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Research Article

Effect of social isolation and kisspeptin analogues (KS6 and KS10) on the expression of KISS1, KISS2, and their receptors in danio rerio

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Abstract

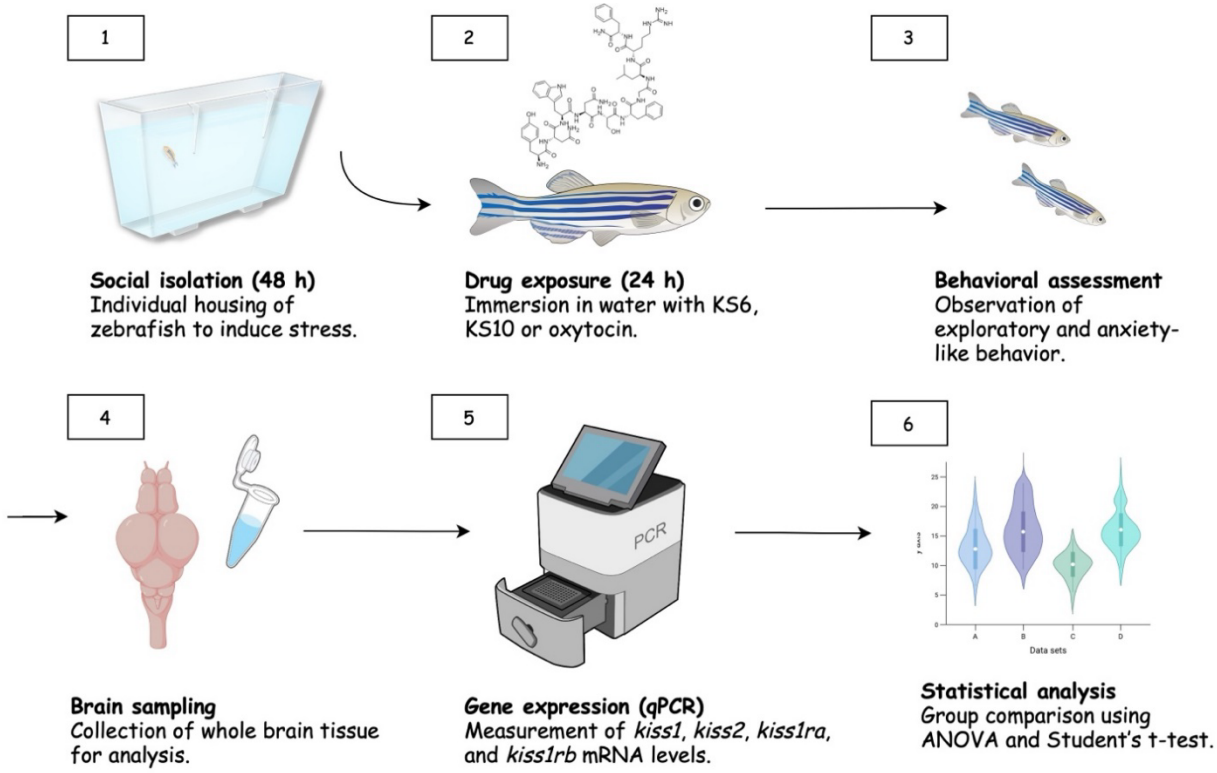
Introduction: The kisspeptin system regulates both reproductive and stress-related neuroendocrine functions. In zebrafish (*Danio rerio*), social isolation disrupts the expression of *kiss1, kiss2*, and their receptors (*kiss1ra, kiss1rb*). This study investigates whether kisspeptin analogues KS6 and KS10 can reverse these effects and compares them with the well-known neuropeptide oxytocin.

Materials and Methods: Adult zebrafish were exposed to 48-hour social isolation and treated with KS6, KS10 (0.1 mg/L), or oxytocin (0.019 IU/L). Total RNA was extracted from whole brains, followed by cDNA synthesis and quantitative PCR targeting kiss1, kiss2, kiss1ra, and kiss1rb. Gene expression was normalized to gapdh. Statistical significance was assessed using one-way ANOVA and Student's t-test.

Results and Discussion: Social isolation downregulated kiss1, kiss2, and kiss1ra, and upregulated kiss1rb. KS6 significantly increased kiss1 expression and normalized kiss1rb levels. KS10 partially restored kiss1 and kiss2, but reduced kiss1ra and further elevated kiss1rb. Oxytocin reversed all isolation-induced changes. KS6 showed the most consistent restorative effects, whereas KS10 demonstrated a more selective receptor profile, suggesting differential downstream signaling.

