BIOPHYSICS AND BIOCHEMISTRY

Effect of Carnosine on *Drosophila Melanogaster* Lifespan

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A positive dose-dependent effect of carnosine (β-alanyl-L-histidine) on the lifespan of male *Drosophila melanogaster* flies was shown. The mean lifespan of male flies receiving 200 mg/liter carnosine approached that of females. At the same time carnosine had no effect on the lifespan of female flies. This positive effect of carnosine probably reflects its protective action against age-related accumulation of free radicals and did not depend on carnosine metabolism in the body. Addition of 200 mg/liter histidine and β-alanine (separately or in combination) had no effect on the mean lifespan of flies.

Key Words: *Drosophila melanogaster; lifespan; carnosine; histidine; β-alanine; aging; oxidative stress; antioxidants*

In excitable tissues, carnosine acts as a mobile buffer of protons, heavy metal ions, and free radicals, thus playing a role of nonspecific protector of biomacromolecules under unfavorable conditions [1,6,7]. Recent studies showed that carnosine improved blood supply to the brain and decreased rat mortality caused by experimental ischemia [8,11]. This agent also increased lifespan of SAMP1 mice characterized by accelerated aging caused by accumulation of free radicals in tissues [12]. Experiments on vertebrates showed that improvement of animal survival is associated with the positive effect of carnosine on metabolism not only in the brain, but also in other tissues. Carnosine produced a protective effect in the brain and muscles (where it is accumulated due to activity of specific enzyme carnosine synthase) and in the liver and blood (where its accumulation is normally inhibited by carnosinases) [4,5].

The aim of the present study was to examine the effect of carnosine and its precursors, β-alanine and L-histidine, on the lifespan of *D. melanogaster*, which possessed no internal carnosine.

MATERIALS AND METHODS

The study was carried out on male and female D-32 *D. melanogaster* flies grown on a standard raisin-yeast medium. Virgin male and female flies were selected under ether anesthesia within the first 3 days of flight. During the experiment (40-45 days) the flies were kept in a thermostat at 25°C in tubes (diameter 1.5 cm, length 10 cm). Every 3-6 days the flies were transferred to fresh nutrient medium, and their viability was evaluated by the ratio of the number of flies to their initial number. The mean lifespan was calculated by the formula:

$$X_m = \sum x_i / n,$$

where x_i is variable (lifespan of i-fly) and n is the total number of variables (number of flies in the sample) [3].

Carnosine, β-alanine, and L-histidine were added to freshly prepared and cooled to 40°C nutrient medium. Control flies were kept on standard nutrient medium. At the beginning of the experiment each group consisted of 80 flies.

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On day 40 of growth on a medium containing 200 mg/liter carnosine the flies were frozen and the content of carnosine, its precursors, and some related compounds was measured in a methanol extract (1:4 v/v) by isocratic reverse phase HPLC with UV detection (λ= 210 nm) [10]. The samples were fractionated on an Ultrasphere-I.P. ODS column (d_p=5 μ, 4.6×250 mm; Beckman) at 1.0 ml/min elution rate; the mobile phase contained 0.1 M NaH_2PO_4 (pH 2.5), 2.5% acetonitrile, and ion-paired reagent (0.1% hexasulfuric acid). Under these conditions the retention times for histidine, carnosine, anserine, N-acetylcarnosine, and carcinine (determined for standard solutions of carnosine and related compound) were 4.66, 7.48, 7.68, 8.19, 8.60, and 15.72 min, respectively.

The results were processed using regression analysis of survival curves. The significance of differences between regression curves was evaluated by Fisher's test (for dispersions) and Student's *t* test (for comparison of regression quotients) at $p \leq 0.05$ [10].

RESULTS

In our experiments, the lifespans for male and female *D. melanogaster* flies were different (Fig. 1, *a*), which was not surprising because females demonstrated higher conservatism and resistance to various environmental factors [2,9]. Carnosine added to the nutrient medium in a dose of 200 mg/liter (the concentration was determined empirically considering the body weight) had no effect on lifespan of female flies. β-Alanine and L-histidine (alone or in combination) in the same concentrations were ineffective.

