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A. V. Egorova, A. F. Gatiyatullina, D. M. Khakimova, et al.

Activation of *Caenorhabditis Elegans* Cholinergic System by Food Deprivation and Octopamine

A. V. Egorova[1,a)], A. F. Gatiyatullina[1,b)], D. M. Khakimova[2,c)], R. R. Shagidullin[1,d)] and T. B. Kalinnikova[1,e)]

[1]*Research Institute for Problems of Ecology and Mineral Wealth Use of Tatarstan Academy of Sciences, 28, Daurskaya str., Kazan, 420087, Russian Federation*
[2]*Kazan Federal University, 18, Kremlevskaya street, Kazan, 420008, Russian Federation*

[a)]egorovanastassia@gmail.com
[b)]gaf9212@gmail.com
[c)]diazkzn@mail.ru
[d)]shagidullin_@mail.ru
[e)]Corresponding author: tbkalinnikova@gmail.com

Abstract. Effects of food deprivation and octopamine on the sensitivity of behavior of nematode *C. elegans* to agonist of nAChRs levamisole and partial inhibition of ACh-esterase by aldicarb were investigated. Either food deprivation or octopamine caused sensitization of swimming as induced by mechanical stimulus to aldicarb and levamisole action, which was revealed in disturbances of coordination of muscles necessary for swimming. These results show that both food deprivation and exogenous octopamine caused activation of *C. elegans* cholinergic system. The joint action of food deprivation and octopamine revealed their additive effects. Therefore, food deprivation caused adaptive activation of cholinergic system via nonidentified mechanism independently from octopamine secretion. Activation of cholinergic system by food deprivation may be an adaptive reaction of *C. elegans* nervous system necessary for movement speed elevation to avoid local unfavorable environment.

INTRODUCTION

A common stress encountered by all animals is lack of available food. Therefore, behavioral responses to food deprivation are the fundamental aspect of nervous system function in all animals and thus could have conserved underlying mechanisms amenable to study in simple model organisms [1–3]. In *C. elegans*, nutrient-sensing pathways have evolved to adjust behavior and metabolic processes to changing dietary resources [3]. The special feature of soil nematodes, in contrast to mammals, is their persistent feeding on bacteria. Therefore, even short-term (minutes and hours) food deprivation for *C. elegans* is a stress inducing several adaptive behaviors [2–4].

It is known that activation of cholinergic system in the brain is a primary central process in the rodents' organisms stress-reaction [5]. Cholinergic system plays an important role in all functions of all multicellular animal organisms, and our previous studies have shown that moderate heat stress rapidly caused adaptive activation of *C. elegans* cholinergic system [6–7].

Starvation constitutes stressful conditions in all animals including *C. elegans*. However, it is still unknown how the cholinergic system of invertebrates responds to food deprivation. Therefore, the aim of this work was to test the hypothesis assuming that cholinergic system of *C. elegans* is involved in adaptive response to food deprivation.

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MATERIALS AND METHODS

C. elegans wild-type strain N2 Bristol was grown at 22°C in Petri dishes with standard Nematode Growth Medium (NGM) and *E. coli* OP50 [6]. Experiments were performed in NG buffer (0.3 % NaCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, and 25 mM potassium phosphate buffer (pH=7.0)). In order to investigate the influence of food deprivation on the steady-state of cholinergic systems worms were previously incubated for 1–4 hours in Petri dishes 40 mm in diameter with NGM with food *(E. coli* OP50) or without it. Worms were individually transferred into glass tubes with 1 mL of NG buffer (pH=7.0). An agonist of nicotinic acetylcholine receptors (nAChRs), levamisole hydrochloride, octopamine and acetylcholine esterase (ACh-esterase) inhibitor aldicarb were added to NG buffer immediately after placing worms into glass tubes. Disturbances of swimming induced by a mechanical stimulus (shaking of the tube containing the nematodes) caused by partial inhibition of ACh-esterase and levamisole were observed after 15 and 30 min of worms' exposure to levamisole or aldicarb at temperature of 22°C. The uncoordinated behaviors were a lack of coordination of body muscle contraction necessary for sinusoidal body movements and inability to sustain swimming during 10 seconds after stimulus.

