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Evaluation of Kisspeptin Transport Across the Blood–Brain Barrier After Intranasal Administration

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ABSTRACT

BACKGROUND: The kisspeptin family (KISS1), encoded by the kiss1 gene, is among the newly identified yet underexplored peptide families in terms of efficacy and intranasal delivery. Kisspeptins are involved not only in reproductive function but also in behavioral, emotional, and cognitive processes. Efficient delivery of kisspeptins to the central nervous system could open new perspectives for their application.

AIM: The work aimed to evaluate the efficacy of kisspeptin-10 transport across the blood–brain barrier after intranasal administration.

METHODS: The study included 45 outbred mice. Animals received kisspeptin-10 intranasally at doses of 0.1, 1, and 10 μg, and intraperitoneally at doses of 1, 10, and 100 μg. Animal behavior was assessed using the open field test, elevated plus maze, and sexual motivation test.

RESULTS: In the present study, stable and dose-dependent effects of kisspeptin-10 on mouse behavior were observed after intranasal administration. Intranasal kisspeptin-10 induced statistically significant increases in sexual motivation, horizontal and vertical locomotor activity, reduced anxiety, and enhanced exploratory behavior in sexually mature male mice. The most pronounced behavioral changes were produced by the 10 μg dose, exerting central effects after intranasal administration compared with the groups receiving intraperitoneal administration. In contrast, intraperitoneal kisspeptin-10 at doses of 1 μg and 10 μg produced virtually no behavioral changes. Increasing the intraperitoneal dose to 100 μg resulted in statistically significant behavioral changes; however, the effect was less pronounced than that observed after intranasal administration of 10 μg.

CONCLUSION: Statistically significant behavioral changes following intranasal administration required concentrations 10-fold lower than those needed for peripheral administration. Given the evident effects of intranasal kisspeptin-10 in each behavioral test, it can be assumed that kisspeptin-10 penetrated the brain, bypassing the blood–brain barrier, and exerted central effects. These findings support the potential feasibility and importance of this delivery route for targeting the central nervous system.

Keywords: kisspeptin; intranasal administration; blood–brain barrier; central nervous system; central nervous system drug delivery systems; sexual motivation; elevated plus maze; open field test; behavioral tests; sexual behavior; mice.

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Оценка транспортировки кисспептина через гематоэнцефалический барьер после интраназального введения

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АННОТАЦИЯ

Обоснование. Семейство пептидов, кодируемых геном *kiss1*, кисспептины (KISS1), — одно из новых неизученных семейств с точки зрения эффективности и интраназальной доставки. Кисспептины участвуют не только в репродуктивной функции, но и в поведенческих, эмоциональных и когнитивных реакциях. Эффективность доставки кисспептинов в центральную нервную систему откроет новые перспективы их применения.

Цель — оценить эффективность кисспептина-10 при транспортировке через гематоэнцефалический барьер после интраназального введения.

Методы. В работе использовали 45 беспородных мышей. Животные получали кисспептин-10 интраназально в дозах 0,1, 1 и 10 мкг; кисспептин-10 в дозах 1, 10, 100 мкг внутрибрюшинно. Поведение животных исследовали с помощью тестов «открытое поле», «приподнятый крестообразный лабиринт» и «половая мотивация».

Результаты. В настоящем исследовании были получены стабильные и дозозависимые эффекты кисспептина-10 на поведение мышей после интраназального введения. Интраназальный кисспептин-10 вызывал статистически значимое повышение половой мотивации, повышение горизонтальной и вертикальной двигательной активности, уменьшение стресса и увеличение исследовательской активности у половозрелых самцов мышей. Наибольшие изменения в поведении вызывала дозировка 10 мкг, оказывая центральное действие на мозг после интраназального введения в сравнении с группами животных после внутрибрюшинного введения, в то время как показатели после внутрибрюшинного введения кисспептина-10 практически не вызывали изменений в поведении при дозах 1 и 10 мкг. Повышение дозировки до 100 мкг внутрибрюшинно показывало достоверное изменение в поведении, однако не такое сильное, как после интраназального введения вещества в количестве 10 мкг.

Заключение. Для статистически значимого изменения поведения при интраназальном пути введения требовались концентрации в 10 раз меньше, чем при периферическом введении. Исходя из очевидных эффектов кисспептина-10 после интраназального введения в каждом тесте, можно предположить, что кисспептин-10 проникал в мозг, минуя гематоэнцефалический барьер и оказывал центральное действие. Данные подтверждают потенциальную возможность и значимость такого способа доставки вещества в центральную нервную систему.

Ключевые слова: кисспептин; интраназальное введение; гематоэнцефалический барьер; центральная нервная система; стратегии доставки лекарственных средств в центральную нервную систему; половая мотивация; приподнятый крестообразный лабиринт; открытое поле; поведенческий тест; половое поведение; мыши.