Conclusion: KS6 effectively reversed social isolation-induced dysregulation of kisspeptin signaling in zebrafish, indicating its potential for modulating neuroendocrine responses to stress. KS10 exhibited selective receptor modulation, which may be valuable for fine-tuned therapeutic strategies. These findings highlight the distinct pharmacological profiles of kisspeptin analogues in stress-related contexts.

Graphical abstract



Keywords

Danio rerio, gene expression, kisspeptin analogues, neuroendocrinology, oxytocin, social isolation, stress response, zebrafish

Introduction

The kisspeptin system is well-conserved across vertebrates and acts as a major regulator of the hypothalamic-pituitary-gonadal (HPG) axis. In mammals, kisspeptins activate the KISS1 receptor (KISS1R) to stimulate gonadotropin-releasing hormone (GnRH) secretion, resulting in the production of gonadotropins and the regulation of puberty and fertility. Mutations in KISS1 or KISS1R lead to severe reproductive phenotypes, such as hypogonadism or precocious puberty (Biran et al. 2008).

In teleosts, including zebrafish (*Danio rerio*), the kisspeptin system is more complex due to gene duplication events. These fish have two kisspeptin ligands, KISS1 and KISS2, and two receptor genes, *kiss1ra* and *kiss1rb*. These paralogs allow functional specialization: *kiss1ra* is primarily expressed in the brain and gonads, while *kiss1rb* is expressed in peripheral tissues, including the kidney and intestines (Biran et al. 2008; Shahjahan et al. 2010). Functional studies revealed that the two receptors have different ligand preferences: KISS1Ra is activated by both KISS1 and KISS2, whereas KISS1Rb shows a significantly greater preference for KISS1. They also differ in their downstream signaling pathways, as *kiss1ra* relies solely on the PKC pathway, while *kiss1rb* utilizes both PKC and PKA pathways (Shahjahan et al. 2010; Onuma and Duan 2012).

The primary role of kisspeptins is the regulation of reproductive functions. In zebrafish, *kiss1* and *kiss2* exhibit tissue-specific expression: *kiss1* is primarily expressed in the hypothalamus, while *kiss2* is more broadly expressed in the hypothalamus, gonads, and kidneys (Biran et al. 2008). Studies have shown that KISS2 is a key regulator of reproductive hormone secretion in zebrafish, with intraperitoneal injections of KISS2 decapeptide stimulating the expression of follicle-stimulating hormone beta (*fshβ*) and luteinizing hormone beta (*lhβ*) genes in the pituitary (Shahjahan et al. 2010; Shi et al. 2010).

In *Danio rerio*, neurons expressing *kiss1* are located along the borders of the hypothalamic preoptic area, which is responsible for the reproductive function of the hypothalamic-pituitary-gonadal axis. *Kiss2* neurons are found in the upper part of the posterior hypothalamus and an area within the posterior thalamus. While these neurons also contribute to reproductive control, they may have more specialized functions, such as responding to environmental changes (Sivalingam and Parhar 2022). Kisspeptins influence processes beyond reproduction, including the modulation of stress. Kisspeptin-8 has been shown to induce anxiety-like behavior in rats by activating the HPA axis and increasing GABA release in the nucleus accumbens (Ibos et al. 2021). Zebrafish exhibit similar behavior and emotional regulation following the administration of kisspeptin analogues, as evidenced by changes in anxiety and exploratory behavior (Ibos et al. 2021).

Social isolation becomes a great stressor and influences behavior in zebrafish with respect to neuroendocrine systems. These animals are innately social and possess neural circuits that are well defined, making them an excellent candidate for modeling social stress based on observable behavior. Prolonged isolation has previously shown to induce significant behavioral changes, including reduced social preference and increased anxiety-like behaviors along with altered exploratory activity (Tunbak et al. 2020). The altered behavior is generally associated with altered expression of reproductive genes and stress-related genes like *kiss1*, *kiss2*, and their receptors (Tunbak et al. 2020; Perova et al. 2024).

Mapping the entire brain of socially isolated zebrafish has indicated increased activity in those brain regions that deal with stress and social behavior. This is in conjunction with reports from mammals, where isolation-induced stress is shown to cause neuroendocrine pathways dysregulation (Tunbak et al. 2020).

