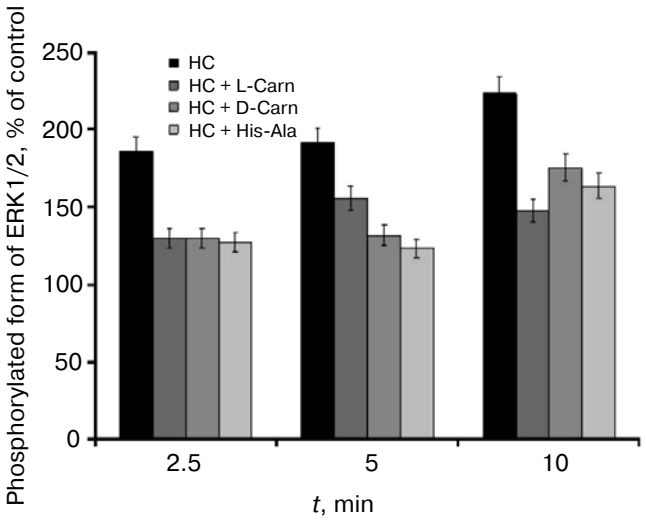

Fig. 5. L-Carnosine (L-Carn) and its analogs D-carnosine (D-Carn) and L-histidyl-β-alanine (His-Ala) (all in the final concentration of 1 mM) prevent phosphorylation of ERK1/2 induced by the neuron incubation with 0.5 mM homocysteine (HC). The phosphorylated form of ERK1/2 in the intact cells is taken as 100%.

carnosine is directly due to its ability to decrease the level of free radicals. The antioxidant activity of carnosine seems to be directly responsible for the cell protection against necrosis, but in the case of apoptosis the problem can be more complicated. Data of RT-PCR (Real Time Polymerase Chain Reaction) suggest that carnosine can induce Mn-SOD, suppress the expression of NFκB, and increase the Bcl2/Bax ratio [102]. We do not know yet the inner mechanism of these features of carnosine; nevertheless, they can be used by brain tissues for counteracting age-related and neurodegenerative changes and also tissue malignization. In the literature there are independent reports about the decrease with age in carnosine contents in excitable tissues [103] and in patients with myodystrophy [104], and there are well-based hypotheses that a decrease in the carnosine level accompanies various neurodegenerative processes [105].

Systematic studies of Japanese researchers from Osaka University have shown that carnosine acts not only on the brain but also on other organs. They described the lowering of increase in blood pressure induced in rats by injection of deoxycorticosterone acetate [106]. Later, it was shown that the regulation of blood pressure was mediated through histamine receptors, and low doses of carnosine (1 μg intravenously) suppressed the activity of sympathetic nerves of the renal system, whereas high doses (100 μg intravenously) activated sympathetic nerves and increased blood pressure. Anserine acted similarly [107]. Later the ability of carnosine to regulate the activity of the sympathetic system of the brown fat tissue was described: low doses of carnosine decreased and its high doses increased the body temperature [108]. The authors suggested that on intensive muscle activity small amounts of carnosine could be released from the muscle tissue into blood and thus contribute to regulation of the sympathetic nervous system.

PRACTICAL APPLICATION

Thus, at the turn of two centuries interest in carnosine significantly increased. During the first decades after

