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Effects of the mitochondria-targeted antioxidant SkQ1 on sexually motivated behavior in male rats

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ABSTRACT

Ample research indicates that age-related neuronal–behavioral decrements are the result of oxidative stress and may be ameliorated by antioxidants. Here we examined effects of mitochondria-targeted antioxidant, SkQ1, on sexual motivation in 12-month-old Wistar and accelerated-senescent OXYS male rats. A change in behavioral activity of a male at a holed transparent partition with a receptive female on the other side was taken as an index of sexual motivation. The social behavior of male in same conditions with ovariectomised (OVXed) female and castrated male was investigated to differentiate sexually and socially motivated behavior. Behavioral response to social stimulus did not depend on age and genotype. No differences were found between 4- and 12-month-old Wistar males when sexual stimulus was presented; however, 12-month-old OXYS males demonstrated a lower propensity for sexual motivation as compared to 4-month-old OXYS rats and 12-month-old Wistar rats. We examined effects of SkQ1 on sexual motivation in 12-month-old male rats following prolonged supplementation begun at 1.5 months of age (10, 50 or 250 nmol/kg daily), a 45-day supplementation begun at 10.5 months of age (50 nmol/kg) and a 3-month supplementation begun at 9 months of age (250 nmol/kg). SkQ1 did not affect locomotor activity; however, it increased the time spent at the partition. A significantly higher measure of the motivational stage of sexual behavior was displayed following chronic preventive treatment at a dose of 50 and 250 nmol/kg by OXYS rats. Chronic therapeutic treatment during 3 months at a dose of 250 nmol/kg was effective in age-accelerated OXYS rats too. These findings suggest an essential role for oxidative stress associated with mitochondrial dysfunction in the decline of sexually motivated behavior of male rats. Recovery from these impairments and/or their prevention enables a fully successful performance of the initial stage of male sexual behavior.

1. Introduction

Aging is associated with a general decline of physiological functions, and those which depend on the central nervous system, such as sexual behavior are the most affected. The critical age at which an impairment of sexual behavior is most likely to occur strongly depends on a range of factors, including genetic background, ecological conditions, social status and stress loads.

Oxidative stress, abnormal ion homeostasis and disturbed energy metabolism are closely linked to aging and aging-related diseases, including changes in the reproductive function. All these three items are tightly associated with mitochondria, a major source of reactive oxygen species (ROS) in somatic cells (Passos et al., 2007). Not only are ROS important for many life sustaining processes in cells and tissues, but also they can induce cell damage and death. It is not surprising that antioxidants are commonly used in anti-aging interventions. However, in most cases antioxidants are not supposed to remove ROS entirely, but only excess ROS, which are generated in the mitochondrial respiratory chain. Furthermore, an antioxidant should not be inactivated by enzymes produced by the organism. Since mitochondria are the primary sites of ROS production within cells, targeting ROS scavengers to these organelles appears to be a particularly effective strategy. One such antioxidant, SkQ1 was recently synthesized (Skulachev, 2005). SkQ1 is a complex of plastoquinone (an antioxidant moiety) and a mitochondria-targeted lipophilic cation, triphenyl(alkyl)phosphonium with decane as an alkyl residue to bind quinone. One of the important advantages of SkQ1 is its rapid reduction by mitochondrial respiratory chain complex I and II, that is, SkQ1 is a reusable regenerating antioxidant (Skulachev, 2007).

A complex like SkQ1, MitoQ, was first used by Murphy for targeted delivery of the antioxidant ubiquinone (CoQ) to mitochondria (Murphy, 2001; Kelso et al., 2002). Cationic quinones SkQ1 and MitoQ may show anti- and prooxidant activity in dependence on concentration. The