Как цитировать

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BACKGROUND

Central nervous system (CNS) diseases are the leading cause of morbidity worldwide and are currently a growing concern, given the increase in population and life expectancy, as well as the COVID-19 pandemic [1]. CNS diseases, including neoplastic, neurodegenerative (Parkinson disease, Alzheimer disease), and mental (depression, bipolar disorder, schizophrenia) disorders, are frequently refractory to drug therapy because of the blood–brain barrier (BBB). The BBB is a microcirculatory system of the brain that strictly regulates ion, molecule, and cell transport between plasma and the brain [2]. The BBB protects the brain from toxins and pathogens; however, it also impairs drug delivery, preventing 98% of small molecules and almost 100% of large molecules from entering the brain [3]. Therefore, novel strategies of drug delivery to the brain are required to treat CNS diseases [4]. Intranasal delivery is currently viewed as an alternative way of drug delivery to the CNS for the treatment of concomitant diseases. Intranasal drug delivery has several advantages over conventional oral administration. It overcomes the key pharmacokinetic barriers associated with oral drug delivery to the CNS, such as gastrointestinal pH, enzymes, first-pass effect, renal filtration, and BBB [5]. The nasal epithelium provides an adequate surface area for drug absorption owing to its high permeability, loose intercellular matrix, and good vascularization [6]. The olfactory and trigeminal pathways of the nasal epithelium provide direct delivery to the brain, improving therapeutic bioavailability in the CNS and reducing peripheral side effects, which enables a rapid therapeutic effect at a lower dose [5]. In terms of patient care, intranasal delivery is a non-invasive approach that is suitable for self-administration and can benefit patients with motor disorders, nausea, gastrointestinal disorders, and/or salivary gland dysfunction (dry mouth).

Kisspeptins, a family of peptides encoded by the *kiss1* gene, are a promising yet understudied family in terms of intranasal delivery [7, 8]. Kisspeptin neurons interact with nitric oxide-synthesizing neurons in the ventromedial hypothalamus, regulating the hypothalamus–pituitary gonadal axis [9]. Kisspeptin expression has been reported in the limbic system, indicating its role not only in reproductive function, but also in behavioral, emotional, and cognitive responses [10]. There are currently no fundamental research addressing the potential use of intranasal kisspeptin delivery.

This study aimed to evaluate the efficacy of kisspeptin-10 transport across the BBB after intranasal administration. The work assessed the effect of various kisspeptin-10 doses on the behavior of experimental animals after intranasal and peripheral administration.

METHODS

Animals

The study used 45 outbred mice weighing 20–30 g from the Rappolovo husbandry (Leningrad region, Russia). The animals were housed in a vivarium in standard plastic cages, with ad libitum access to water and feed. A lighting schedule with lights on between 8:00 and 20:00 was used, at 22 ± 2 °C.

Animal Testing

Animal behavior was assessed using the open field test, elevated plus maze, and sexual motivation test.

Elevated plus maze. This test measures anxiety in laboratory animals. Mice were placed in the center of a plus-shaped maze with four arms (30 cm long and 6 cm wide). Two arms were enclosed by 30-cm high walls, whereas the other two arms were open and exposed to diffuse artificial light. The maze was placed on a stand 40 cm above the floor level. The following parameters were visually assessed for 5 min: time in open arms; number of entries into open arms; time in the central part; and head-dipping in open arms.

Open field test. This test measures general locomotor activity. The open field was a circular arena (80 cm in diameters) enclosed by 30-cm high nontransparent walls. The open field had 16 uniformly distributed holes. The diameter of each hole was 3 cm. The holes were used to assess the willingness to explore (hole exploratory behavior) in rodents. The illumination level in the open field was set at 100 lux. Each test session lasted 3 min. A series of motor acts and postures, which collectively characterize the overall behavior in the open field, were selected based on a behavioral atlas for rodents. The orienting response was assessed by placing a mouse in the open field divided into sections. The following parameters were assessed for 5 min: number of rearings (vertical component of the orienting response), number of crossings (horizontal component of the orienting response), number of sniffings (exploratory component of the orienting response), grooming, freezing, number of boli, and hole-poking (reflecting the exploratory activity component).

Sexual motivation test. Sexual motivation was assessed using a set of four test chambers (15 × 30 cm^2). Each chamber had a stimulating cage with a perforated partition. The perforated partition allowed male mice to smell a potential mate (a female in estrus) while preventing tactile contact or mating. One day before the sexual motivation test, all experimental animals were adapted to testing conditions for 30 min. To assess the behavior, videos were recorded in a dark room with red lighting for 10 minutes. Between test sessions, the test set and partition were cleaned with 3% hydrogen peroxide solution to remove odors. Sexual motivation in each animal was

assessed by the number of attempts to reach a female, time spent near the partition, and approach latency.

Drug Products

The study used kisspeptin-10 (Sigma, USA), which was dissolved in distilled water to three concentrations (0.1 μg/20 μL, 1 μg/20 μL, and 10 μg/20 μL) and administered intranasally. Kisspeptin-10 at doses of 1 μg/0.2 mL, 10 μg/0.2 mL, and 100 μg/0.2 mL was administered intraperitoneally. 0.9% sodium chloride solution was used as a control. Kisspeptin-10 was administered intranasally and intraperitoneally 10–15 min before placing the mice in test sets.