High concentrations of drugs used in this paper (10^{-3} M or more) are explained by a very low permeability of *C. elegans* cuticle for drugs and toxicants [3–4, 8–9].

Experiments were performed from October to December. All experiments were performed in three replications with 30 nematodes in each replication. Statistical analysis was performed using the φ* criterion of Fisher's angle-transformation.

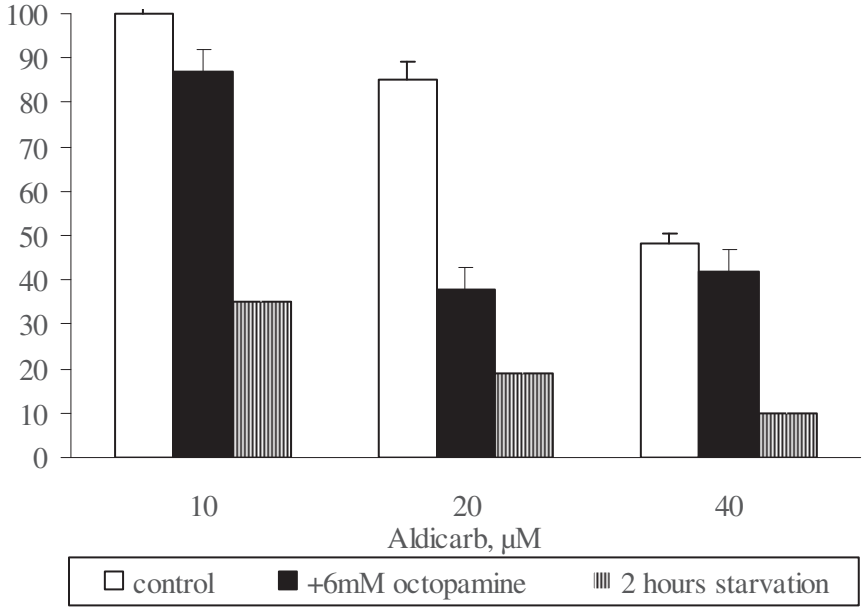

FIGURE 1. The sensitivity of *C. elegans* behavior to partial inhibition of ACh-esterase by. Ordinate: percentage of worms that retain normal coordination after 15-min exposure to aldicarb.

RESULTS AND DISCUSSION

To reveal the possible role of cholinergic system in the adaptive response of *C. elegans* to food deprivation we investigated the influence of food deprivation (1–4 hours) on the sensitivity of *C. elegans* behavior to partial inhibition of ACh-esterase by aldicarb. The data presented in Fig. 1–2 show that short-term (15–30 min) exposure of *C. elegans* to aldicarb (40 μM) caused disturbed nematode swimming, as induced by mechanical stimulus. After such exposure all nematodes sustained the ability to swimming as induced by mechanical stimulus, but body muscle contractions necessary for sinusoidal body movements were uncoordinated. Comparison of behavior sensitivity to ACh-esterase inhibition of worms previously incubated on agar plates with food (*E. coli*) or without it showed that food deprivation (2–4 hours) caused strong sensitization of behavior to aldicarb (Fig. 1–2). Since numerous mutations of genes

regulating acetylcholine (ACh) secretion and nAChRs sensitivity caused changes in *C. elegans* movement sensitivity to aldicarb this sensitivity reflected steady-state of cholinergic synaptic transmission regulating *C. elegans* movement [10–12]. Therefore, sensitization of *C. elegans* movement to aldicarb caused by food deprivation (Fig. 1) show that adaptive response of nematode organism to food deprivation includes activation of cholinergic system.

