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Scientific Article

Comparison of anxiolytic effects of mammalian and bony fish kisspeptins in *Danio rerio*

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In our previous work, we suggested that analogs of mammalian kisspeptin Kiss1 reduce anxiety and phobic reactions novel in *Danio rerio*. The most effective dose for the action of the studied analogs of kisspeptin corresponded to 0.1 mg per 1000 mL of water. In this study, other analogs of mammalian Kiss1 at a dose of 0.1 mg per 1000 mL of water also reduced the anxious behavior of *Danio* fish. The effect of Kiss1 and Kiss2 kisspeptins on the behavior of *Danio rerio* was also evaluated. In the novel test, the number of freezing decreased by two times with the introduction of kisspeptin 10 and by three times after the introduction of the kisspeptin analog. An analog of mammalian kisspeptin reduced the freezing time by two times. The length of the trajectory decreased by two times under the influence of the mammalian Kiss1 kisspeptin analog. With the action of kisspeptin 10, the number of transitions to the upper part of the tank increased by two times. After the introduction of the kisspeptin analog, the number of transitions to the upper part of the aquarium increased by three times. In the predator test, the number and time of freezing decreased by 1.5 times with the action of mammalian kisspeptins. The length of the trajectory after the introduction of kisspeptin bony fish and kisspeptin 10 mammals increased. The length of the trajectory after the introduction of Kiss1 increased by 1.5 times. The length of the trajectory after the introduction of Kiss2 increased by three times. After the introduction of kisspeptin 10, the trajectory increased by two times, and the time spent in the lower part of the tank decreased by two times. Kisspeptins of bony fish also reduced the anxiety and phobic reactions in fish, but to a lesser extent. Thus, kisspeptin 10 and an analog of mammalian kisspeptin in response to the presentation of a predator had more significant effects on anxiety in *Danio rerio* compared with the action of kisspeptin bony fish Kiss1 and Kiss2. Thus, bony fish kisspeptins and mammalian kisspeptins can reduce anxiety and phobic reactions in *Danio rerio*; however, mammalian kisspeptins are the most effective. Bony fish kisspeptin Kiss1 has an anxiolytic effect in contrast to Kiss2, which suggests that it affects fear reduction, and Kiss2 appears to be responsible for social and sexual behavior. The results support the hypothesis that kisspeptins may be involved in the regulation of anxiety and phobic states, apparently to maintain the emotional aspects of reproductive behavior, such as sexual motivation and arousal.

Keywords: *Danio rerio*; Kiss1; Kiss2; kisspeptin 10; mammalian kisspeptin analogs; anxiety; fear.

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Научная статья

Сравнение анксиолитического действия кисспептинов млекопитающих и костистых рыб у *Danio rerio*

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Актуальность. Ранее нами было высказано предположение, что аналоги кисспептина Kiss1 млекопитающих снижают тревожно-фобические реакции на новизну у *Danio rerio*. Наиболее эффективная доза для действия изученных аналогов кисспептина соответствовала 0,1 мг на 1000 мл воды в тесте новизны.

Цель — показать, что другой аналог кисспептина Kiss1 млекопитающих, KS6, в дозе 0,1 мг также снижал тревожное поведение рыбок *Danio rerio*.

Материалы и методы. Оценивалось действие кисспептинов костистых рыб Kiss1 и Kiss2 на поведение *Danio rerio* в тесте новизны.

Результаты. В тесте новизны выявлено, что количество фризингов на фоне введения кисспептина 10 снижалось в 2 раза, после введения аналога кисспепина — в 3 раза. Аналог кисспептина млекопитающих снижал время фризингов в 2 раза. Длина траектории снижалась под воздействием аналога кисспептина Kiss1 млекопитающих в 2 раза. Также на фоне действия кисспептина 10 в 2 раза увеличивалось число переходов в верхнюю часть аквариума, после введения аналога кисспептина — в 3 раза. В тесте с хищником число и время фризингов сокращались на фоне действия кисспептинов млекопитающих в 1,5 раза. Длина траектории после введения кисспептинов костистых рыб и кисспептина 10 млекопитающих увеличивалась. Длина траектории после введения Kiss1 увеличивалась в 1,5 раза, после введения Kiss2 — в 3 раза. После введения кисспептина 10 траектория увеличивалась в 2 раза, время нахождения в нижней части аквариума уменьшалось в 2 раза. Кисспептины костистых рыб также снижали тревожно-фобические реакции у рыб, но в меньшей степени. Таким образом, кисспептин 10 и аналог кисспептина млекопитающих KS6 в ответ на предъявление хищника оказали более значимое воздействие на тревожность у *Danio rerio* по сравнению с кисспептинами костистых рыб Kiss1 и Kiss2. Сделан вывод, что кисспептины костистых рыб и кисспептины млекопитающих способны снижать тревожно-фобические реакции у *Danio rerio*, но наиболее эффективны кисспептины млекопитающих.

