Ethanol Sensitizes the Nervous System of *Caenorhabditis elegans* Nematode to Heat Stress

M. Kh. Gainutdinov, A. Kh. Timoshenko, A. M. Petrov, T. M. Gainutdinov, and T. B. Kalinnikova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 150, No. 7, pp. 62-65, July, 2010
Original article submitted May 29, 2009

Ethanol sensitizes the nervous system of *C. elegans* to heat stress, which manifested in exacerbation of locomotion disturbances induced by exposure to constant temperature 36°C. Adaptation of *C. elegans* to high temperature by heat shock (1 h at 32°C and 1 h at 18°C) or two-hour exposure at 30°C resulted considerably reduced sensitivity of the nervous system to the negative effects of ethanol under conditions of heat stress.

Key Words: *ethanol; C. elegans; heat stress; adaptation*

Increase in season and diurnal maximal temperatures negatively affecting humans and animals is a consequence of global climate warming. Alcohol sensitize the organism to thermal influences and is a known risk factor heat and sun strokes [4,6]. The mechanisms for this sensitization are little studied, because the etiology of heat disorders remains unclear in many instances due to extreme complexity of negative effects of hyperthermia [2,14]. At the same time, it is known that the nervous system is the target for the effects of ethanol on human and animal behavior [10,11], therefore sensitization of the nervous system to heat stress by ethanol can be responsible for potentiation of the effects of hyperthermia on human body. Molecular mechanisms of integrative functions in living organisms and adaptation of the nervous system to stress are highly conservative, therefore simple nervous system of free living soil nematode *Caenorhabditis elegans* is widely used as a convenient model of complex processes taking place in the nervous system of humans under normal and pathological conditions [5,15].

The objective of the study was to demonstrate that ethanol sensitizes the nervous system of *C. elegans* to heat-induced dysfunctions and that adaptation of worms to high temperature protects their nervous system from the negative effects of alcohol under conditions of extreme increase in environment temperature.

MATERIALS AND METHODS

C. elegans, wild strain N2, were cultured at 18°C in Petri dishes with standard nematode medium (SNM) [3] containing *E. coli* OP50. The experiments were carried out on 3-day worms in a temperature-controlled (0.1°C accuracy) water bath at constant temperature; the animals were incubated one by one in 1 ml SNM without agar, peptone, and cholesterol. Thermostability of locomotion was assessed at constant temperature (36°C) by the following parameters: mean time until loosing the ability to mechanically induced (vial shaking) swimming and the mean time to complete, but reversible immobilization of the worms. The worms were adapted to high temperature by 2-h exposure at 30°C or by heat shock (1 h at 32°C and 1 h at 18°C). The data were processed statistically uning Student's *t* test.

RESULTS

The exposure of *C. elegans* to constant high temperature (36°C) resulted in reversible impairments of worm locomotion induced by mechanic stimulus: they lost

Institute of Ecology of Natural Systems, Academy of Sciences of Tatarstan, Kazan, Russia. ***Address for correspondence:*** tbkalinnikova@gmail.com. T. B. Kalinnikova

0007-4888/10/1501-0055

the ability to swim, but still responded to mechanical stimulus by moving a part of the body or the whole body (single movement), and then led to complete immobilization (Fig. 1). Addition of 0.12-1.0% ethanol to the medium aggravated locomotion disturbances induced by high temperature: we observed a decrease in the time to loosing swimming capacity without impairment of the response to stimulus and the time to complete immobilization of worms during exposure to 36°C (Fig. 1). At 18°C, ethanol in a concentration >3% induced locomotion disturbances similar to those observed after exposure to high temperature. Therefore, our experiments revealed not synergism in the effects of ethanol and high temperature on *C. elegans* behavior, but sensitization of the worms to negative effects of hyperthermia in the presence of alcohol. Adaptation of *C. elegans* to high temperature by 2-h exposure at 30°C and heat shock considerably modulated alcohol sensitization of worms to extremely high temperature (Fig. 2). These effects manifested in reduced sensitivity of locomotion thermostability to ethanol (Fig. 2) and in better adaptation to high temperature in the presence of alcohol in the medium (Fig. 3).

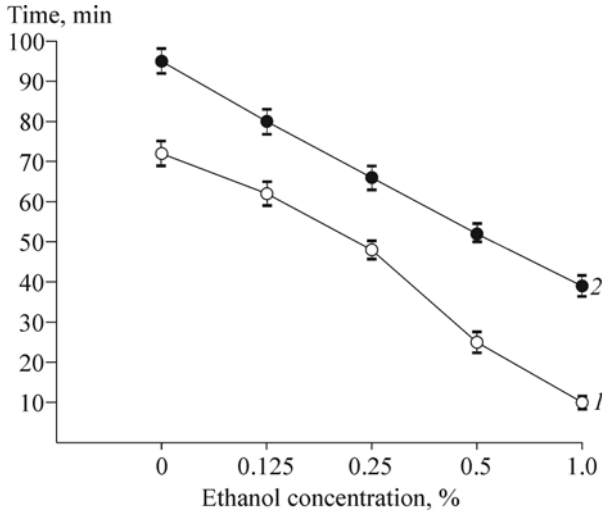

Fig. 1. Sensitization of *C. elegans* locomotion to heat-induced disturbances after addition of ethanol to the medium. *1*) mean time to loosing swimming capacity without impairment of the reaction to mechanical stimulus at 36°C; *2*) mean time to complete immobilization of the worms at 36°C; 30 worms were used in each group.