In male flies carnosine produced a pronounced positive effect on the lifespan. Increasing carnosine concentration from 0.2 to 200 mg/liter increased the number of long-living flies (Fig. 1, *b*). At the early terms of the experiment (<30 days) low and high concentrations of carnosine showed the same efficacy, while on days 35-45 only high concentration of carnosine provided significant increase in the number of flies. Carnosine precursors β-alanine and L-histidine in the highest concentration (200 mg/liter) had no effect on fly survival. The flies grown on carnosine-containing medium were more mobile and healthy than control flies. As the concentration of carnosine in the nutrient medium increased, the male lifespan approached that of females (Table 1).

TABLE 1. Mean Lifespan (MLS, Days) of *D. melanogaster* Males Grown in the presence of Different Concentrations of Carnosine (*M*±*m*)

Experimental series		MLS
Control	males	29.2±1.3
	females	42.4±1.4*
Carnosine, mg/liter	20	36.3±1.4*
	0.2	36.9±1.2*
	0.02	34.9±1.8*

Note. *p<0.05 compared to the control (males).

In control flies, no anserine and carcinine were detected. Trace amounts of carnosine and N-acetylcarnosine approximated limits of HPLC accuracy and did not indicate the presence of these compounds in drosophila tissues. However, the levels of histidine and homocarnosine reached significant values (Table 2).

In flies grown on carnosine-containing medium, no carcinine and anserine were detected, but the content of histidine increased more than 10-fold and a

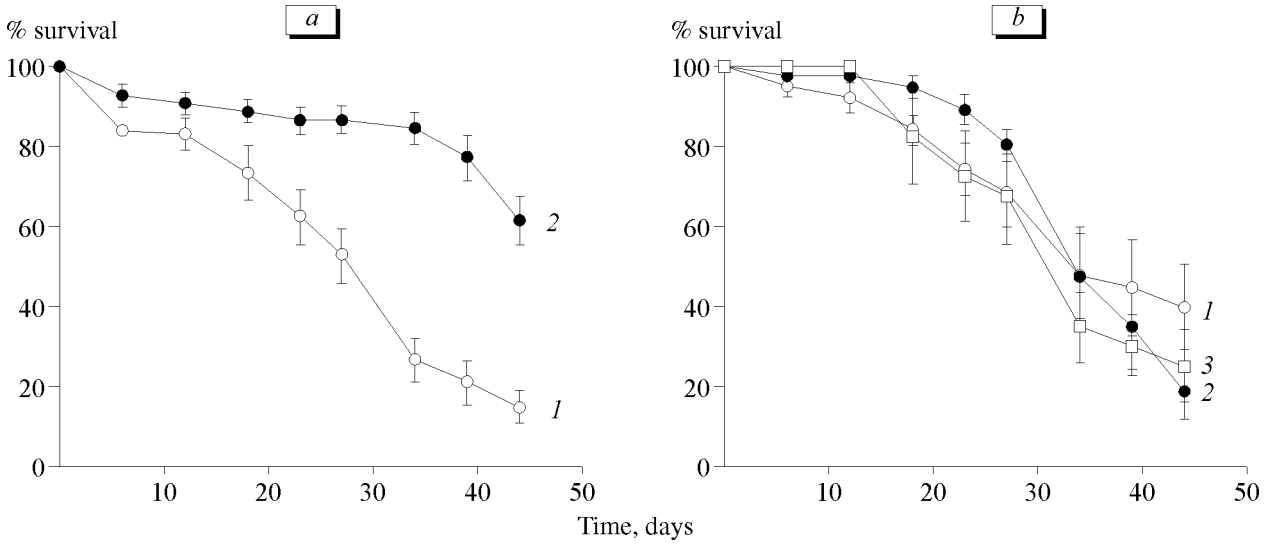

Fig. 1. Lifespan of *D. melanogaster* grown on standard raisin-yeast medium in the absence (*a*) and presence of carnosine in different doses (*b*). *a*: *1*) males; *2*) females; *b*: males grown in the presence of 200 (*1*), 2 (*2*), and 0.2 mg/liter (*3*) carnosine.