Statistical Analysis

The significance of differences was assessed using GraphPad Prism 8.3.4 and one-way analysis of variance (ANOVA). The control and experimental groups were compared using ANOVA. The Kruskal–Wallis nonparametric test was used for intergroup comparisons. Differences were considered significant at $p < 0.05$. Descriptive statistics were used; the data were presented as means and standard deviations.

RESULTS

Sexual Motivation Test

To assess sexual motivation, the time spent near the partition and approach latency were recorded for each animal (Figs. 1, 2, and Table 1). Group 1 (intact group) received no intervention. Groups 2, 3, and 4 received kisspeptin-10 at doses of 0.1 μg, 1 μg, and 10 μg intranasally (in 20 μL of distilled water, 10 μL per nostril), respectively. Groups 5, 6, and 7 received kisspeptin-10 at doses of 1 μg, 10 μg, and 100 μg intraperitoneally (in 0.2 mL of distilled water), respectively. Group 8 received 20 μL of 0.9% sodium chloride solution intranasally (10 μL per nostril; sham intranasal administration group). Group 9 received 0.2 mL of 0.9% sodium chloride solution intraperitoneally (sham intraperitoneal administration group). The control approach latency was 9.1 ± 1.0 s. Intranasal kisspeptin-10 significantly reduced the approach latency. The approach latency in the kisspeptin-10 1 μg group significantly differed from that in the intact group ($p < 0.05$), reaching 4.0 ± 0.4 s. Kisspeptin-10 10 μg reduced the approach latency to 3.4 ± 0.4 s ($p < 0.01$). The maximal dose of intraperitoneal kisspeptin-10 significantly reduced the approach latency compared to the intact group (4.1 ± 0.5 s; $p < 0.05$). Other doses of intraperitoneal kisspeptin-10 (1 and 10 μg) had no significant impact on the control approach latency. Intranasal and intraperitoneal 0.9% sodium chloride solution showed no significant differences with the intact group, indicating a minor increase in anxiety (incorrect manipulations). Therefore, intranasal kisspeptin-10 at a dose of 1 μg reduced the approach latency, as did intraperitoneal kisspeptin-10, but at a 10 times larger dose.

The next step of the sexual motivation test in male mice was to assess the number of attempts to reach a female. Each experimental group received the same drugs as in the previous step. The number of attempts in the intact group was 13.5 ± 1.0. Intranasal kisspeptin-10 at doses of 1 and 10 μg significantly ($p < 0.05$ and $p < 0.01$, respectively) increased the number of attempts

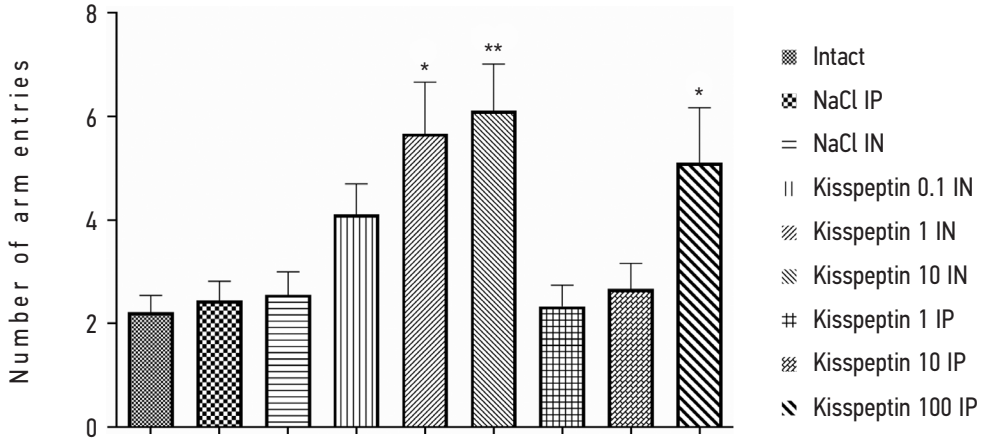

Fig. 1. Number of arm entries in the elevated plus maze. Intact, intact group of animals without intervention; NaCl IP, intraperitoneal administration of 0.9% sodium chloride solution; NaCl IN, intranasal administration of 0.9% sodium chloride solution; kisspeptin 0.1 IN, intranasal administration of kisspeptin-10 at a dose of 0.1 μg; kisspeptin 1 IN, intranasal administration of kisspeptin-10 at a dose of 1 μg; kisspeptin 10 IN, intranasal administration of kisspeptin-10 at a dose of 10 μg; kisspeptin 1 IP, intraperitoneal administration of kisspeptin-10 at a dose of 1 μg; kisspeptin 10 IP, intraperitoneal administration of kisspeptin-10 at a dose of 10 μg; kisspeptin 100 IP, intraperitoneal administration of kisspeptin-10 at a dose of 100 μg.*$p < 0.05$; **$p < 0.01$, differences between intact and experimental animals.