In this context, oxytocin serves as a particularly compelling reference compound for comparison. Its evolutionary conservation and well-established role in social and stress-related behaviors across vertebrates provide an essential framework for understanding neuroendocrine modulation. Recent studies (Akinrinade et al. 2023) underscore its critical involvement in mediating social fear contagion and adaptive responses in zebrafish. These findings align with oxytocin's capacity to regulate the hypothalamic-pituitary-adrenal (HPA) axis and modulate pathways such as GABA release and corticotropin-releasing hormone (CRH) signaling. These neurochemical mechanisms position oxytocin as a robust baseline for evaluating the effects of novel pharmacological agents like kisspeptin analogues.

Furthermore, oxytocin's demonstrated ability to mitigate stress-induced dysregulation of neuroendocrine pathways strengthens its relevance as a comparator. It has been shown to normalize altered expression profiles for genes related to social behavior and HPA axis activity, so it serves as a benchmark to evaluate and test interventions impacting stress by monitoring their effects on reproductive gene expression.

KS6 and KS10 refer to kisspeptin 6 and kisspeptin 10, which are functional fragments of the larger kisspeptin family. These peptides are of particular interest due to their unique receptor-binding profiles and physiological actions. In zebrafish, the administration of these kisspeptin analogues has been shown to influence behavior, highlighting their potential role as mediators between the neuroendocrine system and behavior (Ibos et al. 2021). Differences in the expression of *kiss1ra* and *kiss1rb* between isolated and non-isolated zebrafish provide insights into the mechanisms by which social stress modulates the expression of reproductive and stress-related genes (Tunbak et al. 2020).

The administration of kisspeptin 6 and kisspeptin 10 demonstrated clear anxiolytic and exploratory behavior-enhancing effects under stress conditions caused by novelty (Lebedev et al. 2022). KS6 and KS10 were selected for this study due to their unique receptor-binding profiles and physiological actions. KS6, a shorter fragment of the kisspeptin family, has been shown to exhibit high affinity for the Kiss1Ra receptor, making it particularly effective in modulating stress-related pathways in the brain (Ibos et al. 2021). On the other hand, KS10, a longer peptide, demonstrates a broader range of interactions with both Kiss1Ra and Kiss1Rb receptors, allowing for more complex modulation of neuroendocrine responses (Lebedev et al. 2022). Previous studies have demonstrated that KS6 and KS10 effectively reduce anxiety-like behaviors and enhance exploratory activity in zebrafish under stress conditions, making them ideal candidates for investigating the role of kisspeptins in stress regulation (Ibos et al. 2021; Lebedev et al. 2022).

In comparison to other neuropeptides, such as oxytocin, kisspeptins offer unique advantages in modulating stress responses. While oxytocin is well-known for its role in social bonding and stress reduction, its effects are often limited to specific contexts and may not fully address the complexity of stress-induced neuroendocrine dysregulation (Akinrinade et al. 2023). Kisspeptins, on the other hand, have a broader range of actions, including the regulation of reproductive functions and stress responses, making them more versatile therapeutic agents (Shahjahan et al. 2010). This multi-faceted approach allows kisspeptins to address both the physiological and behavioral aspects of stress, providing a more comprehensive solution compared to oxytocin.

Materials and Methods

Animal selection

The study involved 60 adult zebrafish (*Danio rerio*) aged 6–8 months (young adults, with a lifespan of up to 5 years), reared at the Federal Research Center "Institute of Experimental Medicine" (IEM) (Saint-Peterburg, Russia). The fish were divided into five groups, each consisting of 12 individuals. Testing was conducted on intact animals following a two-week adaptation period in a 40-liter aquarium containing 20–30 fish per tank. Water temperature was maintained at 25–27°C. Standard light conditions were provided (8:00–20:00), simulating 12 hours of light and 12 hours of darkness daily, with a room temperature of 22 ± 2°C. Fish were fed twice daily with a standard diet ("Tropical Flakes", TetraMin, USA).

Social isolation test

Fish were placed individually into 200-mL containers for 48 hours. After this period of social isolation, the effects of kisspeptin analogues (KS6 and KS10) were assessed. Subsequently, fish were returned to 200-mL containers for an additional 24 hours before sampling their entire brain tissue, encompassing all brain structures.

Gene expression analysis

To evaluate the expression of *kiss1*, *kiss2*, and their receptors *kiss1ra* and *kiss1rb*, mRNA was extracted from dissected brain tissues using a standard protocol. The brain tissue was homogenized in 1,000 µL of Trizol and incubated for 5 minutes at 40°C. Afterward, 200 µL of chloroform was added, the samples were mixed, incubated for 5 minutes with gentle agitation, and centrifuged at 13,000 g for 10 minutes to collect the upper aqueous phase. Isopropanol, equal to the volume of the aqueous phase, was added to precipitate RNA. The mixture was incubated overnight at −20°C, followed by centrifugation at 13,000 g for 10 minutes. The RNA pellet was washed with 70% ethanol, dried at 40°C, and dissolved in 50 µL of dH2O containing 1% RNAse inhibitor. cDNA synthesis was then carried out through reverse transcription, and real-time PCR was performed using primers specific to *kiss1*, *kiss2*, *kiss1ra*, and *kiss1rb*. GAPDH was used as the reference gene.