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notable difference between these drugs is the width of the window between their anti- and prooxidant effects. Whereas the anti- and prooxidant concentrations of MitoQ differ less than twofold (300 and 500 nM), this difference for a plastoquinone derivative of decyl triphenylphosphonium named SkQ1 is increased 32-fold (25 and 800 nM) (Skulachev, 2007). The integrated studies summarized in the review by Cochemé et al. (2007) demonstrated that MitoQ has great therapeutic potential for mitochondria-mediated diseases. On the other hand, we have not seen any convincing examples of its use in preventive or therapeutic treatment of age-related dysfunction and diseases. The effects of mitochondria-targeted antioxidants on the reproductive system have not been examined either. It should be noted that data on the effects of classic antioxidants on sexual behavior are sparse. The effects of antioxidants are normally assessed in association with organic disturbances of the reproductive system, including impaired fertility and sperm viability (Paradiso Galatioto et al., 2008). Many studies have demonstrated the ability of antioxidants to prevent disturbances of the reproductive system affected by oxidative stress. The question as to whether antioxidants are able to produce effects on the initial, motivational stage of sexual behavior, which is critical to success in sexual behavior, remains unanswered. This situation could be due to problems with assessing the efficiency of preventive and correction therapy in human patients.

These problems are associated with genetically determined differences in the rates of aging and the differences in the internal supply of antioxidants. Problems exist with how human patients themselves rate their levels of sexual motivation under therapeutic conditions. These problems, however, do not exist when an experimental model for assessment of sexual arousal in rodents is used (Osadchuk and Naumenko, 1981; Amstislavskaya and Popova, 2004). An investigator can monitor the development of specific motivational behavior in rats and mice by placing males in such settings that they can see a receptive female and sense her smell, but not contact her physically in a mating-related manner (Popova and Amstislavskaya, 2002; Amstislavskaya and Popova, 2006). In the present work, we used this model to examine and compare the effects of the mitochondria-targeted antioxidant SkQ1 on sexually motivated behavior in Wistar and accelerated-senescent OXYS rat males. The OXYS rats (Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Science) are known for shortened lifespan and an early development of age-related pathological phenotypes similar to several geriatric disorders observed in humans, including cataract, retinal dystrophy, high blood pressure, an accelerated involution of the thymus. At the age of 3 months, OXYS rats have significantly reduced locomotor and exploratory activities in the open field, increased anxiety in the elevated plus-maze (Loskutova and Kolosova, 2000; Sergeeva et al., 2006) and impairments of learning in the passive avoidance test as compared to Wistar rats (Kolosova et al., 2003, 2006). It was hypothesized that higher sensitivity to oxidative stress observed in OXYS rats as well as senescence acceleration might be caused by mitochondrial dysfunctions (Salganik et al., 1994; Kolosova et al., 2005, 2006). Neither the sexual behavior of OXYS male rats nor how it is affected by antioxidants has previously been studied.

2. Methods

The experimental animals were sexually naive OXYS and Wistar male rats bred and kept at the Institute of Cytology and Genetics, Novosibirsk, under standard vivarium conditions (natural light/dark cycle, temperature: 18–22 °C; relative humidity: 50–65%), with water and food *ad libitum*. The males weaned at the age of 30 days and kept in cages in groups of 4–6 until 4 or 12 months of age. All the procedures performed on the animals were in compliance with the European Communities Council Directive No. 86/609/EES.

The level of social and sexually motivated behavior was assessed in OXYS and Wistar rats aged 4 and 12 months. The effects of inclusion of SkQ1 in the diet on sexually motivated behavior were assessed in 12-month-old Wistar and OXYS male rats. Experimental males received SkQ1 supplementation with food. Three protocols of drug administration were used: one for assessing preventive effects of SkQ1 and two for assessing its therapeutic effects.

According to Protocol 1, males received prolonged supplementation with SkQ1 started from the age of 1.5 months (10, 50 or 250 nmol/kg body weight daily; the animals were tested in July and August). The number of animals (n) was 10 in all groups except for OXYS, 50 nmol/kg ($n=9$).

According to Protocol 2, males received a treatment with SkQ1 for 45 days begun at 10.5 months of age (50 nmol/kg body weight daily; the animals were tested in September). In control Wistar group $n=5$, control OXYS $n=6$ and in groups SkQ1 $n=11$ for both genotypes.

According to Protocol 3, males received a treatment with SkQ1 for 3 months begun at 9 months of age (250 nmol/kg body weight daily; the animals were tested in April), $n=10$ for all groups.

