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RESEARCH ARTICLE

Life span extension in *Drosophila melanogaster* induced by morphine

Tatyana A. Dubiley · Yury E. Rushkevich · Natalya M. Koshel · Vladimir P. Voitenko · Alexander M. Vaiserman

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Abstract The influence of morphine on the life span of *Drosophila melanogaster* fruit flies has been investigated. Morphine hydrochloride (MH) at concentrations of 0.01, 0.05 and 0.25 mg/ml was added to a medium starting from day 5 or 54 of imaginal life. Supplementation with MH starting from day 5 of imaginal life has resulted in significant increases in the mean life span of males at all concentrations studied. In females, a significant increase in life span compared with control was obtained only for those treated with 0.25 mg/ml MH. In flies with MH feeding from day 54, residual life span was significantly increased in both males and females after treatment with 0.05 mg/ml MH. The present data, together with those of our earlier study in mice (Dubiley et al. Probl Aging Longvity 9:331–332, 2000) suggest that morphine supplementation can result in life extension in both vertebrate and invertebrate animal species.

T. A. Dubiley · Y. E. Rushkevich
Laboratory of Endocrinology, Institute of Gerontology, Kiev, Ukraine

N. M. Koshel · V. P. Voitenko
Laboratory of Mathematical Modelling of Aging Processes, Institute of Gerontology, Kiev, Ukraine

A. M. Vaiserman (✉)
Laboratory of Epigenetics, Institute of Gerontology, Vyshgorodskaya st. 67, Kiev 04114, Ukraine
e-mail: vaiserman@geront.kiev.ua

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Abbreviation
MH Morphine hydrochloride

Introduction

Morphine is a naturally occurring substance in the opium poppy, *Papaver somniferum*. Today it remains the most widely used pain killer in contemporary medicine, despite unwanted side effects such as respiratory depression, tolerance, and physical dependence. It is now well accepted that endogenous morphine is present in animals, both in invertebrates and vertebrates. It is a key signaling molecule that plays an important role in down regulation of physiological responses, such as those in the immune system, including immune elements in the central nervous system (Stefano et al. 2000; Glattard et al. 2010).

The morphine stimulation of the μ-opioid receptors causes antinociceptive, hedonic, emotional, as well as autonomic, neuroendocrine and immune responses. Morphine was found to reduce both metabolic rate and body temperature (Endoh et al. 1999; Baker and Meert 2002), induce heat shock proteins expression (Ammon-Treiber et al. 2004),

enhance cell resistance to hypoxia, hyperoxia, nitrosative and metabolic stresses (Kim et al. 2001; Zhao et al. 2006; Cui et al. 2008; Husain et al. 2009; Li et al. 2009), as well as to proinflammatory and proapoptotic stimuli (Lee et al. 2004; Lin et al. 2007; Gwak et al. 2010). Morphine can stimulate the growth and regeneration of the nervous fibers (Brailoiu et al. 2004; Zeng et al. 2007), modulate synaptic plasticity (Morón et al. 2007), induce brain-derived neurotrophic factor expression (Takayama and Ueda 2005), reduce behavioral deficits associated with traumatic brain injury (Lyeth et al. 1993), and protect against development of posttraumatic stress disorder (Bryant et al. 2009). Thus, this alkaloid has a number of effects usually attributed to anti-aging ('geroprotective') activity. Besides, it can protect nervous tissue against a variety of adverse conditions. So, morphine may be a strong candidate to provide protection of brain against risk factors associated with aging and retard the development of age-related pathology.

In our previous work, we studied the effect of morphine on life span of old CBA female mice (Dubiley et al. 2000). The animals received morphine hydrochloride (MH) in their drinking water at 10 mg/kg body weight once a week starting at 850 days of age until death. The log-rank test showed that the survival of MH-treated mice was significantly better than the control ones. There was a trend towards an increase in mean life span and a significant increase in maximal life span after MH administration (1111 ± 14 days in MH-treated vs. 1036 ± 14 days in control, $P = 0.02$). The aim of the study was to determine: (1) whether morphine can extend life span in other species of laboratory animals; (2) whether this effect is sex-specific; (3) its dependence on the timing of intervention.

