Review article

Carnosine and the processes of ageing

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The causes of ageing are usually regarded as multifactorial; thus effective regulation might be achieved by intervention at multiple sites. It has been suggested that the endogenous dipeptide carnosine, also available as a food supplement, possesses anti-ageing activity and may achieve its reported age-alleviating effects via a number of mechanisms. Carnosine’s possible anti-ageing mechanisms are therefore discussed; the evidence suggests that inhibition of the mechanistic target of rapamycin and carbonyl scavenging may be involved.

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Contents

1. Introduction .................................................................................................................................. 00
2. Possible Anti-ageing mechanisms of carnosine ............................................................................ 00
2.1. Rejuvenation of senescent cells and extension of the Hayflick limit: effects at the whole animal level .......................................................... 00
2.2. Telomere shortening ................................................................................................................ 00
2.3. Anti-oxidant activity .................................................................................................................. 00
2.4. Carboxyl scavenging ............................................................................................................... 00
2.5. Regulation of metabolism: suppression of glycolysis and stimulation of mitochondrial activity ........................................................................ 00
2.6. mTOR inhibition ....................................................................................................................... 00
2.7. TGFβ/Smad3 pathway inhibition ............................................................................................. 00
2.8. Activation of proteolysis .......................................................................................................... 00
2.9. Inhibition of translation initiation factor phosphorylation .................................................. 00
2.10. Apoptosis .................................................................................................................................. 00
2.11. Sirtuins ..................................................................................................................................... 00
3. Conclusions .................................................................................................................................... 00

Abbreviations: AD, Alzheimer’s disease; AGES, advanced glycation end products; ALEs, advanced lipoxidation end products; ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; HNE, hydroxynonenal; IGF, insulin growth factor; IGFBP, insulin growth factor binding protein; MG, methylglyoxal; mTOR, mechanistic target of rapamycin; NAD, nicotinamide adenine dinucleotide; NADH, Nicotinamide Adenine Dinucleotide plus Hydrogen; NMR, Nuclear Magnetic Resonance; PD, Parkinson’s disease; RCCs, reactive carbonyl compounds; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; TPI, triose phosphate isomerase; T2DM, type 2 diabetes mellitus.

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1. Introduction

It is generally assumed that ageing is not a single process, but is the result of various persistent deleterious effects which eventually compromise cellular and organism homeostasis. Physiologically, homeostatic dysfunction characterizes cellular and whole animal ageing, ultimately resulting in reproductive failure. When analysed from a biochemical perspective, ageing is usually regarded as multifactorial in both its causality and final outcome: macromolecular dysfunction, in particular deleterious changes in nucleic acids, proteins and lipids appear to accumulate in aged tissues, which may be either causal to, or consequences of, ageing [1]. For example, ageing is associated with increased somatic mutation, progressive homeostatic dysfunction, accompanied by protein modification and lipid peroxidation, which may be attributed to the effects of either exogenous agents or/and interaction with endogenous but potentially deleterious metabolites [1]. Consequently, it can be argued that any effective anti-ageing agent should be pluripotent in order to counteract the various molecular changes which underlie age-related cellular dysfunction.

The endogenous dipeptide carnosine (β-alanyl-l-histidine) is synthesised in muscle and by astrocytes in the brain [2]. In muscle carnosine is predominately located in glycolytic (fast-twitch) muscle than in mitochondria-enriched aerobic muscle; it is degraded back to its constituent amino acids by carnosinases present in a variety of tissues including in plasma and kidney [3].