In all protocols animals were tested at the age of 12 months in April throughout September, as it had been demonstrated previously that there were no season-specific differences in the level of sexually motivated behavior of sexually activated males (Amstislavskaya and Popova, 2004).

The tests were performed from 19:00 to 21:00 local time in a standard plastic cage sized $52\times33\times20$ cm bisected by a perforated transparent partition. Before the testing began, each male was kept in such a cage for 3 days to remove the group effect and to allow the animal to adapt to the cage environment (Amstislavskaya and Popova, 2004). On each testing day, the cage was removed from the rack and taken to the room next door, where behavior was monitored under red light. The animal was given 5 min to adapt to new conditions prior to a 10-minute assessment of its spontaneous activity at the partition. After the assessment was completed, a receptive 4-month-old Wistar female was placed in the neighboring compartment. An event of the male approaching the partition and touching it with its nose or forepaws was acknowledged as an approach. If the male approached the partition but did not touch it, was facing away from the partition, or showed a sideways position, no approach was acknowledged. The time the male spent actively exploring the partition and the number of approaches to it were recorded for 10 min. The following behavioral variables were regarded as the indicators of male sexual motivation. The males would normally approach the partition, smell and touch it with one or two forepaws, and hold or hang on to the partition. Furthermore, the males would thrust their noses into the holes of the partition and even chew it. The total time the males spent at the partition exhibiting the described behavior during the test was calculated and treated as a measure of sexual motivation. The total number of approaches made by the tested males to the vacant neighboring compartment was also calculated.

Additional study provided information that either of these were really the measure of sexual interest vs. social interest. The specificity of the sexual stimulus was demonstrated in a study by comparing the behavioral variables in Wistar and OXYS male rats exposed to an ovariectomised (OVXed) hormone primed receptive female (sexual stimulus), a non-hormone primed OVXed female or castrated male (social stimuli) of Wistar genotype. Social interaction was tested in the same plastic cage and same conditions as in case of receptive female. The behavioral components included the time the male had spent at the partition and the number of approaches to it assessed during 10 min. The number of animals in experimental groups is shown in Table 1.

Estrus in the females was induced by estradiol (pure in peach oil) administered subcutaneously 48 h before testing at a dose of 50 μg per animal, and progesterone administered subcutaneously 4 h before testing at a dose of 1.0 mg per animal. The volume of the liquid injected was 0.15 ml per animal. Estrus was determined by vaginal smear.

Statistical analysis was carried out using a 3-way ANOVA (STATISTICA ver. 6.0) and followed by a post-hoc comparison of group means

Table 1
The number of animals in experimental groups in sexually and socially motivated behavior study.

Partner	4 months		12 months	
	Wistar	OXYS	Wistar	OXYS
Castrated male	10	10	10	11
OVXed female	4	5	10	5
Receptive female	10	5	10	15

(Newman–Keul's test). The independent variables were genotype (Wistar, OXYS), drug (controls, SkQ1) and presence of a partner behind the partition (the vacant compartment, a receptive female, an OVXed female, a castrated male). Data are expressed as means ± standard errors of the means (SEM).

3. Results

3.1. Assessment of age-related changes in the socially and sexually motivated behavior of Wistar and OXYS male rats

To assess age-related changes in social and sexual motivation, the behavior of Wistar and OXYS male rats at the partition without and with partners on the other side was studied at the age of 4 and 12 months (Fig. 1). Overall three-way ANOVA revealed the effects of genotype ($F(1,202)=7.8$, $P<0.01$), presence of a partner behind the partition ($F(3,202)=493.8$, $P<0.001$) and age ($F(1,202)=14.0$, $P<0.001$) on the time that the male spent at the partition. Post-hoc test showed significant increase in time spent close to the partition in the Wistar and OXYS males of different age groups after any partner was put into the cage ($P<0.001$). The interaction between all the factors ($F(3,202)=10.9$, $P<0.001$) makes it possible to analyze behavior characteristics near the partition in the absence of a partner and when another individual was put behind it.