Pharmacological substances

Kisspeptin analogues (KS6 and KS10) obtained from the USA were tested at a dose of 0.1 mg per 1,000 mL of water. This concentration was determined to be the most effective in prior screenings (Golts et al. 2024), as demonstrated in previous studies (Ibos et al. 2021; Lebedev et al. 2022). Specifically, these studies showed that KS6 and KS10 at a concentration of 0.1 mg/L significantly modulated stress-related behaviors and gene expression in zebrafish without causing adverse effects. Oxytocin (Gedeon Richter, Hungary) was used as a comparator at a dose of 3.8 µL (0.005 IU/µL) per 50 mL of aquarium water (0.019 IU/L).

Kisspeptin analogues were administered via water immersion, a validated method for drug delivery in *Danio rerio*. This approach allows for systemic absorption through the gills and intestinal tract, ensuring consistent bioavailability. Previous studies have demonstrated the effectiveness of this method for peptide administration in zebrafish (Lebedev et al. 2022).

Statistical analysis

Each experiment was repeated three times to ensure reproducibility and reliability of the results. For gene expression analysis, 12 fish were used per group, and each group was tested in triplicate.

Sample size (n = 12 per group) was determined using power analysis (GPower 3.1) with $\alpha = 0.05$, 80% power, and an expected effect size of $d = 0.8$. Normality was assessed using the Shapiro–Wilk test, and outlier detection was performed using Grubbs' test ($p < 0.05$). Data points identified as significant outliers were excluded.

Quantitative data were analyzed using GraphPad Prism v.8.0 software. Results are presented as the mean ± standard error of the mean (M ± SEM). Statistical differences between the groups were assessed using one-way analysis of variance (ANOVA). For pairwise comparisons, Student's t-test for independent samples was applied. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

The study revealed the following effects of kisspeptin analogues (KS6, KS10) under conditions of social isolation:

Kiss1 gene expression

- In the group of socially isolated animals, Kiss1 expression decreased three times compared to the control group ($p < 0.05$).
- Treatment with oxytocin resulted in an 8.5 times increase in expression compared to the untreated isolated animals ($p < 0.05$) and a 2.8 times increase compared to the control animals ($p < 0.05$).
- Treatment with KS6 increased Kiss1 expression 7.2 times compared to the untreated isolated animals ($p < 0.05$) and 2.4 times compared to the control animals ($p < 0.05$).
- Treatment with KS10 increased Kiss1 expression 3.31 times compared to the untreated isolated animals ($p < 0.05$), but no differences were observed compared to the control group. Data are shown in Figure 1.

Kiss2 gene expression

- In the group of socially isolated animals, Kiss2 expression decreased 1.83 times compared to the control group ($p < 0.05$).
- Treatment with oxytocin restored Kiss2 expression to the level of the control animals ($p < 0.05$), as did treatments with KS6 and KS10 ($p < 0.05$).
- KS6 showed a slightly greater tendency to increase Kiss2 expression compared to other treatments. Data are shown in Figure 2.

Kiss1Ra gene expression

- In the group of socially isolated animals, Kiss1Ra expression decreased 9.21 times compared to the control group ($p < 0.05$).
- Treatment with oxytocin resulted in a twofold increase in expression ($p < 0.05$).
- KS6 showed only a tendency to increase Kiss1Ra expression, while KS10 decreased it three times compared to the untreated isolated animals ($p < 0.05$). Data are shown in Figure 3.

Kiss1Rb gene expression

- In the group of socially isolated animals, Kiss1Rb expression increased six-fold compared to the control group ($p < 0.05$).
- Treatment with oxytocin reduced Kiss1Rb expression 22 times compared to the untreated isolated animals and three-fold compared to the control group ($p < 0.05$).
- KS6 reduced Kiss1Rb expression 5.8 times compared to th euntreated isolated animals, bringing it to the level observed in the control group.
- KS10 showed only a tendency to reduce *kiss1rb* expression compared to the isolated animals but increased it five times compared to the control group ($p < 0.05$). Data are shown in Figure 4.

