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Cerebrolysin Accelerates Metamorphosis and Attenuates Aging-Accelerating Effect of High Temperature in *Drosophila Melanogaster*

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Abstract

Cerebrolysin® (CBL) is a neuroprotective drug used for the treatment of neurodegenerative diseases. CBL's mechanisms of action remain unclear. Involvement of tryptophan (TRP)–kynurenine (KYN) pathway in neuroprotective effect of CBL might be suggested considering that modulation of KYN pathway of TRP metabolism by CBL, and protection against eclosion defect and prolongation of life span of *Drosophila melanogaster* with pharmacologically or genetically-induced down-regulation of TRP conversion into KYN. To investigate possible involvement of TRP–KYN pathway in mechanisms of neuroprotective effect of CBL, we evaluated CBL effects on metamorphosis and life span of *Drosophila melanogaster* maintained at 23 °C and 28 °C ambient temperature. CBL accelerated metamorphosis, exerted strong tendency ($p = 0.04$) to prolong life span in female but not in male flies, and attenuated aging-accelerating effect of high (28 °C) ambient temperature in both female and male flies. Further research of CBL effects on metamorphosis and resistance to aging-accelerating effect of high temperature might offer new insights in mechanisms of its neuroprotective action and expand its clinical applications.

Keywords

Cerebrolysin; Neuroprotection; Kynurenine; Tryptophan; *Drosophila*; Metamorphosis; Eclosion; High Temperature; Life Span; Aging

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1. INTRODUCTION

Cerebrolysin® (CBL), a mixture of neuropeptides that mimics the action of neurotrophic factors, is used as neuroprotective drug for treatment of neurodegenerative diseases.¹ CBL's mechanisms of action remain unclear. Considering that CBL modulates kynurenine (KYN) pathway of tryptophan (TRP) metabolism,² and that TRP–KYN metabolism is involved in mechanisms of both development³ and aging,^{4–8} one might suggest participation of TRP–KYN pathway in neuroprotective effect of CBL. KYN pathway is a major non-protein route of TRP metabolism. In insects activity of TRP–KYN pathway is peaking up during pupation and just before eclosion resulting in production of brown eye pigment (ommochrome).⁹ Genetic inhibition of TRP conversion into KYN protected against eclosion defect in the *Drosophila* model of Huntington disease.³ We reported that genetic or pharmacological inhibition of KYN formation from TRP prolonged life span of *Drosophila melanogaster* maintained at 23 °C.^{10–13} Life span of flies is temperature dependent, and flies are living faster at the higher temperature.^{14, 15} Inhibition of TRP–KYN metabolism attenuated aging-accelerating effect of high (28 °C) ambient temperature.¹⁶ It was suggested that metamorphosis and adult life span may share some common mechanisms.¹⁷ Current study aimed to evaluate CBL effect on (1) metamorphosis and (2) life span of flies maintained at 23 °C and 28 °C.

2. METHODS

Wild-type stock Oregon of *Drosophila melanogaster* from the collection of V. N. Karazin Kharkiv National University was used in the experiments. Flies were maintained at 23 °C (or 28 °C) in a 12:12 light: dark period on a standard *Drosophila* medium (SM) consisting of sugar, yeast, agar and semolina.

Experiment 1. Effect of CBL on metamorphosis. Eggs were obtained from synchronized egg laying from 60 fertilized flies. CBL (0.02 ml per 4 ml of SM) was added twice during larvae stage. Data from three replicated experiments was used for the statistical analyses. To calculate an average speed of development from egg to imago the time of appearance of each imago was recorded (Experiment 1a). To evaluate CBL effect on eclosion time from pupae to imago, number of remaining pupae was recorded every three hrs after appearance of first imago until all pupae matured to imago (Experiment 1b).

Effect of CBL on viability was estimated by number of filial generation pupae and their lethality as described elsewhere.^{13, 16}

Experiment 2. Effect of CBL on life span of flies. CBL was introduced to larvae (see above). One day old adult flies were collected and maintained either at 23 °C (experiment 2a) or at 28 °C (experiment 2b). Flies were regularly transferred to fresh medium every 3 days. The number of dead flies was recorded every 3 days at the time of flies' transfer to fresh medium.

Statistical analyses. Non-parametric tests were used as indicated in Results.

3. RESULTS

Experiment 1. CBL effect on development from egg to imago.

Average time of development from egg to imago was similar in male and female flies. CBL accelerated development time in both genders (Table I).

CBL did not affect the number of filial generation pupae and their lethality (data not shown).

Experiment 1b. CBL effect on eclosion time from pupae to imago. CBL accelerated the eclosion from pupae to both female and male imago ($p < 0.003$, Mann-Whitney, two-tailed test) (Figs. 1 and 2).

There were no difference in CBL effect on eclosion of female and male imago.

Experiment 2a. CBL effect on life span of flies maintained at 23 °C.

CBL exerted strong tendency to prolong life span ($p = 0.04$) in female but not in male flies maintained at 23 °C (Table II).

Experiment 2b. CBL effect on life span of flies maintained at 28 °C.

Maintenance at 28 °C resulted in shorter life spans of flies (both genders) than at 23 °C.