A two-way ANOVA revealed the effect of age only ($F(1,105)=56.8$, $P<0.001$) on the time that the male spent at the partition with empty compartment. Post-hoc test demonstrated that 12-month-old males of both genotypes spent less time by the partition as compared to 4-month-old males ($P<0.001$).

Separate two-way ANOVAs for the males of each genotype showed that the age of Wistar rats had no effect on time spent by males at the partition ($F(1,108)=0.1$, $P>0.05$) which depended only on the presence of the partner ($F(3,108)=268.5$, $P<0.001$). The 12-month-old as well as 4-month-old Wistar rats demonstrated interest towards the partner behind the partition, and the intensity of such interest was stipulated only by the quality of the stimulus. The maximal increase of this index was caused by the presence of a receptive female behind the partition. The time spent at the partition, behind which OVXed female or castrated Wistar male were put, did not differ in Wistar males of different ages, and the social partner influence was significantly lower as compared with receptive female behind the partition (Fig. 1).

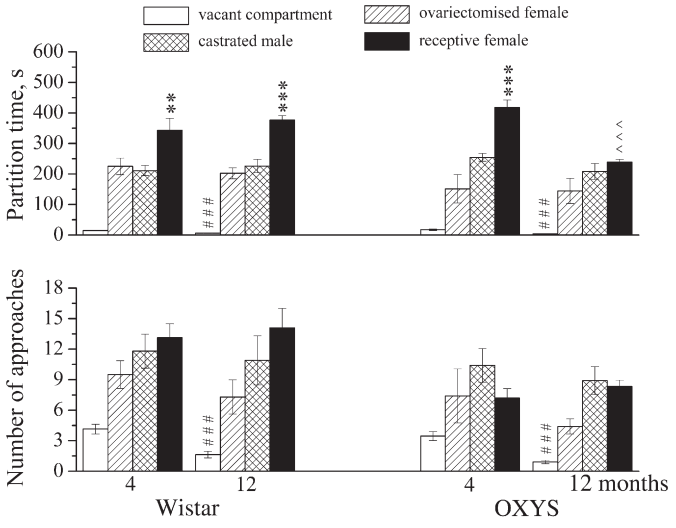

Fig. 1. Level of social and sexual motivation in 4 and 12-month-old Wistar and OXYS rats. $^{**}P<0.01$, $^{***}P<0.001$, compared to animals exposed to OVXed female and castrated male behind the partition, $^{\#\#\#}P<0.001$, compared to 4-month-old animals of the same genotype, and $\wedge\wedge\wedge P<0.001$, compared to 4-month-old OXYS animals. Bars represent means ± SEM.

In OXYS, in contrast to Wistar rats, a 2-way ANOVA revealed an age-dependent ($F(1,21)=5.1$, $P<0.05$) effect of a partner on time spent near the partition ($F(3,94)=243.2$, $P<0.001$). The assessment of sexual motivation in OXYS rats at different ages produced diverse results. OXYS males aged 12 months spent significantly less time at the partition with a receptive female behind it than their 4-month-old counterparts ($P<0.001$). However, the age did not affect the time index of OXYS male behavior when OVXed female or castrated male were put behind the partition. In Wistar males these indicators did not differ significantly either. The fact that draws attention here is that there was no reliable difference in behavioral response of aging 12-month-old OXYS rats to receptive female, OVXed female, or castrated male, which Wistar males had (Fig. 1).

As regard to the other indicator of the behavior by the partition, the number of approaches, a 3-way ANOVA revealed the effects of genotype ($F(1,202)=21.2$, $P<0.001$), the presence of a partner behind the partition ($F(3,202)=67.4$, $P<0.001$), age ($F(1,202)=5.1$, $P<0.05$), an interaction between genotype and presence of a partner behind the partition ($F(3,202)=5.1$, $P>0.01$), and an interaction between age and presence of a partner behind the partition ($F(3,202)=3.4$, $P<0.05$). The main effect of the genotype was that Wistar males approached the partition more often as compared to OXYS males. The presence of any partner behind the partition led to increase in this indicator in rats of both strains as compared to empty neighboring compartment.

12-month-old Wistar and OXYS males made significantly less approaches to the partition than 4-month-old males when the neighboring compartment was vacant ($P<0.001$); while when it was occupied by any partner, the difference was not significant.