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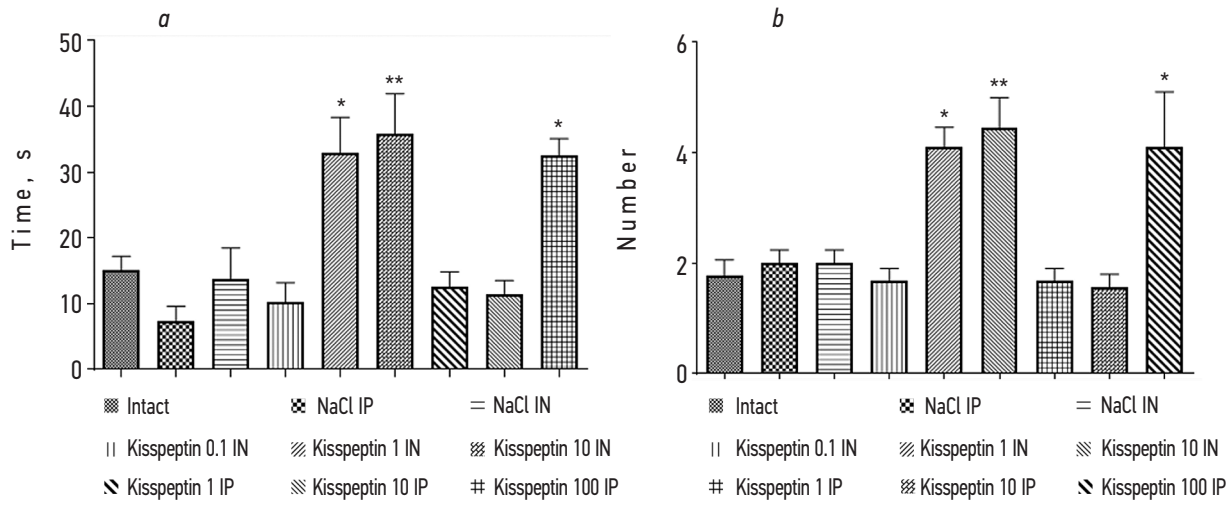

Fig. 2. Elevated plus maze findings: *a*, time in open arms; *b*, head-dipping. Group designations as in Fig. 1. *$p < 0.05$; **$p < 0.01$, differences between intact and experimental animals.

Table 1. Behavior in the sexual motivation test by parameter after intranasal and intraperitoneal administration of 0.9% sodium chloride solution and kisspeptin-10 ($M \pm m$)

Group	Approach latency, s	Number of attempts
Intact	9.1 ± 1.0	13.5 ± 1.0
NaCl IP	8.3 ± 1.1	14.0 ± 1.2
NaCl IN	8.5 ± 1.4	12.5 ± 1.2
Kisspeptin 0.1 IN	6.7 ± 1.1	13.4 ± 1.2
Kisspeptin 1 IN	4.0 ± 0.4*	22.2 ± 2.0*
Kisspeptin 10 IN	3.4 ± 0.4**	22.8 ± 1.8**
Kisspeptin 1 IP	8.7 ± 1.1	11.1 ± 1.2
Kisspeptin 10 IP	8.0 ± 1.3	13.0 ± 2.2#
Kisspeptin 100 IP	4.1 ± 0.5*	15.7 ± 2.1

Note. Intact, intact group of animals without intervention; NaCl IP, intraperitoneal administration of 0.9% sodium chloride solution; NaCl IN, intranasal administration of 0.9% sodium chloride solution; kisspeptin 0.1 IN, intranasal administration of kisspeptin-10 at a dose of 0.1 μg; kisspeptin 1 IN, intranasal administration of kisspeptin-10 at a dose of 1 μg; kisspeptin 10 IN, intranasal administration of kisspeptin-10 at a dose of 10 μg; kisspeptin 1 IP, intraperitoneal administration of kisspeptin-10 at a dose of 1 μg; kisspeptin 10 IP, intraperitoneal administration of kisspeptin-10 at a dose of 10 μg; kisspeptin 100 IP, intraperitoneal administration of kisspeptin-10 at a dose of 100 μg. *$p < 0.05$; **$p < 0.01$, difference between the kisspeptin-10 and intact groups; #$p < 0.01$, difference between intranasal and intraperitoneal kisspeptin at the same dose.

compared to the intact group: 22.2 ± 2.0 and 22.8 ± 1.8, respectively. The number of attempts with intraperitoneal kisspeptin-10 at a dose of 100 μg was 15.7 ± 2.1, which did not differ significantly from the intact group. When comparing intranasal and intraperitoneal kisspeptin-10 at a dose of 10 μg, there was a significant, nearly two-fold increase in the number of attempts to reach a female with intranasal kisspeptin-10. Therefore, intranasal kisspeptin-10 at doses of 1 and 10 μg increased the number of attempts to reach a female by 1.5 times (22.2 ± 2.0 and 22.8 ± 1.8, respectively) compared to the intact group (13.5 ± 1.0). Intraperitoneal kisspeptin10 at doses of 1 and 10 μg had no impact on the number of attempts compared to the intact group. Intranasal and intraperitoneal 0.9% sodium chloride solution (12.5 ± 1.2 and 14.0 ± 1.2, respectively) showed no significant differences with the intact group, indicating a minor increase in anxiety (incorrect manipulations). Intranasal kisspeptin-10

at doses of 1 and 10 μg significantly increased the number of attempts to reach a female, whereas intraperitoneal kisspeptin-10, even at the maximal dose of 100 μg, showed no significant changes in this parameter. Intranasal kisspeptin-10 significantly increased sexual motivation in male mice. Intranasal kisspeptin-10 influenced the sexual behavior of male mice by reducing the approach latency and increasing the number of attempts to reach a female at concentrations 10 times lower than those of intraperitoneal kisspeptin-10. The intraperitoneal dose of 100 μg was insufficient to alter the behavior in the sexual motivation test.