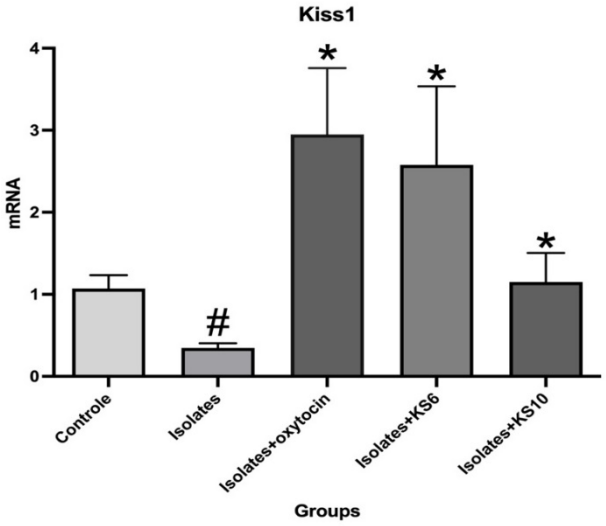


Figure 1. Expression levels of the kiss1 gene in zebrafish brain after social isolation and treatment with kisspeptin analogues or oxytocin. ***Note:*** *#— $p<0.05$ compared to control group, *— $p<0.05$ compared to the social isolation group. Data are presented in arbitrary units, normalized to the expression level of the reference gene glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and calculated as relative units in relation to the average Kiss1 gene expression across groups. Normalization was performed relative to the reference gene (Gapdh). Data are presented as mean ± standard error of the mean.* **Control** – group without social isolation; **Isolates** – social isolation group; **Isolates+oxytocin** – social isolation group treated with oxytocin; **Isolates+KS6** – social isolation group treated with KS6; **Isolates+KS10** – social isolation group treated with KS10.

Expression of kiss1 was significantly reduced in the socially isolated group compared to control. Treatment with KS6 and oxytocin significantly increased kiss1 expression, with KS6 restoring levels comparable to oxytocin. KS10 moderately increased kiss1 expression.

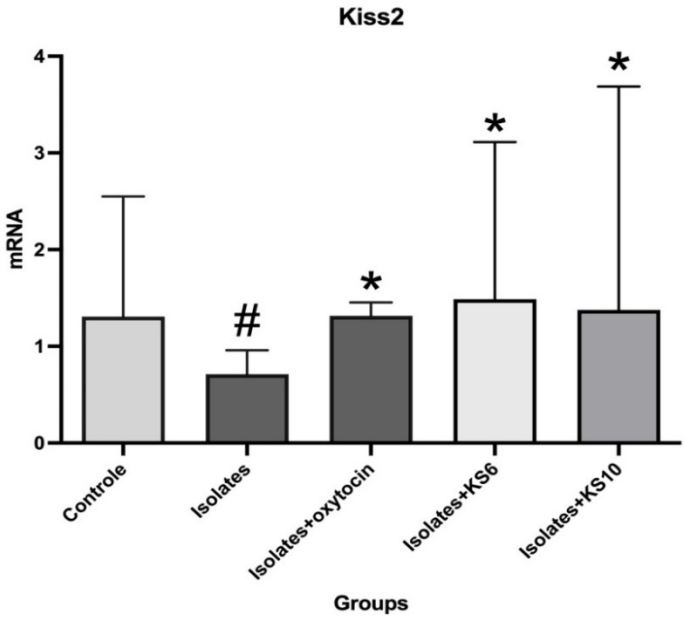


Figure 2. Expression levels of the kiss2 gene in zebrafish brain after social isolation and treatment with kisspeptin analogues or oxytocin. *Note: # — p<0.05 compared to control group, * — p<0.05 compared to the social isolation group. Data are presented in arbitrary units, normalized to the expression level of the reference gene glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and calculated as relative units in relation to the average Kiss1 gene expression across groups. Normalization was performed relative to the reference gene (Gapdh). Data are presented as mean ± standard error of the mean.* **Control –** group without social isolation; **Isolates –** social isolation group; **Isolates+oxytocin –** social isolation group treated with oxytocin; **Isolates+KS6 –** social isolation group treated with KS6; **Isolates+KS10 –**: social isolation group treated with KS10.

Social isolation led to a decrease in kiss2 expression. All treatments (oxytocin, KS6, KS10) restored expression to levels comparable to the control group, with KS6 showing a slightly stronger effect.