Several pieces of evidence suggest a high correlation between life expectancy of mammalian species and muscle carnosine concentration. Carnosine content in human muscle (20–30 mM) was twenty times higher than that found in mice, ten times than in rabbit and three times that in cows [4], such differences approximately consistent with their different lifespans. In humans, lower levels of muscle carnosine were found in elderly individuals compared to younger adults [5]. Carnosine is currently available as a food supplement with no known side effects [8]. Supplementation with carnosine has been shown to have anti-inflammatory, antioxidant, antiglycation and chelating roles, and may act as a buffering agent in skeletal muscle and improve calcium handling [9–12]. Although circulating carnosine levels are affected by the presence of plasma carnosinase in humans [7], long-term supplementation of carnosine results in improved health and/or behavioural outcomes (27–32). Therefore, it could be speculated that chronic supplementation maintains a more constant plasma level of carnosine mainly due to saturation of carnosinase.

Carnosine previously described as enigmatic [2], has been considered to possess anti-ageing properties [13,14] possibly because of its putative pluriotropy, although the precise route or routes whereby the dipeptide achieves this remain(s) to be defined. While few studies have investigated the effect of carnosine on ageing, administration of carnosine to senescence-accelerated mice (SAM-1) increased the mean lifespan by 20% [15] and 50% survival rate by 20% [16]. Carnosine also increased the number of spermatogonia and Sertoli cells in mice [17]. The emphasis of this paper will be to consider those processes, together with their macromolecular bases, which seem to accompany or cause ageing, and discuss in particular whether (and how) carnosine can influence them. Possible areas in which carnosine could exert beneficial effects include suppression of telomere shortening, anti-oxidant activity, anti-AGE activity (carbonyl scavenging), suppression of glycolysis, upregulation of mitochondrial activity, activation of proteolysis, inhibition of tumour cell growth, apoptosis, extension of Hayflick limit, rejuvenation of senescent cells, effects on phosphorylation of translation initiation factors, and effects on mTOR and transforming growth factor (TGFβ)/Smad3.

2. Possible Anti-ageing mechanisms of carnosine

2.1. Rejuvenation of senescent cells and extension of the Hayflick limit: effects at the whole animal level

One of the earliest observations suggesting that carnosine could be regarded as an anti-ageing agent was made by McFarland and Holliday [18,19], who in their Sydney laboratory showed that the dipeptide, when added to cultured human fibroblasts not only extended to so-called Hayflick limit (i.e. the maximum number of times the cells could divide), but also provoked the apparent rejuvenation of senescent cells, although the precise mechanisms by which these effects were achieved have not even now been defined. It is also interesting to note that fibroblast growth was slowed when carnosine was present in the growth medium.

Subsequent to this, the Boldyreov laboratory in Moscow showed that carnosine supplementation of the diet of senescence-accelerated mice (SAM) suppressed some of their symptoms of ageing [20]. More recently, animal and human studies have also revealed beneficial effects of carnosine supplementation with respect to a number of age-related conditions e.g. insulin resistance (2 g/daily for 12 weeks, humans) [21], diabetic kidney disease (60 mg/kg for 20 weeks, rodents) [22], atherosclerosis (2 g/l drinking water for 20 weeks, rodents) [23], Alzheimer’s disease (AD) (10 mM, in vitro) [24], Parkinson’s disease (1.5 g for 30 days, humans) (PD) [25], and wound healing (100 mg/kg for 12 days, rodents) [26]. It is also interesting to note that in humans dietary supplementation with carnosine has produced beneficial outcomes in various aspects of human behaviour and wellbeing [27–32]. Although such phenomenology does not provide any insight into the actual mechanistic route by which the dipeptide induces these effects, these observations nevertheless indicate that dietary carnosine can influence the brain despite the presence of serum carnosinase.

As yet there have been no reports on the effects of carnosine on lifespan on commonly-used model organisms i.e. nematode worms and fruit flies. However, it would be [33] anticipated that addition of carnosine to the diet very early in lifespan may result substantial inhibition of growth due to the negative effects of the dipeptide on glycolysis. In order to examine whether carnosine can affect lifespan in worms and flies it is suggested that the dipeptide be added to the diet only when adult size, i.e. when growth has ceased, has been achieved.