Заключение. Кисспептин Kiss1 костистых рыб оказывает анксиолитическое действие в отличие от Kiss2, что дает основание полагать, что он влияет на снижение страха, а Kiss2, по-видимому, отвечает за социальное и половое поведение. Результаты исследований подтверждают гипотезу о том, что кисспептины могут участвовать в регуляции тревожно-фобических состояний, по-видимому, для поддержания эмоциональных аспектов репродуктивного поведения, таких как половая мотивация и возбуждение.

Ключевые слова: *Danio rerio*; Kiss1; Kiss2; кисспептин 10; аналоги кисспептина млекопитающих; тревожность; тест новизны.

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ЭКО • ВЕКТОР

BACKGROUND

Kisspeptin and its receptors (Kiss-R) were identified in lower and higher vertebrates. Kisspeptin is more frequently considered a behavioral hormone that affects the limbic system, including the hypothalamic–pituitary–gonadal and hypothalamic–pituitary–adrenal neuroendocrine axes [1–4]. In turn, these chains regulate the activity of signaling neurotransmitters and hormones, particularly gonadal steroids and stress hormones [5, 6]. In the central nervous system, kisspeptin acts as an endocrinological regulator of human sexual development and reproductive functions [7, 8]. Structurally, it is a neuropeptide consisting of 145 amino acid residues that undergo proteolytic cleavage to a C-terminal active peptide consisting of 54 residues, which further breaks down into shorter forms, i.e., kisspeptins 10, 13, and 14 [9]. Kisspeptin is encoded by the *Kiss1* gene. For example, two homologous genes (*Kiss1* and *kiss2*) encoding kisspeptin were identified in bony fish, with *Kiss1*, and *kiss2* having a higher affinity for *Kiss-R1* and *Kiss-R2*, respectively [10]. The *Kiss1* gene is a conserved ortholog of the mammalian *Kiss1* gene, whereas the *kiss2* gene was found in hypothalamic nuclei only in nonmammalian vertebrates, including amphibians and bony fish [11]. In *Danio rerio* fish, *Kiss1* and *kissr1* matrix ribonucleic acids (mRNAs) are predominantly expressed in the ventral habenula [12]. In nonmammalian vertebrates, the dorsal and ventral habenulas are homologous to the medial and lateral habenulas in mammals [13]. Kisspeptin is expressed in several regions of the rat central nervous system, including the hypothalamic nuclei (e.g., arcuate nucleus and anteroventral paraventricular nucleus), thalamic nuclei, amygdala, hippocampus, lateral septum, bed nucleus of the stria terminalis, corpus striatum, nucleus accumbens, circumventricular gray matter, and *locus coeruleus* [14, 15]. Similarly, *kiss1r* was localized in rat hypothalamus (e.g., paraventricular, arcuate, and supraoptic nucleus), thalamus, hippocampus, amygdala, septum, corpus striatum, suture nuclei, and cerebral cortex [16, 17]. Evidence reveals that *kiss2* is more efficient than K*iss1*, being the most responsible for reproductive behavior. Results of real-time polymerase chain reaction showed that *Kiss1* neurons were localized in the dorsomedial and ventromedial habenulas, with their nerve fibers projecting into the ventral parts of the interpeduncular nucleus and suture nuclei. In turn, *kiss2r* mRNA was widely expressed in the brain, including the olfactory bulb, terminal medulla, preoptic area, midbrain, hypothalamic nuclei, cerebellum, and spinal cord. *kiss2* neurons are mostly localized in the dorsal and ventral hypothalamus, with neural projections passing to several brain regions such as the preoptic area and ventral hypothalamus. Its wide distribution suggests having multiple functions [18, 19].