3.2. Preventive supplementation with SkQ1

An experiment on prevention of age-related disturbances in male sexual motivation began at 1.5-month age and showed a significant effect of SkQ1 ($F(1,141)=667.9$, $P<0.001$) on the behavior of the 12-month old males in the experimental cage in presence of a female on the other side of the partition (Fig. 2). No effect of genotype on time spent near the partition was found ($F(1,141)=1.0$, $P>0.05$). Prolonged supplementation with SkQ1 (10, 50 or 250 nmol/kg body weight daily) had implications for this parameter ($F(3,141)=3.05$, $P<0.05$) which in the presence of a receptive female is a quantitative measure of the level of sexual motivation. The main effect of SkQ1 was an enhancement in the sexual motivation of the males ($P<0.05$). The interaction between drug treatment and presence of a female behind the partition revealed by a 3-way ANOVA ($F(3,141)=2.9$, $P<0.05$) suggests that SkQ1 had no effect on the amount of time spent by the male at the partition with no female behind; however, SkQ1 increased it whenever a female was present. Post-hoc analysis demonstrated that the dose at which SkQ1 increased sexual motivation in OXYS rats — 50 nmol ($P<0.05$) and 250 nmol ($P<0.01$) (Fig. 2). As a result, the time that the SkQ1-treated early-aging OXYS rats spent at the partition reached the level recorded for the normally-aging Wistar male rats exposed to a sexual stimulus.

The antioxidant treatment had no significant effect on another behavioral measure under study, the number of approaches to the

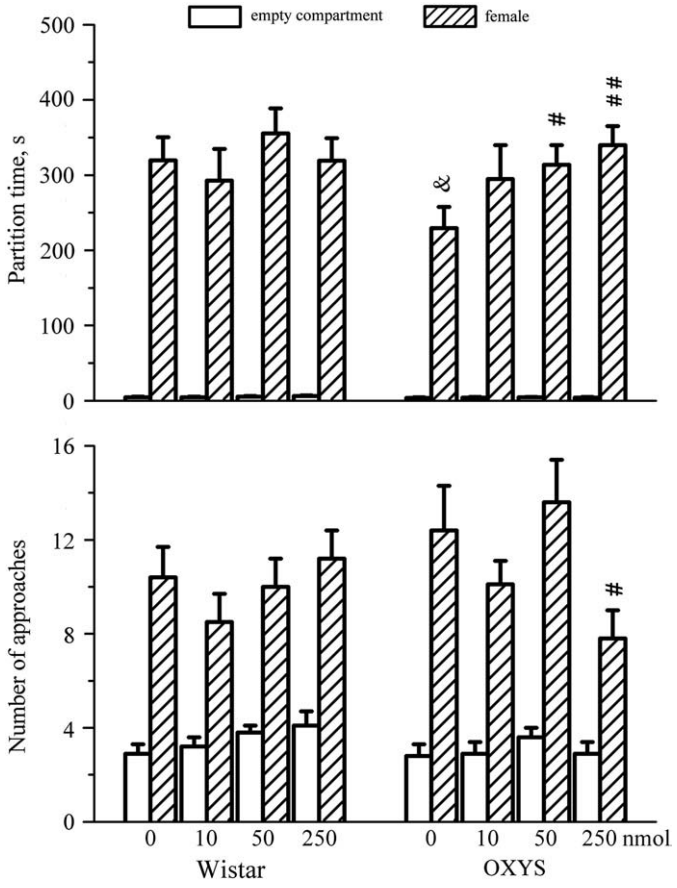

Fig. 2. Effect of preventive treatment with SkQ1 at a dose of 10, 50 or 250 nmol/kg body weight daily begun at 1.5 months of age on sexual motivation in 12-month-old Wistar and OXYS rats. All the means of time at the partition and number of approaches to the partition with a female behind it significantly differ from the corresponding scores obtained when the neighboring compartment was vacant ($P<0.001$). $^{\#}P<0.05$, $^{\#\ \#}P<0.01$, compared to the control animals of the same genotype exposed to a female behind the partition. $^{\&}P<0.05$, compared to the control Wistar animals exposed to a female behind the partition. Bars represent means ± SEM.

