BIOGERONTOLOGY

Opposite Effects of Antioxidants on Anxiety in Wistar and OXYS Rats

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Passive behavior in the open field test and high anxiety in an elevated plus-maze develop in OXYS rats by the age of 3 months and are regarded as manifestations of early aging. We studied the possibility of preventing these disturbances with vitamin E and whortleberry extract (20 mg/kg for 45 days from the age of 1.5 months). Whortleberry extract alone increased horizontal activity and reduced anxiety of OXYS rats, while anxiety in Wistar rats increased significantly after treatment with both preparations, and especially with vitamin E: the number of entries into open arms of the elevated plus-maze and the time spent there decreased. The results necessitate comprehensive evaluation of the aftereffects of long-term use of antioxidants, acknowledged geroprotectors intended for preventive use.

Key Words: *prevention of early aging; behavior; anxiety; antioxidants; OXYS rats*

Prolongation of the life span and improvement of the quality of life in elderly people is the most important task of preventive medicine. More than 20 substances were proven to prolong life span in animals. They were named geroprotectors ("protecting from aging") [1]. In contrast to geriatric agents, intended for the treatment of diseases in elderly people, these preparations are intended for long-term use in young and adult age. For this reason, when evaluating their safety, one should take into consideration not only the direct side and toxic effects, but also the possible delayed aftereffects [15].

Antioxidants are widely used as geroprotectors [14]. Their efficiency seems to cause no doubt: the universal pathogenetic role of oxidative stress (imbalance in ROS generation and detoxication systems) was proven not once. However, new data on the involvement of ROS in physiological processes indicate that the advantages of free radical control, particularly in health, are not so evident. ROS are not only involved in cell metabolism as intermediate products of synthesis and provide the bactericidal activity of phagocytic cells: as signal molecules, they regulate many physiological processes, triggering cell response aimed at fortification of functional reserves [10]. In this connection the efficiency of additional intake of antioxidants becomes an intricate problem, because correct evaluation of their efficiency as prophylactic means is difficult due to individual features of dysfunctions developing with age, different nutrition and basal antioxidant supply. These problems are absent in studies on biological models. Our studies showed unique experimental potentialities of OXYS rats, whose early aging is caused by increased sensitivity to oxidative stress [3,4,9].

Early changes in the cognitive and emotional spheres characteristic of aging humans and animals

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are regarded as manifestations of early aging in OXYS rats. They differ from Wistar rats by low exploratory activity in the open field test, high anxiety in elevated plus-maze (EPM), and disordered capacity to passive avoidance conditioning [5,6], which, according to our data, develop by month 3 of life.

We studied the effects of preventive treatment with antioxidants, vitamin E and whortleberry extract characterized by high antiradical activity [13], on the anxiety and exploratory activity of OXYS and Wistar rats.

MATERIALS AND METHODS

The animals (90 male OXYS and Wistar rats) were kept in cages (3 per cage) at natural light and free access to standard fodder and water. Starting from the age of 1.5 months, some animals received 20 mg/kg vitamin E (α-tocopherol acetate, Uralbiofarm) or 25% whortleberry extract enriched with selenium (Mirtasol; 20 mg of dry substances, including 4.5 mg antocyans and 1 μg selenium (daily requirement); Sibbiotekh) for 45 days. Open field behavior [2] was studied at the age of 3 months. Open field was a square box (100×100 cm, 100 squares) with 40-cm plastic walls, illuminated by 1 shadowless lamp (100 W) positioned at a height of 100 cm above the center of the field. The animal was placed into the corner of the field and its motor activity was observed for 5 min: the number of crossed squares, rearing postures, and grooming reactions were counted. Fecal boluses were counted (indicator of emotional strain).

Forty-eight hours after open field testing, the level of anxiety was evaluated in EPM (time spent in open and closed arms, number of entries into open arms, center, and closed arms, number of rearing postures, grooming reactions, peap-out episodes, and defecations) [2]. All experiments were carried out from 12.00 to 15.00. The results were processed using one-way and two-way ANOVA using Statgraphics software and presented as $M \pm m$.

RESULTS

Horizontal and vertical activities of control OXYS rats in the open field were lower than those of Wistar rats (Fig. 1), the number of defecation acts was higher, attesting to their high anxiety (Fig. 2). Horizontal activity of OXYS rats receiving whortleberry extract increased 1.5 times ($p<0.033$); the effect of vitamin E was negligible (Fig. 1). Both preparations virtually did not modify vertical activity of animals and did not change the number of defecation acts in OXYS rats, but this parameter increased more than 3-fold in Wistar rats treated with vitamin E ($p<0.018$), while the latency of entry into the center (Fig. 3) increased to 5 min (the value corresponding to the period of observation) ($p<0.07$). The preparations did not modify vertical activity.