2.2. Telomere shortening

Cell ageing and cell senescence are frequently associated with shortening of telomeres due to the progressive loss of telomeres which accompanies each cell division [34]. There has only been one publication in which the effect of carnosine on telomere length

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has been examined Shao et al. using cultured rat fibroblasts found that 20 mM carnosine addition resulted in significant decline in telomere length attrition [35]. The mechanism responsible was not investigated however. Possibilities include quenching of deleterious agents such as reactive oxygen (or nitrogen) species which may damage DNA including the telomere regions, or activation of telomerase, although there have been no reports of whether carnosine has any effects on this enzyme.

2.3 Anti-oxidant activity

For many years, it has been thought that the generation of reactive oxygen species (ROS) and their interaction with cellular macromolecules play causative roles in cellular and organismal ageing. More recently however the so-called free radical theory of ageing has been disputed primarily because the presence of effective anti-oxidants do not seem to affect lifespan; in fact suppression of free-radical generation can be deleterious. There are a number of early papers suggesting that carnosine can exert anti-oxidant activity [9,10]. However, it should be pointed out that better anti-oxidants than carnosine do not provoke cellular rejuvenation and lifespan extension observed in carnosine-treated cultured human fibroblasts. Consequently this leads one to doubt that the dipeptide’s effects on senescent cells is a consequence of its anti-oxidant activity.

2.4 Carbonyl scavenging

Reactive carbonyl compounds (RCCs) are a major potential source of macromolecular modification, especially protein–protein cross-linking, due to their reactivity towards amino, imidazole, guanidino and sulphhydryl groups in mostly polypeptides but also in amino-lipids and polynucleotides. The products of the non-enzymic reaction of RCCs with proteins are called advanced glycation end-products (AGEs), whereas RCC reaction with lipids are called advanced lipid peroxidation end-products (ALEs). Sources of reactive carbonyls are not only those common sugars which possess a reactive carbonyl group, e.g. glucose, fructose, galactose, ribose and deoxyribose, but also many glycolytic intermediates such as the phosphorylated forms of these sugars but also the triose phosphates dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, which are highly reactive in their own right, but can also spontaneously decompose into methylglyoxal (MG) [36]. MG is highly toxic whose reaction with cells, organelles and proteins strongly resembles the changes which accompanies ageing in general. Lipid peroxidation provide another source of RCCs by the generation of acrolein and hydroxynonenal (HNE) [37]. It appears that carnosine is very readily glycated by RCCs, and can thereby behave as a sacrificial amino sink to spare proteins and lipids from RCC-mediated damage [38]. Indeed, variously glycated forms of carnosine have been detected in either in tissues and/or urine (e.g. adducts with acrolein, HNE and MG) [39,40]. Direct reaction of carnosine with protein-bound carbonyl groups has also been detected in a model system [41], however such direct protein “carnosinylation” has not been found to occur in vivo, but a “carnosinylated” amino-lipid has been detected using NMR in human muscle [42].

2.5 Regulation of metabolism: suppression of glycolysis and stimulation of mitochondrial activity

A phenomenon called caloric restriction has been shown to delay ageing and the onset of age-related disease in a variety of organisms (rodents, worms, flies, monkeys). It appears that the underlying cause of this effects is related to a decreased frequency of glycolytic activity as intermittent fasting can achieve the same outcome, as can decreased glycolytic flux resulting from suppressed insulin-like-growth factor (IGF) activity or a non-metabolizable sugar molecule such as deoxy-glucose [43]. All these routes which delay ageing involve decreased glycolytic flux and increased mitochondrial activity, possible resulting in lowered potential for Mg generation and enhanced (via mitochondrial action) regeneration of NAD⁺ from NADH thereby ensuring effective metabolism of glyceraldehyde-3-phosphate. It appears that carnosine can exert inhibitory activity towards glycolysis. Studies using tumour cells [44,45] and yeast [46] have shown that carnosine has inhibitory effects on glycolysis, and stimulatory effects on mitochondrial activity have also been shown in yeast [46], cultured neurons [47] and in mouse brains [33]. It is also significant to note that carnosine can selectively inhibit proliferation of transformed cells [33,44,48] which generally rely on glycolysis as major ATP source. However the mechanisms responsible have not been detailed.