Elevated Plus Maze

Intranasal kisspeptin-10 at all three doses increased the number of arm entries compared to the intact group. However, a significant increase was only observed with kisspeptin-10 at doses of 1 and 10 μg, indicating reduced anxiety and increased exploratory activity after intranasal kisspeptin-10 administration (Fig. 1). Intranasal kisspeptin-10 at a dose of 1 μg increased the number of arm entries in the intact group from 2.2 ± 0.3 to 5.7 ± 1.0. Intranasal kisspeptin-10 at a dose of 10 μg significantly increased the number of arm entries to 6.1 ± 0.9. Intranasal kisspeptin-10 showed a dose-dependent effect. There were no differences in the number of arm entries between intranasal (2.5 ± 0.4) and intraperitoneal (2.4 ± 0.3) 0.9% sodium chloride solution and the intact group (2.2 ± 0.3). Intraperitoneal kisspeptin-10 at doses of 1 and 10 μg (2.0 ± 0.3) showed no significant differences with the intact group. Intraperitoneal kisspeptin-10 at a dose of 100 μg significantly increased the number of arm entries to 5.1 ± 1.1 compared to the control group.

The next step was to assess the time in open arms.

Two doses of intranasal kisspeptin-10 increased the time in open arms compared to the intact group, whereas only one dose of intraperitoneal kisspeptin-10 was effective (Fig. 2). Intranasal kisspeptin-10 at a dose of 1 μg provided a significant increase compared to the intact group (32.8 ± 5.4 s vs 15.1 ± 2.1 s).

Intranasal kisspeptin-10 at a dose of 10 μg also significantly increased the time in open arms compared to the intact group (35.8 ± 6.0 s vs 15.1 ± 2.1 s). Only the maximal dose of intraperitoneal kisspeptin-10 significantly increased the time in open arms compared to the intact group (32.5 ± 2.5 s vs 15.1 ± 2.1 s). Intranasal and intraperitoneal 0.9% sodium chloride solution, intraperitoneal kisspeptin-10 at doses of 1 and 10 μg, and intranasal kisspeptin-10 at a dose of 1 μg showed no significant differences with the intact group.

Intranasal kisspeptin-10 at doses of 1 and 10 μg significantly increased head-dipping, likely owing to reduced stress and improved exploratory and locomotor activity. Intranasal kisspeptin-10 at doses of 1 and 10 μg increased this parameter to 4.1 ± 0.3 and 4.4 ± 0.5, respectively, compared to the intact group (1.7 ± 0.2). Intraperitoneal kisspeptin-10 at a dose of 100 μg also provided significant behavioral changes, increasing head-dipping to 4.4 ± 0.5 compared to the intact group.

The intranasal dose of 1 μg and intraperitoneal doses of 1 and 10 μg are likely ineffective, as they did not produce behavioral changes. The intranasal dose of 10 μg resulted in significant changes in all assessed parameters, as did the intraperitoneal dose of 100 μg. Therefore, intranasal kisspeptin-10 produced significant behavioral changes in the elevated plus maze at a ten-fold lower dose than intraperitoneal kisspeptin-10.

Other assessed parameters (time in closed arms and number of groomings) showed no significant intergroup differences.

Open Field Test

Kisspeptin-10 neurons are located in the posterodorsal medial amygdala, which has a role in emotional behavior, sexual motivation, fear, and anxiety. Therefore, we assessed the effect of intranasal kisspeptin-10 on emotional, exploratory, and locomotor behavior in male mice. The findings of the open field test are presented in Figs. 3 and 4. Intranasal kisspeptin10 at a dose of 1 μg provided a minor increase in horizontal locomotor activity (increased number of crossings) compared to the intact group. Furthermore, it improved exploratory activity, with a significant increase in the number of sniffings ($p < 0.05$), hole-poking ($p < 0.05$), and crossings ($p < 0.05$) compared to the intact group.

Intranasal kisspeptin-10 at a dose of 10 μg significantly improved exploratory activity, with an increase in hole-poking ($p < 0.01$), sniffings ($p < 0.01$), rearings ($p < 0.05$), and crossings ($p < 0.01$) compared to the intact group. Intranasal and intraperitoneal 0.9% sodium chloride solution showed no significant differences with the intact group in any of the assessed parameters. There were no significant intergroup differences in the number of wall-supported rearings, number of groomings, and number of boli.