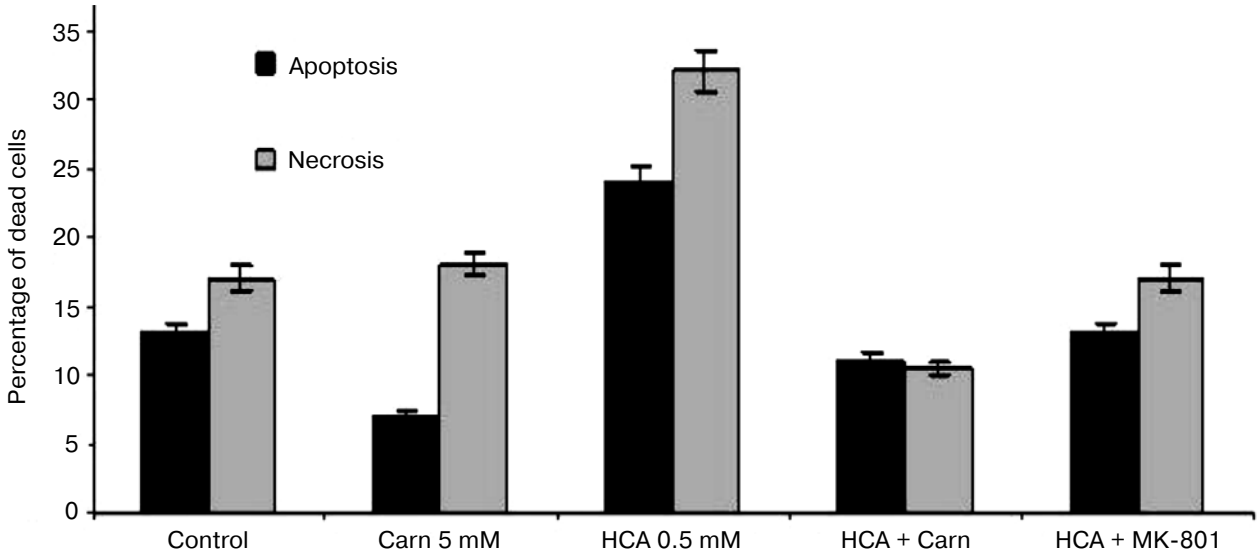

Fig. 6. Carnosine (Carn) prevents apoptosis and necrosis of neurons under conditions of oxidative stress caused by 30-min incubation in the presence of 0.5 mM homocysteic acid (HCA). The toxic effect of HCA is due to activation of NMDA receptors and can be prevented by introduction into the medium of an antagonist of NMDA receptors, MK-801.

the discovery of carnosine, in the literature there were only isolated references concerning this compound, but by the end of the XX century the annual reports about carnosine rapidly became more numerous (Fig. 7). In 2010 the word "carnosine" was cited 400 times, and the total number of publications reached several thousands. In the XXI century it was clear that this compound performs in excitable tissues of vertebrates a specific function of regulating the stationary level of free radicals. And just by this time it becomes clear that free radicals act as secondary messengers especially important for nerve cells [91], and this stimulated additional interest in this compound.

In the XXI century this initially pure scientific problem became a problem of practice. Strictly speaking, separate attempts to use carnosine in medical practice were also described earlier: in 1939-1941 Normark assessed the possibility of using carnosine for electrophoresis in the treatment of joint inflammations (arthritis, arthroses), in 1994-1996 a deceleration of the cataract development and even regression of some kinds were shown, in 1996-1998 a positive effect of 5% carnosine solution was shown in suppression of sinusal rhinitis and other allergic reactions [29].

However, because of absence of a standard method for preparing the substance, difficulties associated with creating pharmaceutics, and tightening of demands to new preparations, there were only few attempts. In 2000 in Russia the Medtekhnika Company received permission to produce biologically active food supplements based on a substance produced by Hamari Chemicals, Ltd. (Japan). By this time in China carnosine-containing drops became a widely used preparation for treatment of cataract, and in some countries carnosine tablets are recommended to promote rehabilitation after a disease and the organism recovery after exhausting physical and psychical loads. A positive effect of carnosine is reported on application in radiotherapy of malignancies: in this case carnosine protects the organism's tissues against toxic effects of free radicals [67].

The experience at hand allowed us to assess the ability of carnosine to increase the efficiency of treatment of human neurodegenerative diseases. In the Research Center of Neurology, Russian Academy of Medical Sciences, in 2004-2008 the standard (double-blind placebo-controlled) testing was performed of carnosine application in patients with disturbances in the brain blood circulation. The patients (42 patients, 32-79-years-old) with ischemic brain stroke were randomly divided into two groups: one group was treated routinely, and the other was given carnosine in addition to the standard therapy. Besides the evaluation of neurological symptomatology, the reaction of the cortical hearing center to paired pulses (potentials P300) was studied and the endogenous antioxidant activity of blood plasma lipoproteins was analyzed. The 20-day treatment resulted in improvement of the neurological symptomatology, discriminating paired pulses, and the recovery of endogenous antioxidant protection in patients given carnosine as an additional treatment [109]. The carnosine effect depended on the dose (it was used in doses of 0.75 and 2 g per day).