The number of entries into open arms of EPM and the time spent there were significantly lower in OXYS rats in comparison with Wistar, this indicating their high anxiety. Whortleberry extract reduced the anxiety of OXYS rats and increased ($p<0.035$) these parameters to a level observed in control Wistar rats (Fig. 2, *a*, *b*). Vitamin E did not modify the anxiety of OXYS rats, but increased it significantly in Wistar rats: the number of entries into open arms and the time spent there decreased by 14.5 times

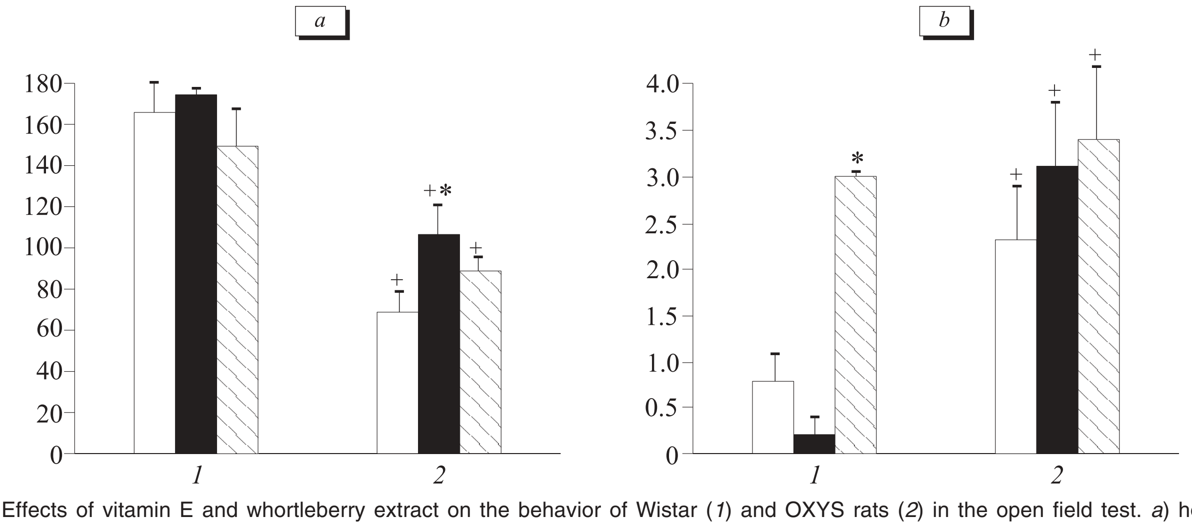

Fig. 1. Effects of vitamin E and whortleberry extract on the behavior of Wistar (*1*) and OXYS rats (*2*) in the open field test. *a*) horizontal activity (number of crossed squares); *b*) number of defecation acts. Here and in Figs. 2, 3: light bars: control; dark bars; animals treated by whortleberry extract; cross-hatched bars: vitamin E treatment. $p<0.05$ compared to *control animals; +significant differences between respective groups of different strains.

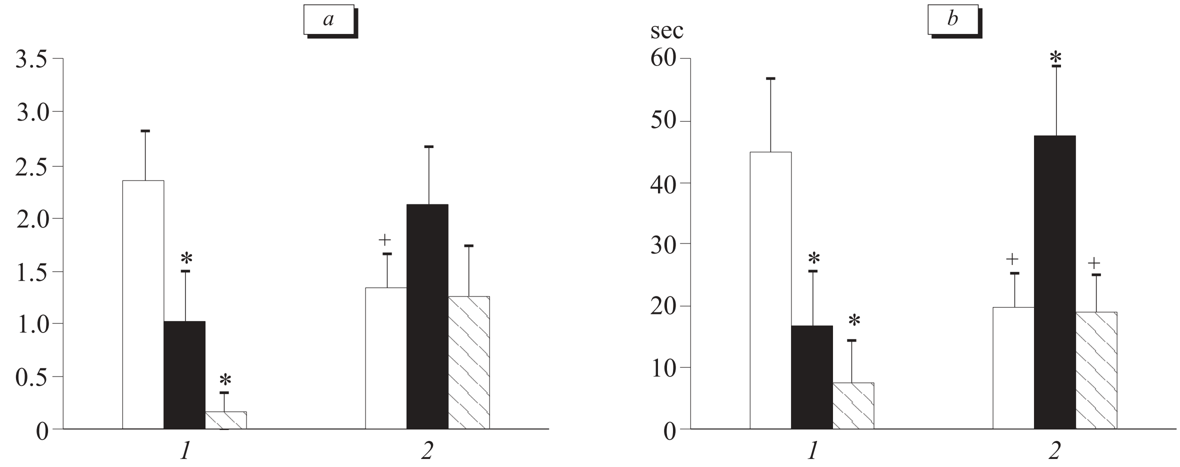

Fig. 2. Effects of vitamin E and whortleberry extract on behavior of Wistar (*1*) and OXYS rats (*2*) in an elevated plus-maze (EPM). *a*) number of entries into open arms; *b*) time spent in open arms.