partition ($F(3,141)=1.76$, $P>0.05$), which implies that the locomotor activity of the males remained the same following treatment with SkQ1 at any dose. Genotype had no effect either ($F(1,141)=0.2$, $P>0.05$). The effect of the presence of a female was the only factor associated with the number of approaches to the partition ($F(1,141)=197.1$, $P<0.001$) (Fig. 2). A weak interaction between genotype and drug ($F(3.141)=2.9$, $P<0.05$) suggests that SkQ1 at the highest dose reduced the number of approaches made by the OXYS rats to the partition (Fig. 2). However, taking into account the high amount of time spent by the OXYS males at the partition (nearly all the test time) this reduction in the number of approaches means that OXYS males spent most of the time at the partition and did not leave this position.

3.3. Estimating the therapeutic potential of SkQ1

A 3-way ANOVA did not reveal any significant effect ($F(1,58)=0.8$, $P>0.05$) of SkQ1 given at a dose of 50 nmol/kg body weight daily for 45 days begun at 10.5 months on the behavioral components of sexual arousal, time spent near the partition in the 12-month-old males (Fig. 3). This behavioral index depended only on the presence of a receptive female ($F(1,58)=224.3$, $P<0.001$) and genotype ($F(1,58)=8.9$, $P<0.01$). In general, Wistar males spent more time at the partition than OXYS males. No significant factor interactions were found. Another behavioral measure, the number of approaches to the partition, was significantly affected only by the presence of a female behind the partition ($F(1,58)=122.5$, $P<0.001$).

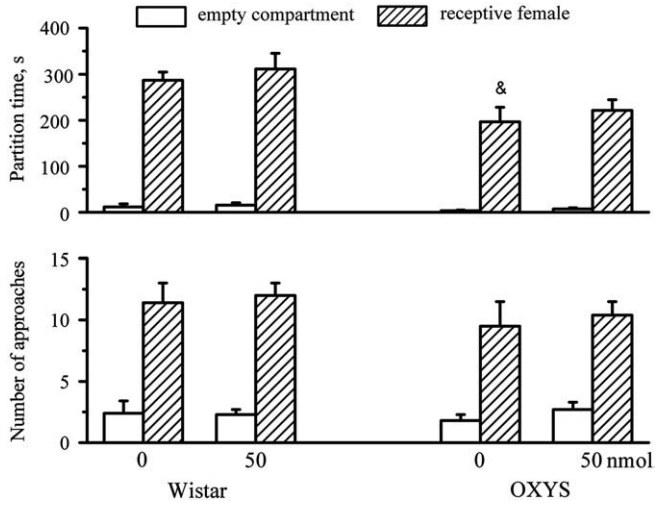

Fig. 3. Effect of treatment with SkQ1 at a dose of 50 nmol/kg daily for 45 days on sexual motivation in 12-month-old Wistar and OXYS rats. All the means of time at the partition and number of approaches to the partition with a female behind significantly differ from the corresponding scores obtained when the neighboring compartment was vacant ($P<0.001$). $^{\&}P<0.05$, compared to the control Wistar animals exposed to a female behind the partition. Bars represent means ± SEM.

A higher dosage of SkQ1 and a more prolonged supplementation (250 nmol/kg body weight for 3 months) had a stronger effect. A 3-way ANOVA revealed that the factors that influenced the amount of time spent by males at the partition were genotype ($F(1,72)=42.1$, $P<0.001$) and presence of a female on the other side ($F(1,72)=899.0$, $P<0.001$). In general, Wistar males spent more time by the partition in the presence of a female, as compared to OXYS ($P<0.001$). An interaction between genotype and drug ($F(1,72)=10.6$, $P<0.01$) suggested that SkQ1 had different effects on the behavior of the males of two strains used (Fig. 4). The antioxidant had no effect on the amount of time spent at the partition by the Wistar rats ($P>0.05$), while the OXYS males treated with SkQ1 spent much more time there than the controls ($P<0.01$). As in the previous experiment, SkQ1 had little effect on another behavioral measure under study, the number of approaches to the partition, which suggests that this antioxidant has no effect on the locomotor activity of the males, no matter whether there was a female behind the partition (Fig. 4). The only factor that