The preoptic area and hypothalamus are important regions for the distribution of pituitary neurons. In the ventral hypothalamus, *kiss2* neurons were thought to be possibly responsible for regulating reproduction. However, whether these *kiss2* neurons project to the pituitary gland is unclear. A recent study found that *kiss2*, but not *Kiss1*, mRNAs were expressed in the pituitary gland of female *Danio* fish. The distribution patterns of these *kiss2*-positive structures were similar to that of *Gnrh3* fibers, whereas *kiss2* cells were in close contact with *Gnrh3* fibers. The *kiss2* gene directly regulates the expression levels of lhβ, fshβ, and prl1 mRNAs in the pituitary gland of female fish [20]. *For example, Kiss1, and kiss2 mRNAs were detected in the pituitary gland of several teleost species. In chub mackerel, Kiss1 mRNAs were detected in both female and male pituitary glands [21]. By contrast, kiss2 mRNAs were expressed in the pituitary gland of grass puffer during spawning [22]. In European sea bass, Kiss1 and kiss2 mRNAs were detected in the pituitary of males and females [23].*

Kisspeptin's role in teleosts is still unclear. However, in mammals, kisspeptin is fairly well known to be involved in at least fear and reproduction reactions. Most likely, kisspeptin in mammals has similar functions to those in fish. Since the pituitary gland is responsible for the production of gonadotropins, which participate in the development and maturation of the sex glands, and, consequently, sex hormone secretion, an acute stressor may decrease the production of sex hormones and the main regulator gonadotropin. Conversely, evidence reveals *Kiss2-R* immunoreactivity in pituitary corticotropes but not in gonadotropes. This study showed that *Kiss2* and *Kiss2-R* signaling directly performed nonreproductive functions and indirectly subordinate reproductive functions in teleosts [24], presenting difficulties at this stage in knowing the functions of the *kiss2* system. For example, in sea bass, *Kiss1* encodes a peptide identical to rodent kisspeptin 10, whereas the *Kiss2* peptide is not identical. A genome database search showed that both genes are present in the genomes of nonplacental vertebrates. These data were consistent with the results of phylogenetic and mapping analyses that *Kiss1* and *kiss2* are paralogous genes that arose from ancestral gene duplication, although *kiss2* was lost in placental mammals. In addition, mRNA analysis showed the presence of *Kiss1* and *kiss2* in the brain and gonads of sea bass, medaka, and *Danio rerio* fish. In the hormone assay, *Kiss2* induced the secretion of luteinizing and follicle-stimulating hormones in sea bass to a greater extent than *Kiss1*. By contrast, *Kiss2* peptide only weakly induced luteinizing hormone secretion in rats, whereas the *Kiss1* peptide was maximally effective [25].

Danio rerio species have recently become a study object for neurobiologists, geneticists, neuropsychopharmacologists, and toxicologists owing to the following advantages: active swimming, adaptation to new environment, short reproductive period, high fecundity, and low production cost. All these made *Danio rerio* animal models for laboratory studies [26]. Currently, behavioral tests for anxiety, stress, and fear are frequently performed on fish. The novelty test of *Danio rerio* revealed signs corresponding to fear, namely, increased number of freezing (immobilization), diving to the

bottom, and decreased number of transitions to the upper and lower parts of the aquarium; however, increased locomotor activity, decreased freezing, and increased number of transitions to the upper part of the aquarium were observed with acclimatization to the new environment [27–29]. The "predator–prey" model has long been used to assess anxiety state. The prey receives information about the predator's location through olfactory, visual, acoustic, and vibratory signals. Studies have revealed sufficient information on predator perception in fish [30, 31]. The combinations of these predator signals induce an anxious-phobic state in fish [32]. Currently, not much data regarding the predator presentation model used on *Danio rerio* are available.

In this study, novelty stress, and predator stress were assessed along with the administration of bony fish kisspeptins and mammalian kisspeptins. The study aimed to examine the comparative characteristics of these peptides to test their effectiveness.

The study used *Kiss1* and *Kiss2* preparations of kisspeptins in bony fish, a novel kisspeptin analog, and *Kiss10* in mammals. In our previous studies [33, 34], the novelty test was used to analyze the behavioral characteristics of fish in response to a stressful situation. In addition, predator–prey stress studies were conducted along with the administration of bony fish kisspeptins and mammalian kisspeptins.

The *study aimed* to investigate the anxiolytic action of mammalian kisspeptins and bony fish kisspeptins in *Danio rerio* fish.

MATERIALS AND METHODS

Animal selection. Tests were conducted on 105 sexually mature *Danio rerio* (zebrafish or striped Danio) fish aged 6–8 months (young sexually mature animals with a life cycle of up to 5 years) from the Aqua Peter Company and Danio rerio (wild type) bred in the Institute of Experimental Medicine. Intact animals were used for testing after 2 weeks of adaptation to the space and aquariums of 40 L of water displacement, with 20–30 animals in each. A water temperature of 25°C–27°C was maintained constantly. Animals were kept under standard light conditions (8:00–20:00) at a temperature of 22°C ± 2°C and fed two times a day with the standard food "Tetramin tropical flakes." Each group contained at least 10–12 fish.