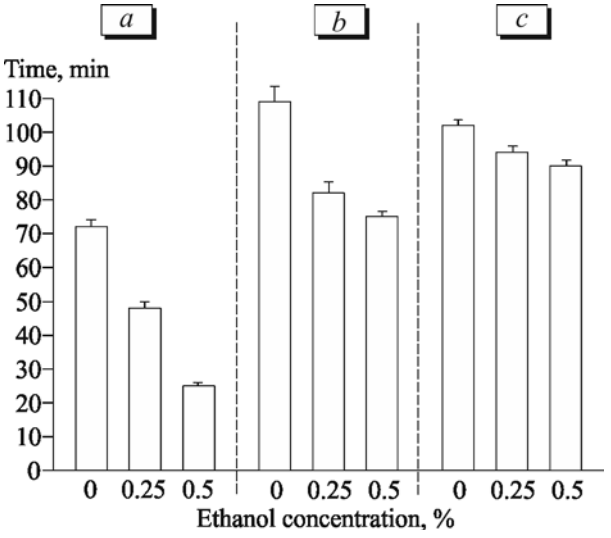

Fig. 2. Effects of adaptation to high temperature on *C. elegans* sensitivity to ethanol under conditions of extreme temperature rise. *a*) control worms; *b*) worms after heat shock; *c*) worms after 2 h exposure at 30°C. Here and at Fig. 3: Ordinate: mean time to loosing swimming capacity without impairment of the reaction to mechanical stimulus at constant temperature 36°C; 30 worms were used in each group.

It is known, that as early as 20 min after addition of ethanol to the medium an equilibrium between alcohol penetration into the *C. elegans* body (limited by low permeability of the cuticle for ethanol) and ethanol metabolism is reached, at which ethanol concentration in the worm body is manifold lower than in the incubation medium [5]. After addition of 400 mM ethanol to the medium, its concentration in the worm body is only 22 mM [5]; and this level is similar to blood alcohol concentrations in human and rodent affecting their behavior [10,11]. At 20°C, ethanol in a concentration range of 0.45-2.0% reduced spontaneous motor activity of *C. elegans* without impairing their locomotion induced by mechanic stimulus [5]. The main mechanism for this reduction is alcohol stimulation of SLO-1 channels regulating secretion of neurotransmitters [5]. For this reason, stimulation of SLO-1 channels is a possible mechanisms of sensitization of the nervous system of *C. elegans* to heat stress. These Ca^{2+}-activated potassium channels with high conductivity are expressed in all types of neurons in *C. elegans* [7], and their stimulation with ethanol, therefore, can modify some forms of behavior, including its sensitization to heat stress. This sensitization occurs at alcohol concentrations (0.12%) below those effective for reduction of spontaneous motor activity in worms. At the same time, there are another possible mechanisms of potentiation of high temperature effects on the worm nervous system by ethanol, because in rodent nerve cells ethanol affects not only Ca^{2+}-activated high-conductance K^+-channels [7], but also GABA, serotonin, and NMDA receptors [10,11,15], which are also expressed in simple nervous system of *C. elegans*.

The increase in thermostability of invertebrate behavior after high-temperature exposure or other stress exposures, including hypoxia, is determined by adaptation processes improving resistance of integrative functions of the nervous system to thermal influences, which do not affect neuronal viability, but disturb interneuronal and neuromuscular synapses [1,9,12,13].