Intraperitoneal kisspeptin-10 at doses of 1 and 10 μg produced no significant behavioral changes. Intraperitoneal kisspeptin-10 at a dose of 100 μg significantly increased horizontal activity, rearings, hole-poking, and crossings ($p < 0.05$) compared to the intact group. Other parameters did not differ significantly from the intact group.

Therefore, intranasal kisspeptin-10 produced significant, dose-dependent changes in emotional, exploratory, and locomotor behavior compared to the intact group. Intraperitoneal kisspeptin-10 at ten times the intranasal dose had a significantly lower impact on behavior, confirming the efficacy of intranasal kisspeptin-10 delivery to the CNS at lower doses compared to peripheral administration.

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DISCUSSION

This is the first work to confirm the possibility of intranasal kisspeptin-10 delivery to the CNS, with a dose-dependent effect. Intranasal administration at a dose of 1 μg resulted in enhanced horizontal and vertical locomotor activity in the open field test, including crossings, sniffings, and hole-poking, compared to the intact group. Intranasal kisspeptin-10 at a dose of 10 μg additionally provided a significant increase in horizontal activity and wall-supported rearings ($p < 0.01$).

Intraperitoneal kisspeptin-10 at doses of 1 and 10 μg showed no significant differences, whereas the dose of 100 μg increased hole-poking, crossings, rearings, and horizontal activity compared to the intact group ($p < 0.05$).

Therefore, the open field test showed a significant increase in exploratory and locomotor activity after intranasal administration of the test substance, with a dose-dependent effect. Intraperitoneal administration showed significant differences in four of eight assessed parameters, whereas intranasal administration at a ten-fold lower dose showed differences in five of eight assessed parameters, which were more significant.

In the elevated plus maze, intranasal kisspeptin-10 at a dose of 0.1 μg produced no significant changes. Intranasal kisspeptin-10 at a dose of 1 μg significantly increased two parameters compared to the intact group: time in open arms and head-dipping ($p < 0.05$). Intranasal kisspeptin-10 at a dose of 10 μg significantly ($p < 0.01$) increased three parameters (number of arm entries, time in open arms, and head-dipping), indicating a dose-dependent effect of kisspeptin-10. The dose of 10 μg was the most effective.

Intraperitoneal kisspeptin-10 at doses of 1 and 10 μg had no effect on behavior. However, the dose of 100 μg increased the time in open arms, head-dipping, and number of arm entries ($p < 0.05$), which is comparable with intranasal kisspeptin-10 at a dose of 1 μg.

An increase in these parameters is associated with reduced anxiety and/or increased exploratory activity after intranasal administration of kisspeptin-10.

Other assessed parameters (time in closed arms and number of groomings) showed no significant intergroup differences.

Therefore, intranasal kisspeptin entered the CNS, significantly altering behavior in the elevated plus maze. The effects of intraperitoneal kisspeptin-10 are comparable