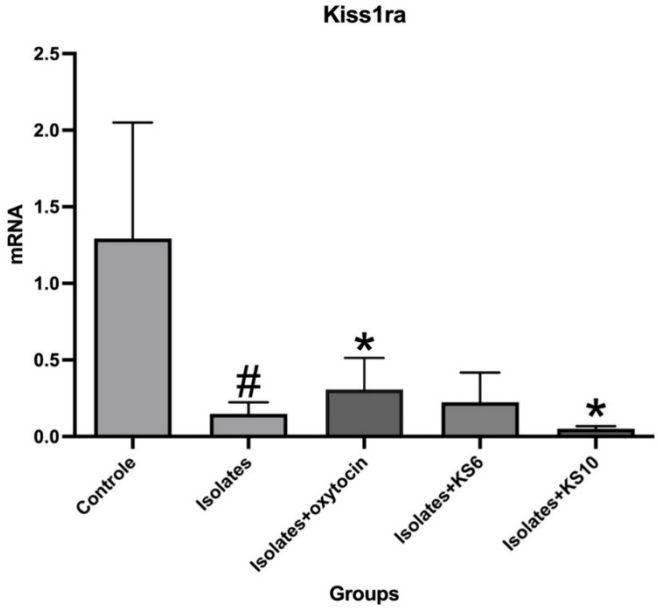


Figure 3. Expression levels of the kiss1ra gene (kisspeptin receptor A) after social isolation and pharmacological treatment in zebrafish. ***Note:*** *# — p<0.05 compared to control group, * — p<0.05 compared to the social isolation group. Data are presented in arbitrary units, normalized to the expression level of the reference gene glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and calculated as relative units in relation to the average Kiss1 gene expression across groups. Normalization was performed relative to the reference gene (Gapdh). Data are presented as mean ± standard error of the mean.* **Control –** group without social isolation; **Isolates –** social isolation group; **Isolates+oxytocin** – social isolation group treated with oxytocin; **Isolates+KS6 –** social isolation group treated with KS6; **Isolates+KS10 –** social isolation group treated with KS10.

Social isolation significantly downregulated kiss1ra. Oxytocin partially restored its expression. KS6 showed a non-significant upward trend, while KS10 further reduced kiss1ra expression.

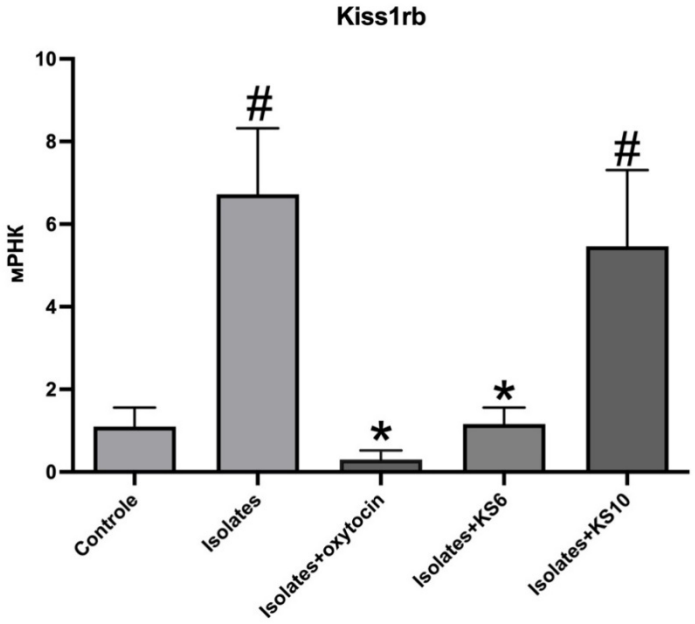


Figure 4. Expression levels of the kiss1rb gene (kisspeptin receptor B) in zebrafish brain under social isolation and after treatment. ***Note:*** *# — $p<0.05$ compared to control group, * — $p<0.05$ compared to the social isolation group. Data are presented in arbitrary units, normalized to the expression level of the reference gene glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and calculated as relative units in relation to the average Kiss1 gene expression across groups. Normalization was performed relative to the reference gene (Gapdh). Data are presented as mean ± standard error of the mean.* **Control –** group without social isolation; **Isolates** – social isolation group; **Isolates+oxytocin –** social isolation group treated with oxytocin; **Isolates+KS6 –** social isolation group treated with KS6; **Isolates+KS10 –** social isolation group treated with KS10.

Social isolation induced a strong increase in kiss1rb expression. KS6 and oxytocin significantly reduced kiss1rb levels, with KS6 normalizing them to control values. KS10 slightly reduced expression but remained elevated relative to control.