One possible explanation of the beneficial effects of glycolytic inhibition may derive from observations made decades by Gracy et al. [49]. They observed that the glycolytic enzyme triose-phosphate isomerase can become metabolically compromised as a consequence of its catalytic activity. It was shown that repeated catalysis promotes the spontaneous deamination of certain asparagine residues within the protein resulting in loss of dimeric status and catalytic activity due to the proteolysis of the monomers. Such a decline in triose phosphate isomerase (TPI) activity then allows the accumulation of dihydroxyacetone-phosphate (strong glycating agent in its own right) but which can spontaneously decompose into MG [50]. It is also pertinent to note that the presence of beta-amyloid (which accumulates in the AD brain) also induces TPI inactivation and thus increases MG generation potential and macromolecular glycation [51].

It has been shown that mitochondria in non-exercising muscle generate more ROS than mitochondria in exercising muscle [52], thus cessation of glycolysis may enhance mitochondrial ATP synthesis activity and thereby promote a decrease in ROS generation (Table 1).

2.6 mTOR inhibition

The mammalian target of rapamycin (mTOR) is a major control element of cellular metabolism [53]. Essentially, stimulation of mTOR activates glycolysis to generate not only ATP but also precursors of the many building blocks for cell reproduction. In contrast, mTOR inhibition, suppresses glycolysis and upregulates mitochondrial activity. Rapamycin, an immune-suppressor, has been shown to exert substantial anti-ageing effects in a number of organisms, from yeast to rodents. It has previously been suggested [54] that

<table>
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<th>Table 1 Possible anti-ageing effects of carnosine.</th>
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<tr>
<td>Observed property of carnosine</td>
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<tr>
<td>Helps maintain telomere length</td>
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<tr>
<td>Scavenges ROS and RNS</td>
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<tr>
<td>Scavenges reactive carbonyl species</td>
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<tr>
<td>Inhibition of initiation factor phosphorylation</td>
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<td>Inhibition of glycolysis</td>
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<tr>
<td>Stimulation of mitochondrial activity</td>
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<td>Activation of proteolysis</td>
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<td>Rejuvenates senescent cells</td>
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carnosine could be a rapamycin mimetic (rapalogue) and there is some recent evidence which supports this speculation [55].

2.7. TGFβ/Smad3 pathway inhibition

TGF-β/Smad3 has an important role in cellular senescence partly through ROS production [56]. Ageing and ageing related chronic diseases are associated with an increase of TGF-β/Smad3 signalling and expression [57,58]. TGF-β, a growth-inhibitory cytokine, also inhibits telomerase in cell cultures [59]. There is evidence that 20 mmol/l of carnosine for 14 days carnosine has a potential to suppress TGF-β production (and signalling) most likely via inhibition of Smad2/3 pathway [60]. It is not known if carnosine has inhibitory effects on the TGFβ/Smad3 pathway in all tissues or if it can affect ageing-related processes via this pathways [61].

Some evidence suggests that decreased IGF-1 and insulin signalling may favourably impact longevity [62] and that raised levels of IGF-1 are associated with a longer perceived age, independent of confounding factors, which was evidenced mainly by skin wrinkling [63]. Administration of 5 g/l carnosine in drinking water for 4 weeks has been shown to increase circulating IGF-1 and decrease IGFBP1 in db/db mice [64].