There is a similarity of the opioid systems in vertebrates and insects (Scharrer et al. 1988). Opioid receptors have been characterized in *Drosophila* neural tissue (Santoro et al. 1990). In a number of studies, a similarity of biological effects of morphine in vertebrates and invertebrates has been reported (Zabala et al. 1984; Kavaliers et al. 1986, 1987; Jaffe and Blanco 1994; Dyakonova et al. 2002). Therefore, insects may be useful models for testing the impact of morphine on aging and longevity. In the present study we evaluated the effects of morphine on life span in *Drosophila melanogaster*.

Materials and methods

Stock and cultivation methods

In the experiment, the wild type *Oregon-R* of *D. melanogaster* stock was used. The flies were reared and maintained in glass vials (7.0 cm height × 2.5 cm diameter) containing 3 cm^3 of the ordinary cornmeal–sugar–yeast–agar medium at 25°C, 75% relative humidity. The room was lit for 12 h a day, from 8 a.m. to 8 p.m. Egg collections were performed on sufficient quantity of animals (~50 10–12-day-old flies per vial) to obtain a number of eggs adequate for the entire study from a single 1-h oviposition. To avoid unfavorable effects of larval crowding, i.e. increased duration of development and decreased imaginal body size (Economos and Lints 1984), larval density in each vial was standardized to 50–60 larvae per vial.

Drug administration

Stock solution of MH (Macfarlan Smith Ltd., UK) was diluted in distilled water at 10 mg/ml. Fruit flies were exposed to 0.01, 0.05, and 0.25 mg/ml of MH once a week starting from day 5 or 54 of imaginal life. Day 54 was chosen because at this age a sharp increase in mortality rate of the flies took place. It was considered to be a marker of the onset of the aging process. The doses used reflect the final concentration of MH in the culture medium and were selected in our preliminary experiments (unpublished data).

Longevity test

The flies for longevity test were collected within 24 h after emergence, etherized, separated according to sex, and placed in groups of 25 of the same sex to vials, containing 3 ml culture medium. Control and experimental vials were placed in the incubator, at temperature 25°C. The flies were transferred to new vials containing medium three times a week. Dead flies were removed, and the number of dead flies was recorded at the same terms until the last death. Five replicates (123–125 flies in total) were used for life span testing in each group. One male and four female were lost during the transfers. In all control and experimental groups, mean residual life span in flies surviving beyond 54 days of age was calculated (five

replicates, 94–98 flies in total, for each male group, and five replicates, 104–117 flies in total, for each female group).

Statistics

The effect of MH on life span was assessed by the analysis of variance (ANOVA). The post-hoc Duncan's multiple range test was used to determine significant differences between groups.

Results and discussion

Treatment with MH starting from day 5 of imaginal life caused a beneficial effect on the flies' longevity. Two-way ANOVA showed no significant effect of sex [$F(1,987) = 1.20$; $P = 0.27$], but a significant effect of MH treatment [$F(3,987) = 6.92$; $P < 0.001$] on the life span of the flies. There was also a significant interaction between sex and MH treatment: $F(3,987) = 5.00$; $P < 0.01$. Supplementation with MH starting from day 5 resulted in significant increase of the male mean life span at all concentrations studied (Table 1, Fig. 1). In females, significant increase of life span compared to control was obtained only for those treated with 0.25 mg/ml of MH.

Supplementation with MH since 54 days of age also resulted in significant effect on the flies' life span. Two-way ANOVA, showed no effect of sex [$F(1,826) = 1.86$; $P = 0.17$], however, there was a significant effect of MH treatment [$F(3,826) = 7.11$; $P < 0.001$] on the life span of flies. No interaction between sex and MH treatment was detected: $F(3,826) = 2.41$; $P = 0.07$. Residual life span was significantly increased in both male and female flies treated with MH at 0.05 mg/ml (Table 2, Fig. 2). No significant differences were observed between control flies compared to MH treated flies at 0.01 and 0.25 mg/ml.