Novelty stress test. For novelty assessment, a standard viewing aquarium (trapezoidal in shape, 1.5-L displacement, 15 cm high, and 7 cm wide) was used to evaluate anxiety–phobic reactions in *Danio rerio* [35, 36]. The aquarium was 22 and 28 cm long at the base and top, respectively. This design allows for observation of the vertical and horizontal movements. Since this behavioral test is based mainly on the instinct to seek protection from an unfamiliar environment by diving to the bottom [37, 38], the aquarium was divided by a line into two equal parts, i.e., upper and lower. Fish were first placed in a 200-mL measuring beaker with a dissolved pharmacological substance (or water) for 5 min, then in a pre-start aquarium with water (10×10×10 cm^3) for 5 min, and in a viewing aquarium for 6 min, where motor activity during the experiment (fish track length), number of transitions to the upper and lower halves of the aquarium, and time spent therein were recorded. The number and time of freezing (immobilization) patterns per experiment, which are commonly observed during novelty stress and reflect the anxiety level of the animal, were automatically scored [39]. Behavior was recorded automatically using the EthoVision XT7 system (Noldus, Netherlands), which allows both digital recording of readings and visual control of the fish's video track.

Predator-prey test. The test is similar to posttraumatic stress exposure in rats. Intact animals were used for the experiment after 2 weeks of adaptation to the space and aquariums of 40 L of water displacement with 20–30 fish in each. The water temperature of 23°C–25°C was maintained constantly. Animals were kept under standard light conditions (8:00–20:00) at a temperature of 22°C ± 2°C and fed two times a day with the standard food Tetramin tropical flakes.

All animal manipulations were approved by the Local Ethical Committee of the Institute of Experimental Medicine (Minutes No. 12 of September 26, 2019).

A standard viewing aquarium, which was utilized to evaluate anxiety–phobic responses in zebrafish (1.5-L displacement, trapezoidal in shape, 15 cm high, and 7 cm wide), was used to assess the predator stress test. The aquarium was 22 and 28 cm long at the base and top, respectively. In this case, fish were placed in a 200-mL measuring beaker with a dissolved pharmacological agent for 5 min, then in a pre-start aquarium (10×10×10×10 cm^3) with the predator *Hypsophrys nicaraguensis* for 5 min, and in a viewing aquarium for 6 min, which is usually used to assess stimulus novelty. *Kiss1*, *Kiss2*, *Kiss10*, and *KS6* were dissolved in a measuring cup at a dosage of 0.1 mg/L.

Pharmaceuticals. *Kiss1* (pyroglut-NVAYYNLNNSFGLRY-NH_2), *Kiss2* (FNYNPFGLRF-NH_2) of the bony fish synthesized in the Department of General Pathology and Pathophysiology, *Kiss1* mammalian kisspeptin analog of Cloud Clone (USA) *KS6* (differed from *Kiss1* by the terminal fragment), and kisspeptin 10 (Tyr-Asn-Trp-Asn-Ser-Phe-Gly-Leu-Arg-Phe-NH_2) of mammals (State Research Institute of Highly Pure Biopreparations, Russia) were used for pharmacological analysis. All preparations were dissolved in water at a dosage of 0.1 mg/L.

Statistical analysis. The statistical significance of differences was assessed using GraphPad Prism 8.4 (GraphPad Software, USA) and one-factor analysis of variance (ANOVA). One-factor ANOVA was conducted to compare the control group (CG) and the experimental group. The results obtained by analyzing biological materials were determined by Student's *t*-test. The Newman–Keuls criterion for group comparison was used among nonparametric criteria. Differences were considered statistically significant at $p < 0.05$. Data are presented using descriptive statistics such as the arithmetic mean and the error of the mean.

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RESULTS

In the novelty stress test without a predator, *Kiss10* and the *Kiss1* analog of the mammalian *Kiss1* kisspeptin Clone (USA) *KS6* were statistically significant in the "number of freezing." Table 1 shows a significant reduction in the number of freezing in the experimental group compared with the control group. Kisspeptins of bony fish insignificantly reduced this pattern. In addition, *KS6* significantly reduced the freezing time and increased the number of transitions to the top of the aquarium. Moreover, exposure to *Kiss10* increased the number of transitions. However, bony fish kisspeptins reduced anxiety–phobic reactions in fish but to a lesser extent.