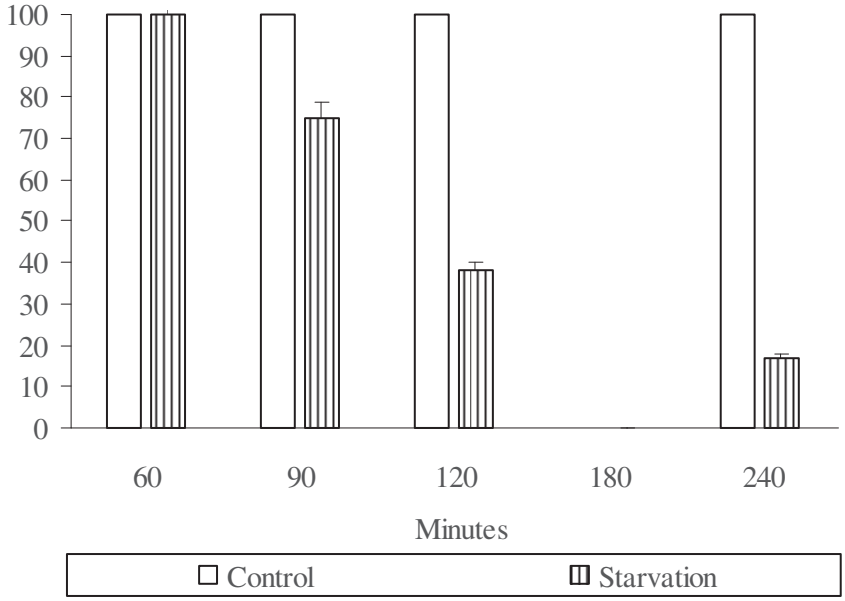

FIGURE 2. The dependence of the sensitivity of *C. elegans* behavior to aldicarb on the time of preliminary starvation. Ordinate: percentage of worms that retain normal coordination after 15-min exposure to aldicarb (10 μM).

Octopamine, a functional analogue of mammalian norepinephrine, has been implicated in the responses associated with the absence of food in such invertebrates as insects and worms [13]. Therefore, activation of *C. elegans* cholinergic system caused by food deprivation (Fig. 1) can be a consequence of octopamine secretion. In accordance with this hypothesis exogenous octopamine in concentration 6 mM which is effective for changing of *C. elegans* behavior [2], caused sensitization of *C. elegans* behavior to aldicarb (Fig. 1). However, octopamine was less effective for sensitization of *C. elegans* behavior to aldicarb than food deprivation: consequently 2-fold and 4-fold decrease of effective concentrations of aldicarb respectively (Fig. 1). Moreover, effects of octopamine and food deprivation were additive (Fig. 1). Therefore, it is evident that secretion of octopamine can't be a general mechanism of activation of C. elegans cholinergic system caused by food deprivation.

Two possible mechanisms of cholinergic system activation by food deprivation are stimulation of ACh release from neurons regulating *C. elegans* movement and sensitization of nAChRs in postsynaptic neurons or muscles. In both cases activation of cholinergic system is revealed in the sensitization of *C. elegans* behavior to partial inhibition of ACh-esterase [6, 11–12]. To reveal the possible role of n-cholinergic signaling in the activation of *C. elegans* cholinergic system by food deprivation and exogenous octopamine we investigated their influence on the sensitivity of *C. elegans* behavior to nematodes nAChRs agonist levamisole. Levamisole caused disturbances of *C. elegans* behavior similar with aldicarb action since hyperactivation of nAChRs is the reason of behavior disturbances caused by abnormally high ACh level [11–12]. The data presented in Fig. 3 show that both food deprivation and exogenous octopamine caused sensitization of nematode behavior to levamisole since levamisole concentration necessary to disrupt coordination of nematodes swimming were considerably lowered by octopamine and food deprivation. Levamisole is the most potent agonist of nematodes nAChRs, and changes in *C. elegans* behavior sensitivity to levamisole caused by numerous mutations are explained as a result of changes in nAChRs sensitivity [9]. Therefore, it is evident that sensitization of *C. elegans* behavior to levamisole caused by food deprivation and octopamine is a consequence of modulation of nAChRs sensitivity both by activation of octopamine receptors and by the absence of food (*E. coli*) in environment.