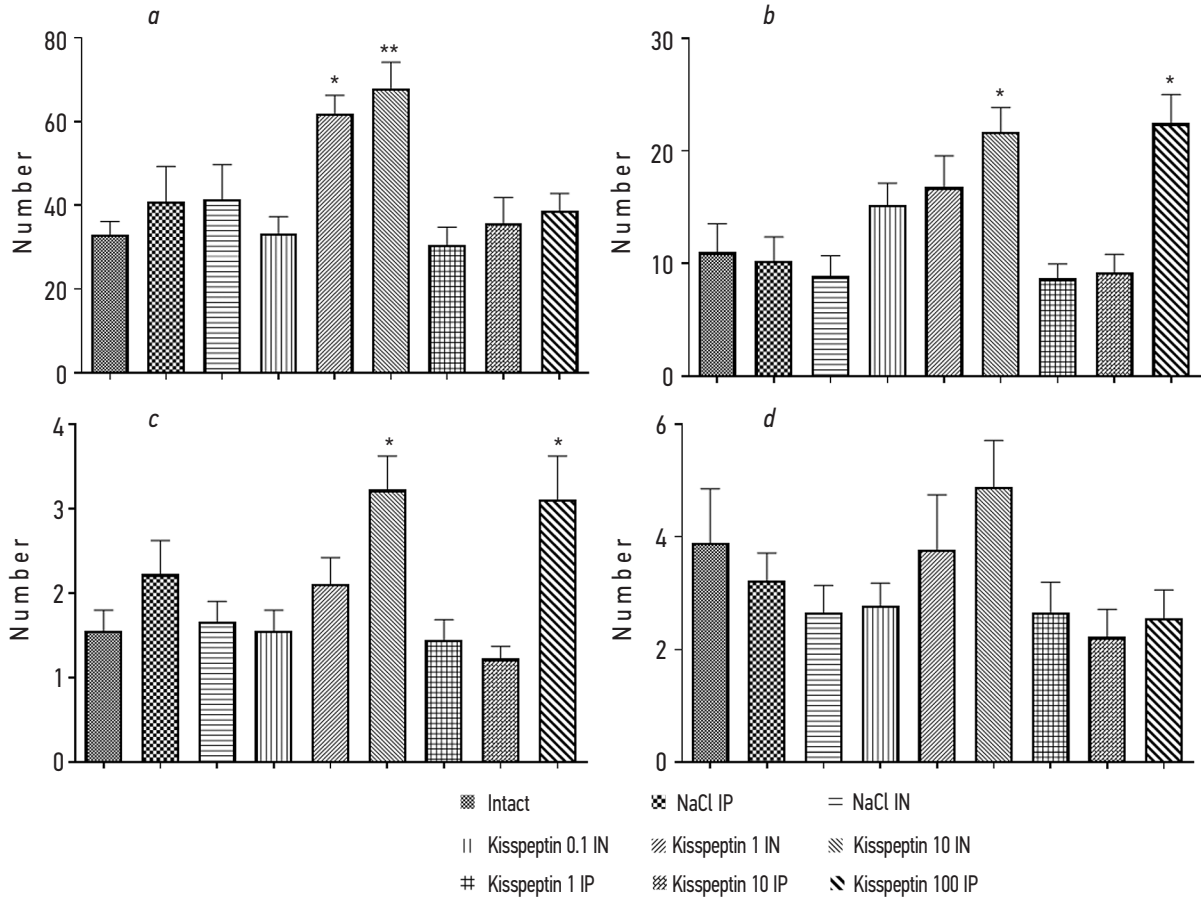

Fig. 3. Open field test findings: sniffings (*a*), locomotion (*b*), rearings (*c*), and wall-supported rearings (*d*). Designations as in Fig. 1. *$p < 0.05$; **$p < 0.01$, differences between intact and experimental animals.

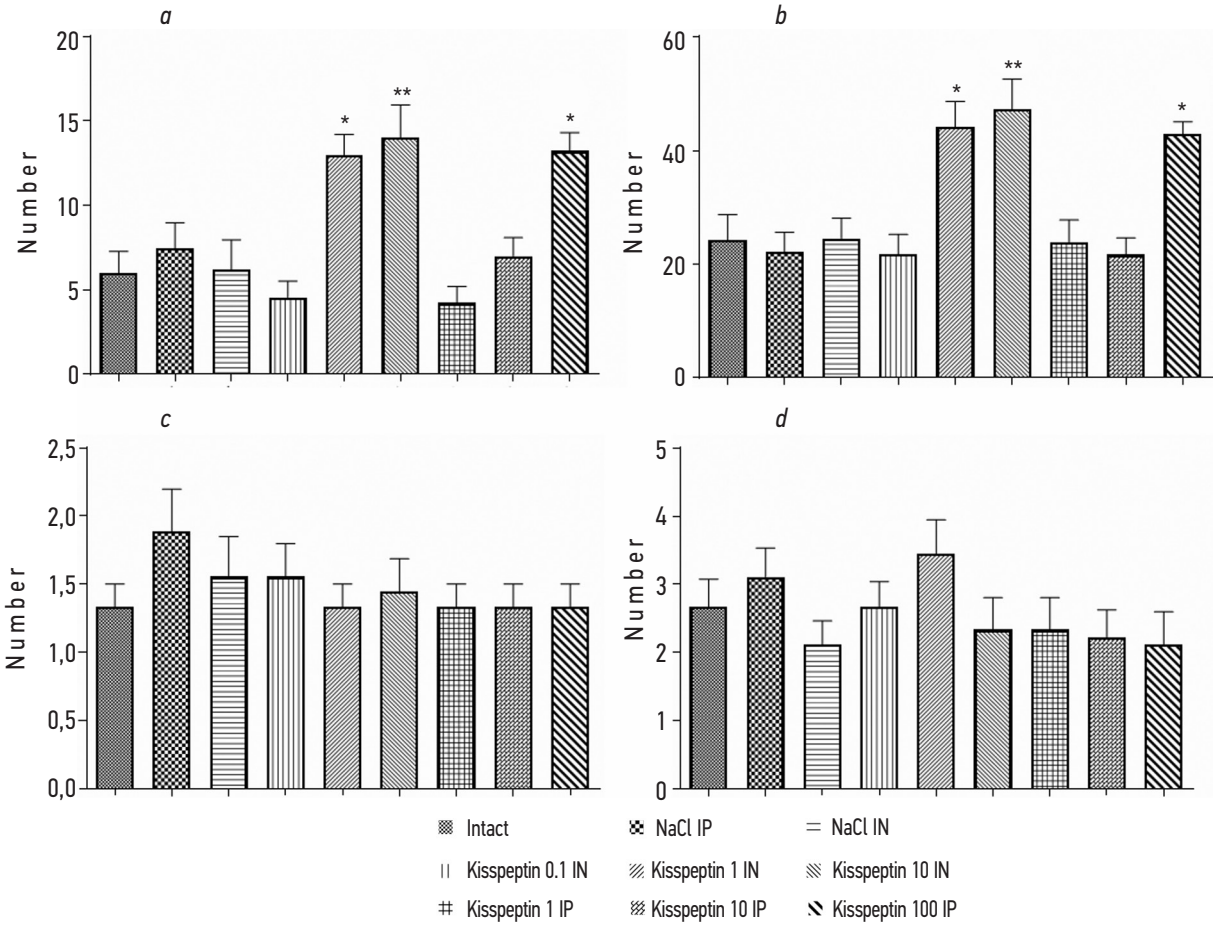

Fig. 4. Open field test findings: hole-poking (*a*), crossings (*b*), number of boli (*c*), and grooming (*d*). Group designations as in Fig. 1. **p* < 0.05; ***p* < 0.01, differences between intact and experimental animals.

to those of intranasal kisspeptin-10 at a dose of 1 μg. Intranasal kisspeptin-10 at a dose of 10 μg was the most effective.