The effects of KS6 and KS10 on socially isolated zebrafish (*Danio rerio*) demonstrated significant changes in the expression of genes associated with stress and reproductive behavior. KS6 consistently enhanced the expression of *kiss1* and normalized *kiss1rb* levels, highlighting role of KS6 in activating central stress-response pathways and restoring neuroendocrine balance. These findings align with earlier studies in mammals, which have shown that kisspeptin analogues can stimulate neuropeptides like corticotropin-releasing hormone (CRH), a marker of hypothalamic-pituitary-adrenal (HPA) axis activation (Lebedev et al. 2022; Sivalingam and Parhar 2022).

In contrast, KS10 presented a more complicated regulatory profile. It raised *kiss1* expression slightly, but reduced it further by showing a modulatory rather than increaser-type of action on *kiss1ra* and *kiss1rb*. KS10 reduced *kiss1ra* expression, which possibly indicates that it is associated with receptor-specific signaling pathways that differ from those activated by KS6. This selectivity of modulation points to the fact that there is significant diversity of receptor-ligand interactions as far as the kisspeptin system is concerned, thus making the role it plays in stress and reproductive adaptation of high complexity (Tunbak et al. 2020; Lebedev et al. 2022).

Moreover, the fact that KS6 and KS10 both reinstated *kiss2* expression strongly attests to the evolutionary conservation of this paralog in modulating homeostatic responses to stress. In fact, when one compares them in terms of the ability to stimulate *kiss2* expression, KS6 was slightly better than KS10, indicating a more potent role in neuroendocrine control.

Interestingly, the contrasting effects of KS6 and KS10 on *kiss1ra* and *kiss1rb* expression suggest a balancing act between stress adaptation and reproductive functions. KS6's ability to normalize *kiss1rb* expression while enhancing *kiss1ra* highlights its dual role in stress mitigation and reproductive regulation. On the other hand, KS10's tendency to increase *kiss1rb* expression compared to controls may indicate its potential as a modulator rather than a direct activator of stress-related pathways (Tunbak et al. 2020; Ibos et al. 2021).

The results suggest a differential impact of KS6 and KS10 on receptor interactions, potentially linked to variations in their binding affinities or downstream signaling pathways. KS6's broader influence on neuroendocrine pathways, as evidenced by its consistent effects on *kiss1* and *kiss2*, supports its potential therapeutic value in addressing stress-induced

dysregulation. Meanwhile, the nuanced role of KS10 may offer opportunities to explore selective modulation of specific receptors for targeted outcomes.

The therapeutic potential of kisspeptin analogues is further supported by recent findings in mammalian models. For example, it was demonstrated that KS10 effectively reduces sexual dysfunction in rats exposed to acute psychogenic stress, suggesting that kisspeptin-based interventions may have broader applications in managing stress-related neuroendocrine dysregulation (Lebedev et al. 2022). This aligns with our observations in zebrafish, where KS6 and KS10 showed significant effects on stress-related gene expression, further underscoring the potential of these analogues as therapeutic agents for stress-induced disorders.

Contrastingly to oxytocin, which provides stabilization of stress and reproduction gene expression, KS6 complements this aspect significantly at the level of *kiss1* and *kiss1rb* rebalancing, thereby signifying the advantage of a combined oxytocin and kisspeptin analogue treatment for therapeutic purposes. Although KS10 demonstrates less consistent effects on its own, it may act as a modulator in situations where receptor-specific signaling is desired.

Conclusion

Kisspeptin analogues KS6 and KS10 exerted significant modifications on the expression of genes involved in stress and reproductive behavior under social isolation conditions in zebrafish. Specifically, the enhancement of *kiss1* and *kiss1ra* expression, along with normalization of *kiss1rb* levels, indicated the potential role of KS6 in strongly activating central stress-response pathways and restoring the neuroendocrine balance. In contrast, KS10 exhibited a more complex and selective profile by showing distinct effects from KS6 in terms of its modulation of *kiss1ra* and *kiss1rb*.

Both compounds were administered via water immersion at a concentration of 0.1 mg/L, which had previously been identified as the most effective dose in behavioral screening assays without inducing adverse effects. For comparative purposes, oxytocin was used at 0.019 IU/L. These concentrations were selected based on earlier dose-finding experiments and provided reliable modulation of gene expression in the current model.

The restoration of *kiss2* expression by both analogues suggests an evolutionarily conserved role for this gene in managing homeostatic responses to stress. The differential effects observed between KS6 and KS10 underscore the complexity of the kisspeptin system and highlight the potential of these analogues as therapeutic agents. KS6, with its pronounced effects on stress adaptation and reproductive regulation, shows promise as a potential candidate for future research into treatments for stress-related disorders. KS10's modulating role may also hold therapeutic value, particularly in fine-tuning neuroendocrine responses.