TABLE 2. Content (ng/mg Tissue) of Carnosine and Its Metabolites in *D. melanogaster* Grown on a Medium with Carnosine (200 mg/liter) for 40 Days

Compound	Control	Carnosine
Histidine	5.67±0.52	64.73±2.66
Carnosine	0.77±0.08	3.22±0.36
N-acetylcarnosine	0.35±0.04	0.45±0.05
Homocarnosine	2.76±0.31	11.50±1.15

distinct carnosine peak was revealed (Table 2). N-acethylcarnosine was present in trace amounts, while the homocarnosine peak increased 4-fold. These data show that carnosine is hydrolyzed in drosophila tissues contributing to the increase in histidine content. Increased content of homocarnosine suggests that histidine released after carnosine hydrolysis is partly utilized and interacts with GABA.

This assumption was confirmed by the data on the content of carnosine, histidine, and homocarnosine in flies grown in the presence of various carnosine concentration (0.2, 2, and 200 mg/liter). Addition of carnosine to the nutrient medium increased its content in flies, though this increase was not dose-dependent (Fig. 2). At the same time, dose-dependent increase in histidine and homocarnosine content in flies confirmed active metabolism of carnosine in drosophila tissues. Here we did not elucidate the question whether carnosine metabolism occurred in the digestive tract or in tissues of *D. melanogaster*. However, accumulation of histidine and homocarnosine in flies grown on carnosine-containing medium suggests that it can penetrate fly tissues and can be metabolized. In our opinion carnosine metabolism was a secondary factor, while prolonged lifespan is a direct effect of this compound. This conclusion was supported by the fact that dose-effect curves for accumulation of carnosine metabolites and prolongation of drosophila lifespan did not coincide (Fig. 1, *b*; Fig. 2). It should be also noted that even minimum carnosine concentration (0.2 mg/liter) had a positive effect on fly lifespan and this effect increased in a dose-dependent manner (Table 2).

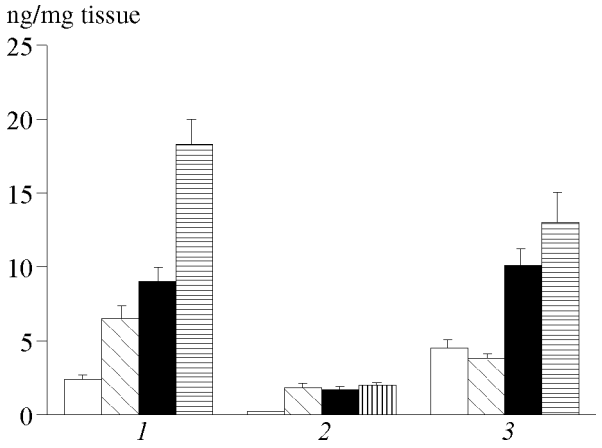

Fig. 2. Content of histidine (*1*), carnosine (*2*), and homocarnosine (*3*) in *D. melanogaster* grown on a medium containing 0.2 (oblique hatching), 2 (dark bars), and 200 mg/liter (vertical hatching) carnosine. Open bars: control.

The positive effect of carnosine can be explained by protection of macromolecules against free radicals, which are accumulated with age and are a factor causing death. This assumption is confirmed by the fact that carnosine produce a selective effect on the lifespan of male flies possessing weaker defense mechanisms compared to females [2,9]. It can be assumed that carnosine improves tissue capacity to prevent the accumulation of signs of aging in *D. melanogaster* males and increases their lifespan.

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