In the predator exposure test, kisspeptin decreased the freezing time in both fish and mammals; however, *Kiss10* and *KS6* were statistically significant. In comparison with the CG, freezing under the influence of these drugs was reduced by two times. Simultaneously, the length of the fish's trajectory increased; however, whether motion reactivity may be considered a positive effect of the drug, or whether it is still determined by the fear response, is unclear. In particular, fish-derived kisspeptins did not affect the preference of fish to be at the top of the aquarium compared with the CG. In this case, the fish preferred to be in the lower part, whereas *Kiss10*-, and *KS6*-treated fish had significantly decreased stay in this area. If the number of freezing was assessed, all kisspeptins lowered this parameter, although no statistically significant preparations were identified. Furthermore, the number of movements increased in all groups compared with the CG. The results revealed that *Kiss10* and *KS6* had the strongest effect in response to predator presentation (Table 2).

DISCUSSION

An ecosystem, as a basic natural unit, includes a set of organisms interacting with each other and occupying certain levels in the food chain. The interaction between the predator and the prey, or two-order consumers, is the most common type of relationship. This model is most commonly used by experimenters as one of the stressors that involve a threat from a predator when present [40–42] or the odor of a predator [43–45]. While predator–prey relationships between mammals are still one of the most common research topics, similar interactions between herbivorous, and predatory fish have not gained as much popularity. In an aquatic system, chemical signals are the primary means by which fish detect a predator and assess the possibility of predation [46, 47]. Predator-specific signals allow the prey to develop adaptive defense mechanisms. These most frequently include behavioral, morphological, and physiological changes [46, 48–52]. In response to a predator signal, the prey exhibits a set of short-term behavioral responses such as decreased activity or freezing [51], decreased feeding intensity, stealth displays, and environmental changes [49, 53, 54]. Currently, a distinct lack of information exists on the sensory pathways by which the prey processes the predator's odor. This is partly explained by the lack of intensive studies on fish pheromones. Olfaction and touch are the main sensory pathways for detecting chemicals present in the aquatic environment [55]. Three types of olfactory receptor neurons (ORNs) exist in fish, namely, ciliated, microvillous, and cryptocytic cells, which are assembled into rosettes in the olfactory epithelium. These ORNs project to tubules located in specific regions within the olfactory bulb, resulting in tubules with the same chemosensitivity located next to each other. Chemical information is then transferred from the olfactory bulb through mitral cells to the forebrain, where higher-order olfactory information is processed [56, 57]. ORNs are sensitive to different classes of odors; accordingly, food odors, pheromones, and alarm signals are predominantly processed by separate pathways [56–58]. Exposure to predator odors alters various cognitive traits related to behavior. For example, exposure to a predator's odor may promote learning in general [59–61]. Although exposure to predation risk may

Table 1. Effect of Kiss1, Kiss2, Kiss10, and KS6 (0.1 mL/L) on the behavior of *Danio rerio* fish in the novelty stress test without presenting a predator

Таблица 1. Действие Kiss1, Kiss2, Kiss10, KS6 (0,1 мл/л) на поведение рыб *Danio rerio* в тесте стресса новизны без предъявления хищника

Group	Number of freezing, *n*	Freezing time, s	Trajectory length, cm	Time at the bottom of the aquarium, s	Number of transitions to the top of the aquarium
Control	81.38 ± 4.95	41.35 ± 2.3	1643 ± 289.8	213.9 ± 32.46	20.67 ± 6
Kiss1	61.33 ± 3.61	35.92 ± 1.52	1310 ± 205.8	275.3 ± 22.67	34.67 ± 8
Kiss2	64.25 ± 6.67	38.85 ± 1.75	1792 ± 476	210.6 ± 44.83	30.33 ± 6.8
Kiss10	46.17 ± 11.15*	28.42 ± 7.96	1163 ± 155.6	224.4 ± 38.58	44.17 ± 5.5*
KS6	29.67 ± 4.88***	18.92 ± 5.520**	663.6 ± 188.6*	183.1 ± 84.21	42.0 ± 6.0*

Note: *$p < 0.05$; **$p < 0.005$; ***$p < 0.0001$ relative to the control group.