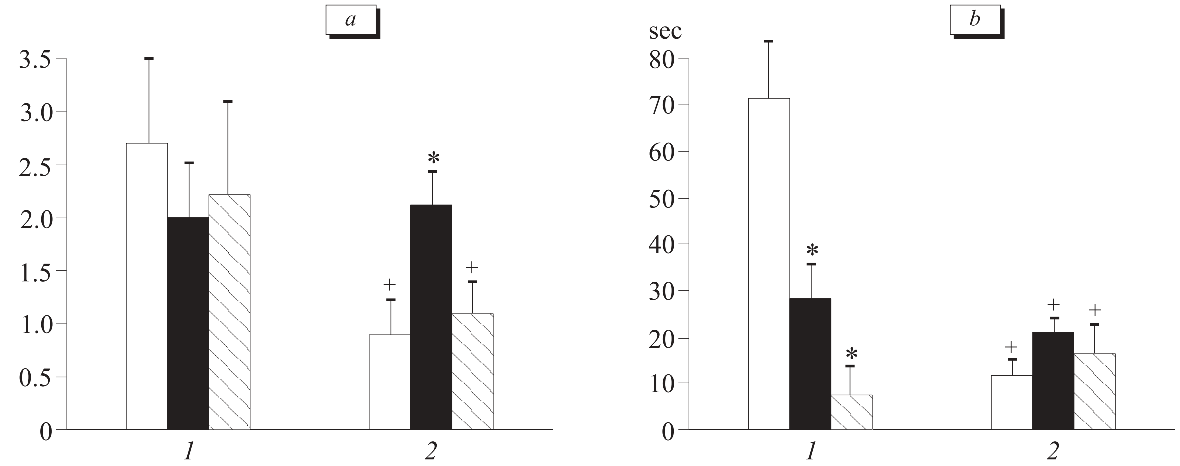

Fig. 3. Effects of vitamin E and whortleberry extract on behavior of Wistar (*1*) and OXYS rats (*2*) in EPM. *a*) number of entries into the center; *b*) time spent in the maze center.

($p<0.023$). The effect of whortleberry extract was similar, but less pronounced ($p<0.07$). None preparations changed the number of entries into the EPM center in Wistar rats, but significantly decreased the time spent there (Fig. 3, *a*, *b*). On the other hand, whortleberry extract increased the number of entries into the center in OXYS rats ($p<0.03$).

These results are a part of complex studies, aimed, in particular, at verification of the capacity of antioxidants to modulate the development of cataract and maculodystrophy in OXYS rats [7]. By the moment of evaluation of animal behavior, changes in the retina and lens of different severity were detected in 70% control OXYS rats. Vitamin E reduced, and whortleberry extract completely prevented their development. Analysis of correlations showed that the behavior of OXYS rats did not depend on the severity of changes in the lens, while animals with more pronounced alterations in the retinal macular area spent more time in open arms of EPM ($r=0.362$, $p<0.015$, $n=45$). These results suggest that the behavior of OXYS rats and the effects of antioxidants are not related to changes in vision acuity.

Anxiety is an emotional state emerging under conditions of indefinite hazards and manifesting by expectation of an unfavorable course of events, which under natural conditions helps to adapt to changing environmental conditions. However, high basal anxiety can become a cause of excessive stress-reactivity and injuries. Presumably, it is responsible for increased reaction of OXYS rats to mental stress [4]. The present findings indicate that vitamin E is little effective, while whortleberry extract had a favorable impact on behavioral characteristics and reduced the anxiety of OXYS rats. On the other hand, both preparations, especially vitamin E, increased it in Wistar rats with normal level of anxiety.

Recent studies indicate that not all effects of vitamin E are due to its antioxidant characteristics.

Vitamin E through signal transduction pathways mediated by ROS, protein kinase C, and phosphoinositol-3-kinase is involved in the regulation of gene expression and is essential for platelet proliferation and aggregation, NADPH-oxidase activity, *etc.* [8].The decrease in the content of vitamin E modifies expression of various genes in the brain, causing changes in neuronal plasticity in tocopherol-binding protein knockout mice [11]. Presumably, the antioxidant effect on anxiety, detected in this study, is determined by a complex of mechanisms, which remain to be investigated. On the other hand, these results indicate the hazards of uncontrolled use of little studied antioxidants, because even vitamin E, whose properties have been studied during more than 80 years, can cause unexpected negative reactions.

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