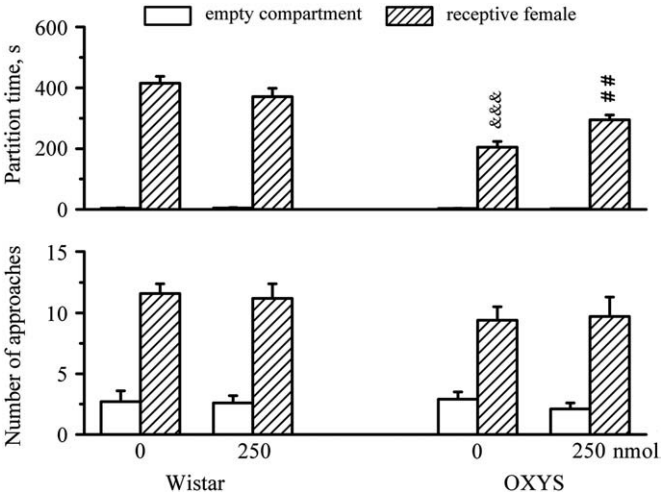

Fig. 4. Effect of treatment with SkQ1 at a dose of 250 nmol/kg daily for 3 months on sexual motivation in 12-month-old Wistar and OXYS rats. $^{\#\ \#}P<0.01$, compared to the control OXYS animals exposed to a female behind the partition; $^{\&\&\&}P<0.001$, compared to the control Wistar animals exposed to a female behind the partition. Bars represent means ± SEM.

had an effect on this measure was the presence of a female behind the partition ($F(1,72) = 122.4$, $P<0.001$).

4. Discussion

The processes that make an animal search sexual contact with another animal are usually grouped under the term of sexual motivation (Agmo, 1999). Ample experimentation demonstrates that motivations are powerful forces. Animals driven by initial motivation can not be stopped, even by difficult obstacles (wall, partition or even electrified grid). Method for quantitative assessment of sexual motivation without sexual interaction was employed in studies on female rats (Meyerson and Lindstrom, 1973), and the same procedure was later also used in males (Hetta and Meyerson, 1978). A crucial difference from earlier procedures was that no sexual interaction was possible (Agmo et al., 2004).

Our studies demonstrated that introduction of any partner (a receptive female, an OVXed female or a castrated male) behind the partition to the tested male showed that the amount of time spent at the partition and the number of approaches to it significantly increased. However, when OVXed female or castrated male was behind the partition, this time parameter was notably less as compared to time spent near a receptive female. Furthermore, the character of behavior differed distinctly, signs of aggression appeared and the males even tried to bite the opponent separated by the partition. These results are in a good agreement with data obtained in mice in our earlier studies, which demonstrated the behavioral differences at the partition in males exposed to a receptive female, a non-receptive female, or a male (Popova and Amstislavskaya, 2002; Amstislavskaya and Popova, 2004). The specificity of the sexual stimulus was confirmed by the hormonal response of the hypothalamic–pituitary–testicular system: exposure to a male or a diestrous female had no stimulating effect on blood testosterone level, in contrast to exposure to a receptive female (Amstislavskaya and Popova, 2004).

During the experiment the increase of time spent by male at the partition contacting an OVXed female or a castrated male most probably shows animal determination to communicate and its social interest in the partner behind the partition. We must admit that this kind of interest did not change with aging. The introduction of sexual stimulus, a receptive female behind the partition, increased the amount of time spent by young males of both strains two times more than in case of social stimulus. However, sexual motivation intensity of 12-month-old males depends on genotype. While the 12-month-old Wistar males had the same level of sexual motivation as the 4-month-old Wistar males, the 12-month-old OXYS male rats had a reduced level of sexual motivation compared to both 4-month-old OXYS males and 12-month-old Wistar males. In this case there was no difference between the intensity of behavioral response to sexual stimulus and the one of communicative response. We consider such changes as the evidence of accelerated senescence in the OXYS rats.