Table 2. Effect of Kiss1, Kiss2, Kiss10, and KS6 (0.1 mL/L) on the behavior of *Danio rerio* fish in the novelty stress test with the presentation of a predator

Таблица 2. Действие Kiss1, Kiss2, Kiss10 и KS6 (0,1 мл/л) на поведение рыб *Danio rerio* в тесте стресса новизны с предъявлением хищника

Group	Number of freezing *n*	Freezing time, s	Trajectory length, cm	Time at the bottom of the aquarium, s	Number of transitions to the top of the aquarium
Control	104.7 ± 15.7	53.14 ± 7.38	608.7 ± 96.19	326.6 ± 22.92	9.6 ± 4.2
Kiss1	61.86 ± 12.7	33.43 ± 5.51	993.2 ± 143.6*	352 ± 4.95	23.86 ± 5.2
Kiss2	69.71 ± 10	34.93 ± 5.02	1810 ± 499.8*	350.3 ± 4.55	11.43 ± 4.2
Kiss10	61.3 ± 5.13*	34.36 ± 2.8*	1108 ± 208.8	185.7 ± 11.75***	15 ± 2.6
KS6	62.93 ± 5.8*	32.8 ± 2.9*	1135 ± 191.9*	188.9 ± 12.69***	24 ± 5.6

Note: *$p < 0.05$; **$p < 0.005$; ***$p < 0.0001$ relative to the control group.

enhance cognitive traits related to predator recognition, other cognitive functions, such as spatial learning, may be impaired [62]. Thus, if mammals produce a characteristic set of persistent behavioral responses to a single exposure by a predator, this stress will cause similar changes in fish as a confirmation of the hypothesis of common genes responsible for the development of affective disorders among different evolutionary chains [63].

Previous studies have shown that the novelty stress test is sensitive to anxiety–phobic reactions in *Danio rerio*. Our studies confirmed that the response to the novelty of being placed in a viewing aquarium demonstrates typical behavioral patterns in *Danio rerio* (zebrafish). The fish reacted by diving to the bottom, freezing, and having decreased locomotor behavior [33, 36, 39]. Freezing was frequently observed, with quite high number, and time per experiment, as was the time the fish spent at the bottom of the aquarium. The results obtained largely agree with the literature [29, 64].

In the analysis of the behavioral activities of lower vertebrates, predator-related stress showed the most striking reaction compared with novelty stress. However, these techniques represent anxiety–phobic reactions quite well, which suggests that fish behavior may be considered a screening model for the development of new drugs that normalize mental state. In this study, kisspeptin preparations were examined, which were hypothesized to have anxiolytic effects. In the comparative analysis, kisspeptins indeed inhibit the anxiety–phobic state of fish after both novelty and predator stresses. The present study showed that the number of kisspeptin-induced freezing and freezing time decreased in models of novelty stress and predator stress in comparison with the CG. The number of transitions to the top of the aquarium increased. However, no significant difference in the time the fish were in the lower part of the aquarium was found when compared with the CG. *Kiss10*, the mammalian kisspeptin analog of *KS6*, exhibited the most characteristic signs of anxiolytic effect. The highest number of statistically significant indices was found in *KS6*. In addition, bony fish kisspeptins reduced anxiety patterns, but to a lesser extent. *Kiss2* in teleosts, which predisposes fish to sexual behavior (Table 2), has a minor anxiolytic effect and does not differ significantly from the CG; however, some evidence reveals that fear reduction leads to mate-seeking. Thus, the hypothesis that these drugs have these expected effects was confirmed. Nevertheless, their effectiveness for further application is still unclear, providing a reason to continue the study in lower vertebrate biochemistry.

CONCLUSIONS

1. Bony fish kisspeptins and mammalian kisspeptins reduced anxiety–phobic responses in *Danio* fish; however, mammalian kisspeptins were more effective.

2. The results support the hypothesis that kisspeptins may be involved in the regulation of anxiety–phobic states, apparently to maintain emotional aspects of reproductive behavior such as sexual motivation and arousal.

3. Compared with *Kiss2*, *Kiss1* kisspeptin has anxiolytic effects, suggesting that *Kiss1* affects fear reduction, whereas *Kiss2* appears to be responsible for social and sexual behavior in *Danio rerio* fish.

ADDITIONAL INFORMATION

Authors contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study. The contribution of each author: V.A. Golts, A.A. Blazhenko, V.A. Lebedev, A.A. Bayramov, P.P. Khokhlov, E.R. Bychkov, S.S. Purveev, S.V. Kazakov — manuscript drafting, writing and pilot data analyses; A.A. Lebedev, P.D. Shabanov — general concept discussion.

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