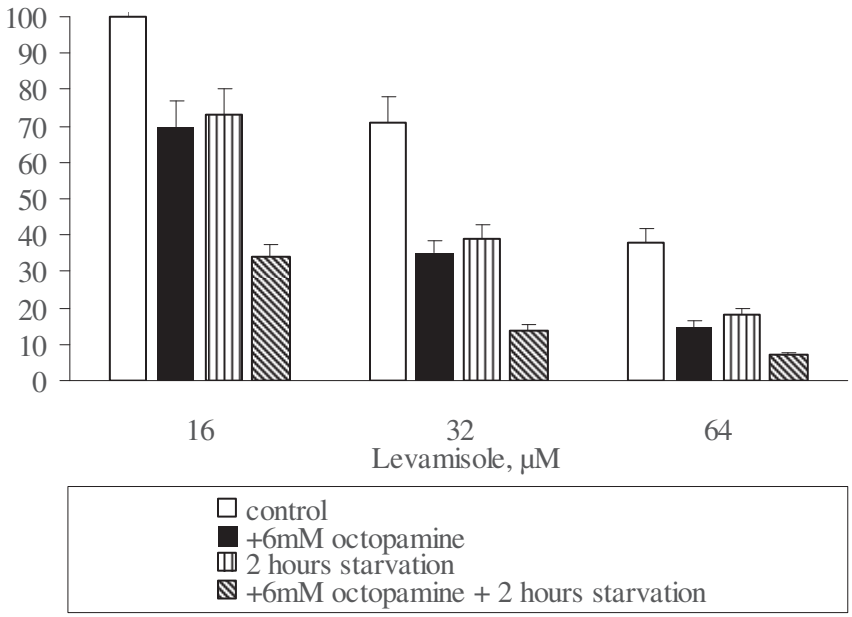

FIGURE 3. The influence of octopamine and short-term starvation on the *C. elegans* sensitivity to levamisole. Ordinate: percentage of worms that retain normal coordination after 15-min exposure to levamisole.

CONCLUSION

It is known that activation of cholinergic system in the brain via stimulation of ACh secretion by neurons is a primary response of mammalian organism to stress [11]. Since both moderate heat stress [6, 8] and food deprivation (Fig. 1–3) caused activation of *C. elegans* cholinergic system it is evident that this activation is an adaptive response to stressful environmental conditions not only in mammals but also in simple nematode organism. Mechanisms of cholinergic system activation by stress can be different in mammals and nematodes. In mammals stress stimulates ACh secretion from brain neurons [11] whereas in *C. elegans* either heat stress or food deprivation caused sensitization of nAChRs (Fig. 2) [6]. However, we do not rule out that food deprivation caused both sensitization of nAChRs and stimulation of ACh secretion by neurons since heat stress caused strong sensitization of C. elegans behavior to levamisole accompanied with moderate behavior sensitization to partial inhibition of ACh-esterase [6] while food deprivation in contrast caused strong behavior sensitization to aldicarb accompanied with moderate sensitization to levamisole (Fig. 1–2).

It is known that food deprivation subject to its duration (minutes or a few hours) caused in *C. elegans* the broad repertoire of adaptive changes of behavior and metabolism [2–4, 13]. It is evident that activation of cholinergic system did not refer to adaptive changes of behavior in the first minutes of food lack in the environment [4] since displayed only after 120-min incubation without *E. coli* (Fig. 3). Therefore, it is possible that activation of cholinergic system which is revealed in changes in sensitivity of locomotion to the rise of ACh level in organism (Fig. 1–3) is a mechanism not only of adaptive behaviors, but also of adaptive changes of nematodes metabolism by food deficiency. To answer this question additional researches are needed.

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