One possible explanation of the life-extending effect of MH administration can be the decrease of metabolic rate of the flies. Gene expression profiling studies performed in mice reveal that a single morphine dose predominantly reduces expression of genes involved in metabolic function (Loguinov et al. 2001). Depressant effect of acute morphine administration on oxygen consumption has also been reported (Endoh et al. 1999). In our previous studies, old mice who were receiving morphine with drinking water once per week during 9 months exhibited 15% decrease in basal rate of oxygen consumption and higher life span in comparison with age-matched controls (unpublished data).

Beneficial effect of MH on the flies' longevity can also be attributed to the decrease in reactive oxygen species production and improved protection from oxidant injury. Morphine was found to inhibit NADPH oxidase—one of the main intracellular sources of superoxide (Qian et al. 2007). This alkaloid has also been shown to produce a significant increase in the level of free ubiquitin, which suggests that it reduces the amount of oxidized proteins targeted for degradation (Rambhia et al. 2005). In numerous cell culture studies, morphine was demonstrated to have a protective effect against oxidative and nitrosative stresses. For example, morphine prevents glutamate-induced death of primary rat neonatal astrocytes through modulation of intracellular redox (Lee et al. 2004), inhibits doxorubicin-induced reactive oxygen species generation and nuclear factor kappaB transcriptional

Table 1 Mean life span (in days) in control flies and flies treated with MH starting from day 5 of imaginal life

Concentration of MH (mg/ml)	Males		Females	
	n	Mean life span ± SEM	*n*	Mean life span ± SEM
Control	125	63.00 ± 1.73	125	67.69 ± 1.35
0.01	125	72.82 ± 1.61** (+15.6)	123	67.57 ± 1.31 (-0.02)
0.05	124	69.96 ± 1.33* (+11.0)	124	70.44 ± 1.48 (+4.1)
0.25	125	69.33 ± 1.70* (+10.0)	124	74.02 ± 1.30* (+9.4)

* $P < 0.01$, ** $P < 0.001$

n Total number of flies in each group

In parentheses—percentage change compared to control

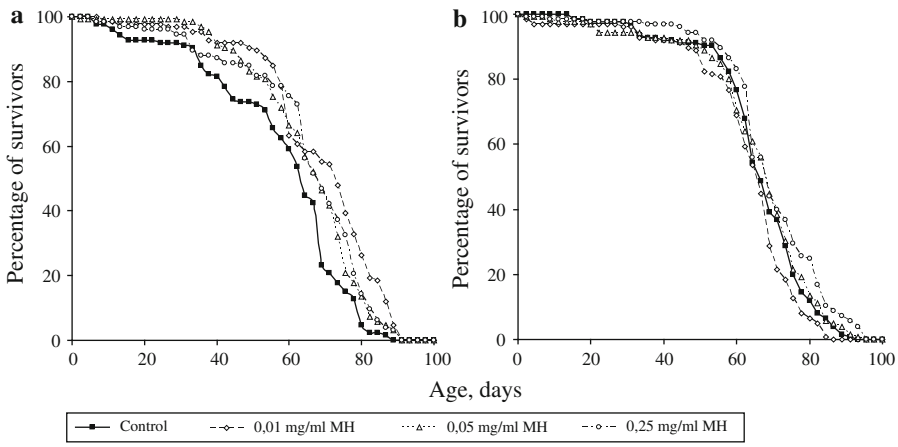

Fig. 1 The survival curves of male **a** and female **b** *D. melanogaster* after treating with MH starting from day 5 of imaginal life and control flies

Table 2 Residual life span (in days) in control flies and flies treated with MH starting from day 54 of imaginal life

Concentration of MH (mg/ml)	Males		Females	
	n	Residual life span ± SEM	*n*	Residual life span ± SEM
Control	94	18.48 ± 0.93	114	19.74 ± 0.89
0.01	95	21.47 ± 1.07 (+16.2)	115	19.96 ± 0.92 (+1.1)
0.05	97	22.79 ± 1.02** (+23.3)	117	22.96 ± 0.89* (+16.3)
0.25	98	20.82 ± 1.09 (+12.7)	104	17.18 ± 0.90 (−13.0)