In the sexual motivation test, intranasal kisspeptin-10 produced changes in both assessed parameters. Intranasal kisspeptin-10 at doses of 1 and 10 μg significantly reduced the approach latency and increased the number of attempts to reach a female compared to the intact group ($p < 0.05$ and $p < 0.01$, respectively). Intraperitoneal administration produced significant changes in only one parameter and only at a dose of 100 μg. There was a decrease in approach latency ($p < 0.05$) compared to intact mice. The effect of kisspeptin after peripheral administration could be explained by its indirect influence on sexual behavior or partial penetration into the CNS. Therefore, kisspeptin10 could enter the brain after intranasal administration and influence the CNS, increasing sexual motivation in adult male mice by both parameters. Intranasal kisspeptin-10 significantly increased sexual motivation in male mice, whereas intraperitoneal administration produced changes in only one parameter. Our findings confirm the possibility of kisspeptin delivery to the brain following intranasal administration.

All three behavioral tests showed significant changes after intranasal administration of kisspeptin-10, with a dose-dependent effect. In contrast, intraperitoneal kisspeptin-10 at doses of 1 and 10 μg showed no significant behavioral changes. The intraperitoneal dose of 100 μg resulted in significant behavioral changes; however, the effect was less pronounced than that after intranasal administration of 10 μg. Overall, intranasal administration can be used for kisspeptin delivery to the CNS, bypassing the BBB, and requires further research. Kisspeptin was effective at lower doses when administered intranasally.

CONCLUSION

Intranasal delivery has numerous advantages, including the ability to bypass the BBB. This work was the first to demonstrate the central action of kisspeptin-10 in the brain following intranasal administration compared to intraperitoneal kisspeptin and intranasal or intraperitoneal 0.9% sodium chloride solution, with a dose-dependent effect. Intranasal kisspeptin-10 demonstrated a persistent dose-dependent effect on behavior in mice. Intranasal

kisspeptin-10 significantly increased sexual motivation, improved horizontal and vertical locomotor activity, reduced stress, and enhanced exploratory behavior in adult male mice. Given the evident effects of intranasal kisspeptin-10 in each behavioral test, it can be assumed that kisspeptin-10 penetrated the brain, bypassing the BBB, and exerted central action. Our findings confirm the possibility and significance of intranasal delivery for regulating sexual behavior and influencing emotion-mediated processes (for example, reducing anxiety).

Another advantage of intranasal delivery is the low dose of active substance. We used three intranasal doses of kisspeptin-10, which were ten times lower than intraperitoneal doses. In contrast to intranasal administration, even high intraperitoneal doses did not cause significant behavioral changes.

Intranasal drugs for the treatment of CNS diseases are a promising area in modern pharmacology. However, there are several factors limiting their use, including the impossibility of intranasal administration of some drugs and difficulties in achieving steady concentrations and precise dosing. Therefore, further fundamental research into intranasal delivery is required.

ADDITIONAL INFO

Author contributions: M.V. Litvinova, A.A. Lebedev, E.R. Bychkov: investigation, formal analysis, writing—original draft; P.D. Shabanov: conceptualization, writing—review & editing. All the authors approved the version of the draft to be published and agreed to be accountable for all aspects of the work, ensuring that issues related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. М.В. Литвинова, А.А. Лебедев, Е.Р. Бычков — получение результатов, анализ данных, написание статьи; П.Д. Шабанов — разработка общей концепции, рецензирование статьи. Все авторы одобрили рукопись (версию для публикации), а также согласились нести ответственность за все аспекты работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой ее части.

Этическая экспертиза. Проведение исследования одобрено локальным этическим комитетом ФГБНУ «Институт экспериментальный медицины» (протокол № 2/23 от 15.06.2023).

Источник финансирования. Исследование выполнено в рамках государственного задания ФГБНУ «Институт экспериментальный медицины» FGWG-2025-0020 «Поиск молекулярных мишеней для фармакологического воздействия при аддиктивных и нейроэндокринных нарушениях с целью создания новых фармакологически активных веществ, действующих на рецепторы ЦНС».

Раскрытие интересов. Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных с третьими лицами (коммерческими и некоммерческими), интересы которых могут быть затронуты содержанием статьи.

Оригинальность. При создании настоящей работы авторы не использовали ранее опубликованные сведения (текст, иллюстрации, данные).

Доступ к данным. Все данные, полученные в настоящем исследовании, доступны в статье.

Генеративный искусственный интеллект. При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.

Рассмотрение и рецензирование. Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали два внешних рецензента и член редакционной коллегии.

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