It should be emphasized that in the behavioral study of males with empty nearby compartment the amount of time that 12-month-old Wistar and OXYS males spent at the partition and the number of approaches were significantly less than those of their younger conspecific males. These data suggest general decreasing of locomotor activity with aging. However, the behavioral manifestation of sexual motivation in 12-month-old rats does not seem to depend on the decreasing of general locomotor activity. Indeed, OXYS males have a decrease in it with aging, but 12-month-old Wistar rats have unchanged sexual motivation, as compared to 4-month-old ones. Besides, we should underline the fact that the number of approaches to the partition is a less specific behavioral characteristic, and it did not change with exposure to different partners. These results correspond to the ones obtained earlier in mice (Amstislavskaya and Popova, 2004). Apparently, the number of approaches to the partition reflects mainly the general locomotor activation of an animal in the presence of a partner.

Thus, our experimental results confirm the adequacy of time spent by a male at the partition with a receptive female behind it as the behavioral index to estimate the intensity of sexual motivation. This behavioral index allows revealing aging disturbances at the initial stage of sexual behavior, male sexual motivation, in senescence-accelerated rats in our study. According to literature, considerable changes in the mating-related behavior of rats can occur for the first time at the age of 11 months. At this same age, latency to mount, latency to intromission and latency to ejaculation, which are all measures related to sexual behavior, become prolonged. Additionally, a slight decrease in blood testosterone levels was observed at the same age (Smith et al., 1992). Nevertheless, the manifestation time of these abnormalities seems to depend on animal genotype, because there is no decrease in sexual motivation intensity in 12-month-old Wistar males, unlike OXYS, towards a receptive female. The differences between these two strains might be regarded as the differences in their aging rates (Loskutova and Kolosova, 2000).

In this work, we assessed the preventive and therapeutic potential of the mitochondria-targeted antioxidant SkQ1 as well as its ability to interfere with age-related changes in the initial stage of sexual behavior in accelerated-senescent OXYS rats. The obtained data demonstrate that SkQ1 has no effect on rat general locomotor activity at empty nearby compartment. However, SkQ1 increases the main measure of sexually motivated behavior in male rats, the amount of time they spend at the partition with a receptive female behind. This effect depends on SkQ1 dosage, administration protocol, duration of supplementation, and genotype. Noteworthy, the activating effect of SkQ1 on sexually motivated behavior was stronger in the accelerated-senescent OXYS male rats, whose sexual motivation wanes earlier than Wistar rats'. In OXYS males, preventive administration of SkQ1 was efficient at a dose of 50 and 250 nmol/kg. In Wistar rats, the effect of the drug was not significant. The drug also has a therapeutic potential. Treatment with SkQ1 for 3 months at a dose of 250 nmol/kg enhanced sexual motivation in OXYS males considerably, but had no effect in Wistar rats. Interestingly, treatment at a dose of 50 nmol/kg for 1.5 months beginning from the age of 10.5 months had no effect on sexually motivated behavior in the males of either strain. This failure is possibly due to either too low drug dose or too short supplementation period because the increase in the duration of treatment up to 3 months and in the dosage up to 250 nmol/kg had a positive effect.

In summary, our studies demonstrate that prolonged supplementation with the mitochondria-targeted antioxidant SkQ1 prevented early impairment of sexual motivation in accelerated-senescent OXYS male rats and did not change sexual motivation in 12-month-old Wistar rats, whose sexual activity was as high as their younger conspecifics'. Moreover, our study revealed a therapeutic potential of SkQ1, its ability to restore compromised sexual motivation in OXYS male rats. The question about the exact target site of this antioxidant in an organism is still under consideration and requires a special investigation approach, because the age-related disturbances in the critical component of the reproductive function, sexual behavior, result from functional changes in the neurochemical and hormonal mechanisms of the reproductive system both centrally and peripherally and can be regarded as an integrative measure of sexual behavior. Based on the results obtained, the pathogenesis of the decreased sexually motivated behavior in male rats may involve oxidative stress associated with mitochondrial dysfunction. Recovery from these impairments and/or their prevention allows a fully successful performance of the initial stage of male sexual behavior.

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