These findings suggest that exposure to kisspeptin analogues may influence neuroendocrine pathways regulating stress and social behavior. The differential effects of KS6 and KS10 highlight their complexity in interacting with the kisspeptin system. This complexity may lead to different therapeutic applications for these analogues in the future. Future directions should include examining the exact molecular underpinnings of different kisspeptin analogues, their receptor-binding dynamics, and dose-dependent effects. Finally, the potential of these molecules for therapies targeting stress-related disorders and social dysfunctions should provide valuable insights for their translational applications.

Additional information

Conflict of interest

The authors declare the absence of a conflict of interests.

Funding

The work was carried out within the framework of the State task of the Ministry of Education and Science of Russia "Search for molecular targets for pharmacological intervention in addictive and neuroendocrine disorders to create new pharmacologically active substances acting on CNS receptors", code FGWG-2025-0020.

Data availability

All of the data that support the findings of this study are available in the main text.

Ethics Statements

The methods used in the work were approved by the Ethical Committee for the Care and Use of Animals of the Institute of Experimental Medicine (Minutes No. 21/5 dated February 26, 2015), Saint-Petersburg, Russia.

References

- Akinrinade I, Kareklas K, Teles MC, Reis TK, Gliksberg M, Petri G, Levkowitz G, Oliveira RF (2023) Evolutionarily conserved role of oxytocin in social fear contagion in zebrafish. Science 379(6638): 1232–1237. https://doi.org/10.1126/science.abq5158 [PubMed]
- Biran J, Ben-Dor S, Levavi-Sivan B (2008) Molecular identification and functional characterization of the kisspeptin/kisspeptin receptor system in lower vertebrates. Biology of Reproduction 79(4): 776–786. https://doi.org/10.1095/biolreprod.107.066266 [PubMed]
- Golts VA, Perova AP, Lebedev AA, Lizunov AV, Nadbitova ND, Bychkov ER, Purveev SS, Shabanov PD (2024) Effect of kisspeptin analogues on exploratory behavior in zebrafish. Experimental and Clinical Pharmacology 87(12): 3–7. https://doi.org/10.30906/0869-2092-2024-87-12-3-7
- Ibos KE, Bodnár É, Bagosi Z, Bozsó Z, Tóth G, Szabó G, Csabafi K (2021) Kisspeptin analogues modulate stress-induced behaviors in zebrafish. Biomedicines 9(2): 112. https://doi.org/10.3390/biomedicines9020112
- Ogawa S, Parhar IS (2018) Biological significance of kisspeptin-kiss 1 receptor signaling in the habenula of teleost species. Frontiers in Endocrinology (Lausanne) 9: 222. https://doi: 10.3389/fendo.2018.00222. PMID: 29867758; PMCID: PMC5949316.
- Onuma TA, Duan C (2012) Regulation of the endocrine and growth systems by the kisspeptin/GPR54 system in fish. The FASEB Journal 26(7): 2941–2950. https://doi.org/10.1096/fj.11-201095 [PubMed]
- Perova AP, Golts VA, Lebedev AA, Bychkov ER, Beznin GV, Kosyakova GP, Shabanov PD (2024) Comparative effect of kisspeptin analogues on gene expression under social stress in zebrafish. Clinical Pharmacology & Drug Therapy 22(2): 76–80. https://doi.org/10.25557/2310-0435.2024.02.76-80
- Shahjahan M, Doi H, Ando H, Kitahashi T, Parhar IS (2010) Role of kisspeptin in fish reproduction. General and Comparative Endocrinology 169(1): 48–57. https://doi.org/10.1016/j.ygcen.2010.07.009 [PubMed]
- Shi Y, Zhang Y, Wang Y, Wang Y, Wu J, Xu Q (2010) Kisspeptin and gonadotropin regulation in zebrafish. Biology of Reproduction 83(1): 63–74. https://doi.org/10.1095/biolreprod.109.080044
- Sivalingam M, Parhar IS (2022) Neuroendocrine regulation by kisspeptin in teleosts: multiple functions beyond reproduction. Frontiers in Neuroendocrinology 64: 100951. https://doi.org/10.1016/j.yfrne.2021.100951
- Tunbak H, Vazquez-Prada M, Hollis V, Williams A, Aryal A, Calvigioni D, Westmoreland T, Randlett O (2020) The impact of social isolation on brain activity in zebrafish. eLife 9: e55863. https://doi.org/10.7554/eLife.55863

Author Contribution

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