2.8. Activation of proteolysis

Many studies have shown that ageing is accompanied by an intracellular accumulation of altered protein forms, due to either increased generation of altered proteins and/or a decreased ability for their elimination via either autophagy or/and the ubiquitin/proteasome system [65]. There have been few studies of the effect of carnosine on intracellular proteolysis (see Fig. 1). Some years ago it was shown that, using a pulse-label technique, growth of cultured human fibroblasts for many cell generations had no effect on the degradation of rapidly turning-over proteins (labelled for between 30 min to 2 h) whereas proteolysis of proteins labelled for 3 days was enhanced in the carnosine-cultured cells. Carnosine did not however enhance proteolysis when added to labelled cells not previously treated with the dipeptide [66]. These results suggest that growth with carnosine upregulates proteolysis of slowly turning-over proteins by either enhancing their susceptibility to degradation or upregulating the proteolytic apparatus responsible, most probably the autophagic/lysosomal system. It is possible that, as mTOR also regulates autophagy, these findings may derive from effects of the dipeptide on mTOR regulatory complex. These observations also suggest that carnosine may not affect the ubiquitin/proteasomal apparatus. It should be pointed out that these observation have not been independently verified or refuted.

2.9. Inhibition of translation initiation factor phosphorylation

The rate at which proteins are synthesised can affect the onset of ageing, as methionine restriction [67] and decreased availability of translation initiation factor [68] have been shown to delay ageing onset and extend lifespan. The resulting lowered protein synthesis rate will also decrease error-protein generation; protein biosynthesis is not perfect (the error frequency has been estimated at 3 codons in every 10,000 translated [69]), thus any method which decreases protein synthesis may decrease the generation of error-protein, thereby decreasing the error-protein load (of any origin) which the homeostatic proteolytic apparatus is required to deal with [70]. Decreased initiation frequency may also decrease the demand for synthesis of amino acid from glycolytic pathway precursors which may in turn result in decreased MG formation due to the lowered glycolytic flux [71].

Carnosine has been shown to inhibit the phosphorylation of the translation initiation factor elf-4E [72] which results in slowed translation, which would in turn decrease the error-protein generation too, although how carnosine inhibits phosphorylation was not determined. As mTOR can also control protein synthesis, it is possible that carnosine’s effects on elf-4E phosphorylation might be mediated via mTOR.
2.10. Apoptosis

There are contradictory findings with regard to carnosine's effects on apoptosis. Depending on the system studied and the dipeptide concentration employed, evidence for carnosine provoking [73] or preventing [74] apoptosis can be found. Interestingly, recent studies have indicated that apoptotic elimination of "sick" or pre-apoptotic cells can extend the lifespan of any accompanying undamaged cells [75], which could possibly indicate that carnosine's ability to enhance fibroblast lifespan and provoke apparent cellular rejuvenation of supposedly senescent cells could result from the selective elimination via apoptosis of otherwise terminal cells. However, this speculation remains to be evaluated.

2.11. Sirtuins

The so-called silent information regulators or sirtuins have been shown to regulate the onset of age-related dysfunction by facilitating NAD-dependent histone deacetylation [76]. As yet, however, there have been no reports of any effect of carnosine on sirtuin activity, although it has been speculated that carnosine could participate in the scavenging of acetyl-ADP-ribose (a strong glycating agent) which is a product sirtuin-mediated protein deacetylation.

3. Conclusions

It is clear from the above summary that the mechanisms or mechanisms responsible for carnosine apparent ability to affect cellular lifespan and onset of age-related change remain enigmatic. As noted in the introduction, it is likely that a number of processes which the dipeptide can influence are likely to be involved. Prime candidates are mTOR inhibition (i.e. carnosine behaving as a rapamycin mimetic), inhibition of TGFβ/Smad3 pathway and suppressing the deleterious effects of reactive carbonyl compounds (RCCs) towards susceptible macromolecules, especially proteins and amino-lipids, by forming adducts with RCCs which are then selectively removed from the cell to eventually be excreted in urine. Therefore, investigating the effect of carnosine's clinical outcome in rigorous clinical trials and assessing the effects on key molecular mechanisms of ageing and chronic diseases in humans is urgently needed to confirm or refute the suggested evidence.

Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

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Contributors

ARH wrote the first draft. EB and Bdc contributed to writing of the manuscript. All authors reviewed and approved the manuscript.

Provenance and peer review

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