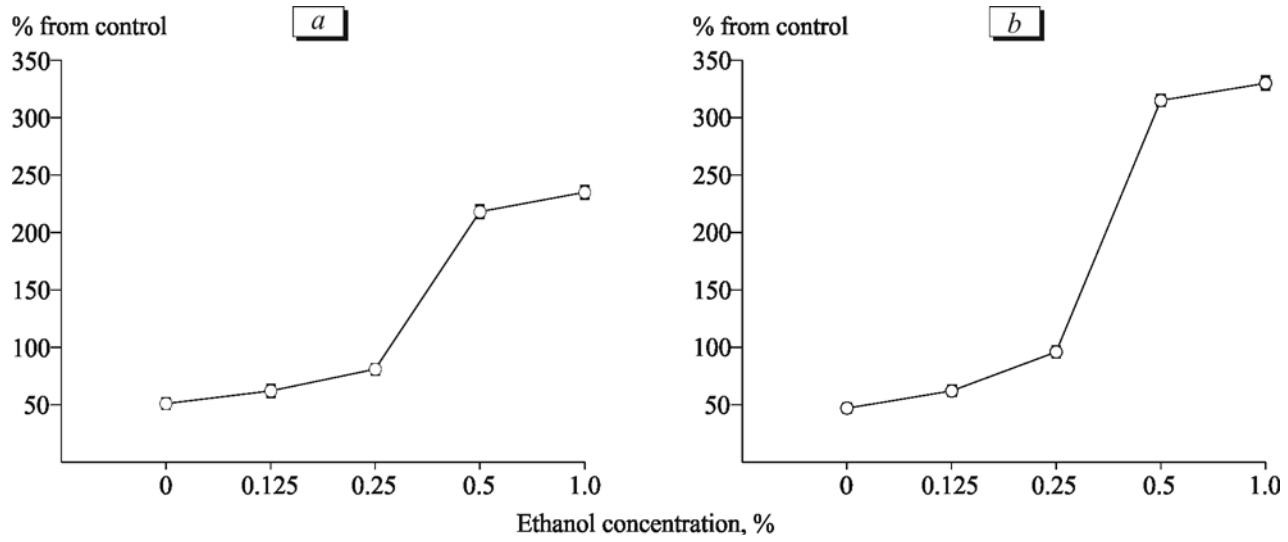

Fig. 3. Effects of ethanol on the efficiency of adaptation to high temperature. *a*) heat shock, *b*) exposure at 30°C.

Pronounced effect of heat shock and the short-term moderate temperature rise up to 30°C on the sensitivity of locomotion thermostability to alcohol (Fig. 2 and 3) suggests that adaptation of the worm nervous system to temperature stresses also includes an increase in its resistance to negative effects of alcohol manifesting under conditions of extreme temperature rise. This stress reaction of *C. elegans* nervous system may be a consequence of processes induced by stress in invertebrate nervous systems and manifest in increased thermostability of synaptic connection and reduced permeability of glial and neuronal membranes for K^+ ions [1,9,12,13]. The decrease in neuronal membrane permeability for K^+ ions can be responsible for reduced sensitivity to alcohol after heat shock and exposure at 30°C, if stimulation of SLO-1 K^+-channels is the target for ethanol action on thermostability of locomotion.

Ethanol appears not only as widely spread "medication" with numerous side effects, but also as important component of dwelling place of such invertebrates as *Drosophila*, *etc*. [11]. In this connection, alcohol sensitization of *C. elegans* nervous system to heat stress revealed in our study may appear in invertebrates living in media characterized by fermentation processes as well as extreme rises of environmental temperature, seasonal or diurnal. Examples of such invertebrates include *Drosophila* [5] and closely related to *C. elegans Caenorhabditis drosophilae* [8].

It can be hypothesized that alcohol also potentiates the negative effects of hyperthermia on complex nervous systems and this can explain sensitization of humans and rodents to heat stroke by alcohol [4,6]. In this case, *C. elegans* can be a suitable model for the study of complex effects of hyperthermia and alcohol on the nervous system.

REFERENCES

1. T. B. Kalinnikova, A. Kh. Timoshenko, and T. M. Gainutdinov, *et al.*, *Zhurn. Evolyuts. Biokhim. Fiziol.*, **42**, No. 5, 457-462 (2006).
2. A. Bouchama and J. P. Knochel, *N. Engl. J. Med.*, **346**, No. 25, 1978-1988 (2002).
3. S. Brenner, *Genetics*, **77,** No. 1, 71-94 (1974).
4. *Centers for Disease Control and Prevention (CDC)*, *MMWR Morb. Mortal. Wkly Rep.*, **47**, No. 23, 473-476 (1998).
5. A. G. Davies, J. T. Pierce-Shimomura, H. Kim, *et al.*, *Cell*, **115**, No. 6, 655-666 (2003).
6. H. Green, J. Gilbert, R. James, and R. W. Byard, *Am. J. Forensic Med. Pathol.*, **22**, No. 2, 196-199 (2001).
7. M. Gruss, M. Henrich, P. Konig, *et al.*, *Eur. J. Neurosci.*, **14**, No. 8, 1246-1256 (2001).
8. K. Kiontke, *Fundam. Appl. Nematol.*, **20**, 305-315 (1997).
9. M. K. Klose and R. M. Robertson, *Integr. Comp. Biol.*, **44**, 14-20 (2004).
10. S. J. Mihic, Q. Ye, M. J. Wick, *et al.*, *Nature*, **389**, No. 6649, 385-389 (1997).
11. R. W. Peoples and F. F. Weight, *Proc. Natl. Acad. Sci. USA*, **92**, No. 7, 2825-2829.
12. R. M. Robertson, *Integr. Comp. Biol.*, **44**, 21-27 (2004).
13. R. M. Robertson, *J. Therm. Biol.*, **29**, 351-358 (2004).
14. H. S. Sharma, *Indian J. Med. Res.*, **121**, No. 5, 621-623 (2005).
15. F. W. Wolf and U. Heberlein, *J. Neurobiol.*, **54**, No. 1, 161-178 (2003).