CBL increased life span of female and male flies maintained at 28 °C in comparison with flies maintained at 28 °C on SM without CBL (Table II).

4. DISCUSSION

Cerebrolysin is a neuropeptide with anti-inflammatory action.^{18–20} Clinical and experimental studies suggested its effectiveness in aging-associated conditions: memory impairment²¹ and diabetes.²² However, to the best of our knowledge, cerebrolysin effect on longevity has not been evaluated.

Our study revealed two new effects of CBL: acceleration of metamorphosis and protection against aging-accelerating effect of high environmental temperature.

The rate of development in insects is under hormonal control. However, it may be suggested, that differences between individuals or stocks in speed of development are connected not only with different level of hormones, but also with rate of genes activation or inactivation as a response on hormonal stimulus that depends on many factors such as rate of hormones transport into a cell, rate of nucleus-cytoplasmic transport etc. So, if a factor alters developmental time, it may alter hormonal status or may influence intensity of metabolic processes. Further studies might elucidate what nature of cerebrolysin-mediated acceleration of development.

Is acceleration of eclosion good or bad for an organism? Fast developing individuals get advantage for earlier reproduction in distributing populations. Larval density and competition are also must be taken into account. When larvae are crowded, fast developing

individuals and, consequently, with small adult body size should be favored. When breeding sites are scarce and larval density is low (competition is weak), individuals with longer developmental times and larger body sizes are expected to be favored.²³ In any case, possibility of modulating developmental rate of insects by cerebrolysin shown in this study may be important in practical aspect.

The direction of correlation between eclosion and life span is not clear. Some studies observed that early emergers were often long-lived¹⁷ while other found no unequivocal relationship between pre-adult developmental time and adult longevity.²⁴

The effect of an external factor on these parameters may be different: for example, melatonin that prolongs life span, also increased the eclosion rate.²⁵ In this study cerebrolysin accelerated development rate, but had no influence on life span at normal, 23 °C temperature at which flies were kept.

We found that cerebrolysin attenuated aging-accelerating effect of high temperature in *Drosophila melanogaster* model. These results are in line with the data of Sharma et al. which showed that in rats pre-treatment with cerebrolysin before heat stress markedly attenuates hyperthermia induced stress reaction and so protects against heat shock-induced neurotoxicity.²⁶

As one of the possible mechanisms of observed protective effect of cerebrolysin against aging-accelerating action of high temperature we consider potential cerebrolysin-induced changes in TRP–KYN metabolism, taking into account that CBL was shown to modulates this pathway of tryptophan metabolism.² In our earlier experiments genetic and pharmacological inhibition of TRP–KYN pathway extended life span of *Drosophila* and enhances stress-resistance.^{10–13, 16}

Our data suggest the protective effect of cerebrolysin against life time exposure to high environmental temperature. Further studies might elucidate the role of TRP–KYN pathway in neuroprotective effects of cerebrolysin.

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Fig. 1.
CBL effect on eclosion from pupae to female imago.

Fig. 2.
CBL effect on eclosion from pupae to male imago.

Table I

CBL effect on development time from egg to imago (in hours).

	Standard medium (SM)	SM+CBL
Females	246.85±19.50 [*] <i>N</i> = 155	215.73±10.39 <i>N</i> = 181
Males	247.44±20.01 [*] <i>N</i> = 171	220.92±14.26 <i>N</i> = 173

(^{*}) *Note:* $P < 0.0001$ in comparison with corresponding CBL-treated flies (mean±standard deviation); (Mann-Whitney two-tailed test).

Table II

CBL effect on life span (in days) of flies maintained at 23 °C and 28 °C.

	Female	Male
23 °C Standard	32.50±1.86	32.39±1.29
medium (SM)	(n = 42)	(n = 66)
23 °C CBL	37.04±1.07	33.78±1.78
	(n = 105)*	(n = 46)
28 °C Standard	29.24±1.06	28.89±0.65
medium (SM)	(n = 50)**	(n = 65)***
28 °C CBL	31.57±0.99	32.23±1.06
	(n = 53)#	(n = 39)#

Notes: Mean±SE;

(*) $p = 0.04$ in comparison with 23 °C females flies (SM) and males (CBL) (Mann-Whitney, one-tailed test);(**) $p = 0.03$ in comparison with 23 °C females flies (SM) (Mann-Whitney, one-tailed test);(***) $p < 0.0001$ in comparison with 23 °C males flies (SM) (Mann-Whitney, two-tailed test);(#) $p < 0.0001$ in comparison with 28 °C females and males flies (SM) (Mann-Whitney, two-tailed test).

Table III

CBL effect on life span (in days) of flies maintained at 28 °C.

	SM	SM+CBL
Females	10.78+0.9 [#] <i>n</i> = 36	13.5+1.2 ^{**} <i>n</i> = 82
Males	8.58+0.6 <i>n</i> = 56	10.22+2.3 [*] <i>n</i> = 92

([#]) Notes: $p < 0.003$ in comparison with males flies maintained on SM without CBL;

(^{*}) $p < 0.01$;

(^{**}) $p < 0.03$ in comparison with flies of corresponding gender maintained on SM without CBL (unpaired *t*-test with Welch correction).