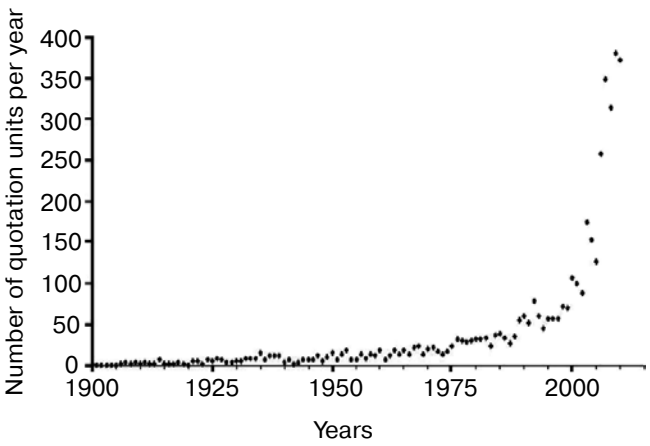

Fig. 7. Quotation of the word "carnosine" in the world literature in 1900-2010 (after data of PubMed and of personal card index of S. E. Severin with indicated publications in Russian journals not shown in the PubMed system).

Another example is represented by an increase in the treatment efficiency of the Parkinson's disease on combination of the standard therapy with carnosine (1.5 g daily) [110]. In this case 30-day therapy resulted in a decrease in the stationary level of oxidized proteins and lipids in blood lipoproteins, an increased resistance to Fe^{2+}-induced oxidation of blood plasma lipoproteins, a decrease in the activity of thrombocytic MAO B, and an increase in the activity of erythrocytic Cu/Zn-SOD. All these processes occurred on the background of a stable decrease in neurologic symptomatology. The symptomatology level determined by the UPDRS (Unified Parkinson's Disease Rating Scale) on the combination of carnosine with the standard therapy decreased from 39.2 to 24.9 marks, whereas on the standard protocol it decreased to 32.5 marks.

Interesting results were obtained on carnosine use by athletes. A group of volunteers took carnosine (2 g per day) during three weeks and was tested with a bicycle ergometer. The athletes' endurance increased by 18% [111]. Then further success in understanding the role of carnosine in work performance came from the Belgian laboratory of Derave [112, 113]. He developed noninvasive approaches using magnetic resonance tomography for quantitative determination of carnosine content in athletes' muscles [113].

It was found that the work capacity of athletes could be increased not only by carnosine but also by β-alanine [113]. The daily dose of 5 g β-alanine already after 5-6 weeks resulted in a significant improvement in sport results accompanied by an increase in the carnosine content in phasic muscles. And the accumulation of carno-

sine in muscles pronouncedly correlated with the increase in the work capacity of the athletes.

This suggested, first, that the carnosine synthesis in the muscle tissue could be limited not by expression of the corresponding enzyme but by availability of the substrate (β-alanine) and, second, that the need for this dipeptide could be determined by the functional exercise of the organ, as it had been earlier supposed by Severin [28, 83].

The mystery of biological activity of carnosine appears as a result of comparison of its accumulation in excitable tissues of vertebrates and the presence of specific enzymes of metabolism. During the century after the discovery of carnosine by Gulevitch, a variability of biological effects caused by unique properties of the molecule was revealed. Carnosine was shown to act in the brain and in cardiac and skeletal muscles as an important physiological buffer, chelator of variable valency metals, and regulator of the level of reactive oxygen species. What property of the molecule is the most important for the special tissue under study seems to depend on its functional state.

Carnosine is able to regulate osmotic pressure, to bind toxic products of lipid oxidation (as noted above for 4,2-hydroxynonenal), and to prevent oxidation of proteins and nucleic acids. Its ability to soften the excitotoxic features of glutamate receptors of NMDA class suggests its intervention in intracellular signaling and makes promising its application for treatment of neurodegenerative diseases associated with a pronounced oxidative stress. The use of carnosine occurred to be promising for recovery of the nervous and immune systems under conditions of extreme physical and psychological loads.

All this attracts attention to carnosine and related compounds as natural regulators of oxidative metabolism in brain and muscle tissues. The broadening of our knowledge about functions of this molecule known for a long time opens new opportunities for its practical application.

I am grateful to all colleagues who actively participated in the studies on the biological role of carnosine and provided for its successful application.

This work was supported by the budget of Lomonosov Moscow State University, the Research Center of Neurology of the Russian Academy of Medical Sciences, by projects of the Russian Foundation for Basic Research, of Fulbright and Fogarty (USA), DAAD (Germany), JSPS (Japan), by the Ministry of Science and New Technologies of Russian Federation, and by the project for supporting leading scientific schools of Russia.

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