* $P < 0.05$; ** $P < 0.01$

n Total number of flies in each group

In parentheses—percentage change compared to control

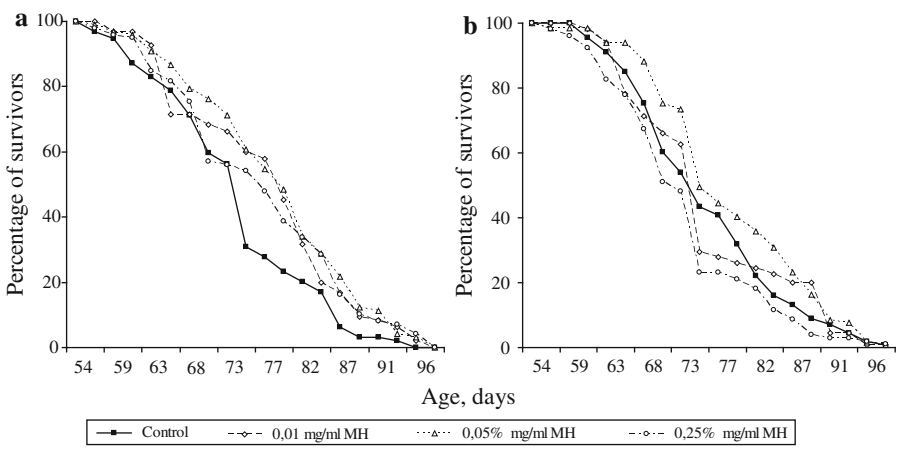

Fig. 2 The survival curves of male **a** and female **b** *D. melanogaster* after treating with MH starting from day 54 of imaginal life and control flies

activation in neuroblastoma SH-SY5Y cells (Lin et al. 2007), protects primary rat astrocytes from cytotoxicity of nitric oxide species, including NO and peroxynitrite (Kim et al. 2001). Morphine inhibits nonenzymatic lipid peroxidation in rat brain mitochondria (Das and Ratty 1987) and effectively protects them against oxidative damage induced by in vitro anoxia-reoxygenation (Feng et al. 2008). Morphine

has also been shown to be a strong inducer of heat shock protein 70 as well as a moderate inducer of heat shock protein 27 (Ammon-Treiber et al. 2004). These molecular chaperones play an important role in the deterrence of protein damage during aging and their expression is required for longevity (Calderwood et al. 2009). It has been found that heat shock proteins have a great influence on aging and that they are involved in pathways of longevity determination in *Drosophila* (Morrow and Tanguay 2003).

The adaptive behavioral effects of MH treatment could also contribute to flies' longevity. Morphine administration was found to result in increase in the locomotory activity of American cockroaches (Kavaliers et al. 1987), in analgesic effect in praying mantis (Zabala et al. 1984), and in memory consolidation in crickets (Jaffe and Blanco 1994). Adaptogen action of morphine can be especially important for survival in old insects which have a small amount of the opioid receptors in nervous tissue (Chapman et al. 1984).

In the present study, influence of the morphine starting from day 5 of imaginal life on the female mean life span was less pronounced than on male. The diminished efficiency of the life-extending manipulations in fruit flies females has been observed repeatedly (Khazaeli et al. 1997; Le Bourg and Minois 1997; Anisimov et al. 1998; Izmailov and Obukhova 1999). The reason why life-extending effects in females are not observed at young age may be because females usually have a high egg-laying activity at this age. Remarkably, in our study late-in-life supplementation with 0.05 mg/ml of MH leads to significant life extension not only in male but also in female flies (Table 2). For these females, fecundity is not at play because egg-laying at 54 days of age is nearly absent. We suggest that age-related decline of fecundity may allow to come to light a life-extending capacity of MH in female *D. melanogaster*.

The present data, together with those of our earlier study in mice (Dubiley et al. 2000) indicate that activation of opioid receptors can result in life extension in both vertebrate and invertebrate animal models, suggesting early evolutionary development of opioid involvement in pathways of longevity determination. A beneficial effect of MH on the flies' life span is age-, sex-, and dose-dependent. Further studies are required to determine the mechanisms underlying this effect in both mammal and non-mammal species.

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