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Research Article

The effect of metformin on behavioral characteristics and *Bdnf* gene expression of old rats with sex differences

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Abstract

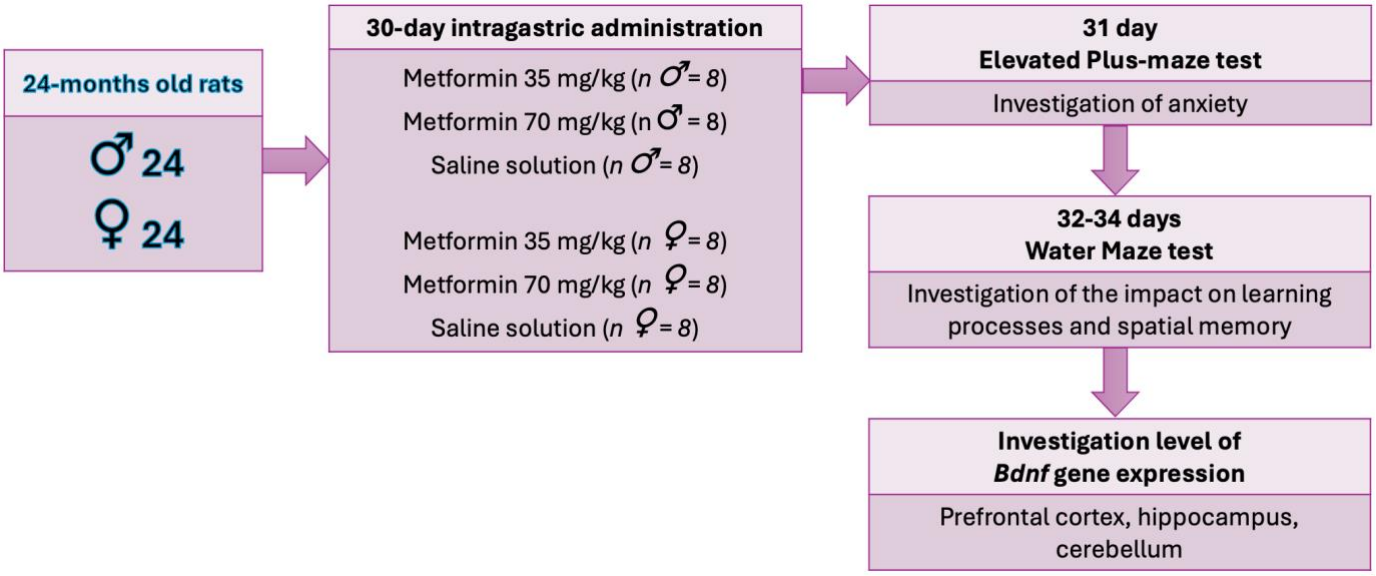
Introduction: Ageing is a key risk factor for neurodegenerative diseases, so aging heightens the urgency of developing safe geroprotectors. Metformin is a compound of significant interest owing to its ability to modulate the pathogenesis of aging and related cognitive decline. Thus, **the aim of the work** was to study the effects of metformin on behavior and *Bdnf* gene expression in the brains of aged rats with consideration of sex differences.

Materials and Methods: The study was performed on 48 old (24 months) *Wistar* rats divided into groups ($n=8$): males and females receiving metformin (35 or 70 mg/kg/day) or saline (control) for 30 days. Anxiety (Elevated Plus maze test) and spatial memory (Water maze test) were assessed. The relative level of *Bdnf* gene expression was determined by real-time PCR.

Results: Metformin at a dose of 70 mg/kg exerts an anxiolytic effect in males in the Elevated Plus maze test, increasing the duration of stay in the open ”arms” of the maze by 3.5 times ($p=0.0443$) and reducing the time of closed ”arms” by 1.5 times ($p=0.0161$) compared to those in the control group. Males also showed an improvement in spatial memory after the introduction of metformin at doses of 35 and 70 mg/kg in the Water Maze test: the duration of swimming in the platform zone increased by 1.7 times (35 mg/kg, $p=0.04$) and 1.5 times (70 mg/kg, $p=0.03$), respectively, compared to such in the control group. At a dose of 35 mg/kg, metformin causes a 3-fold increase in *Bdnf* gene expression in males in the prefrontal cortex ($p=0.047$) compared to such in the control group. In females, *Bdnf* gene expression in the cerebellum positively correlates with spatial memory ($p=0.039$), which emphasizes its role in cognitive functions.

Conclusion: A 30-day metformin treatment exerted pronounced anxiolytic (70 mg/kg) and mnemotropic effects (35 and 70 mg/kg) in aged male rats, correlating with elevated prefrontal cortex *Bdnf* gene expression. In females, spatial memory improvement correlated with cerebellar *Bdnf* gene upregulation.

Graphical Abstract



Keywords

anxiety, behavioral tests, *Bdnf* gene expression, cognitive function, metformin, old rats

Introduction

The aging process is an irreversible decrease in physiological functions, which leads to age-related changes in the central nervous system (Markowicz-Piasecka et al. 2017; Mohammed et al. 2021; Hafizova et al. 2025) and is a risk factor for developing neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, etc. (Markowicz-Piasecka et al. 2017; Hafizova et al. 2025). Currently, active search is underway for drugs capable of influencing the pathogenesis of aging to reduce the severity of age-related behavioral and cognitive impairments, as well as improve the quality of patient's life (Ashrostaghi et al. 2015; Fan et al. 2019; Mohammed et al. 2021). Drugs with proven safety attract special attention, and metformin is one of the most studied candidates for the role of such a drug (Markowicz-Piasecka et al. 2017; Hafizova et al. 2025). The effects of metformin in small doses, as a potential geroprotector, are directed at the main pathogenetic links of the aging process and determine its potential ability to inhibit various behavioral disorders associated with pathological aging (Markowicz-Piasecka et al. 2017; Mohammed et al. 2021; Hafizova et al. 2025). It is known that neurotrophic factor (*Bdnf*) plays an important role in the realization of these disorders. Recent studies have shown the importance of *Bdnf* in the pathophysiology of a number of symptoms accompanying the processes of physiological aging: cognitive impairment, complexity of learning processes, increased anxiety, and eating disorders (Gudasheva et al. 2017). Moreover, it is known that its level may vary in males and females. Thus, the **aim of the work** was to experimentally study the effect of metformin on the behavioral characteristics and expression of the *Bdnf* gene in the brain structures of old rats, considering sex differences. Scientists believe that metformin in small doses that do not have a hypoglycemic effect affects the behavioral and cognitive functions of old animals (Markowicz-Piasecka et al. 2017; Mohammed et al. 2021; Hafizova et al. 2025). The rationale for choosing low doses of metformin is due to the fact that in type 2 diabetes mellitus, the drug is used in high doses and side effects may occur, especially in elderly patients (Hafizova et al. 2025). Thus, reducing the dose of metformin may help reduce the incidence of side effects in elderly patients as a potential geroprotector.

Materials and Methods

Animals

The experiments were conducted on old male rats weighing 550-600 g and female rats weighing 250-300 g of the *Wistar* line (24 months of age (not reproductive)). The animals at the age of 2.5 months were obtained from the Stolbovaya branch of the Federal State Budgetary Institution Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency (Russia). They were kept in standard conditions, in natural light on fully balanced feed, in compliance with the international requirements of the European Convention for the Protection of Vertebrates Used in Experimental Research, as well as in accordance with the Rules of Good Laboratory Practice approved by Order of the Ministry of Healthcare of the Russian Federation № 199n dated January 4, 2016. Experimental studies were carried out with the approval of the Local Ethics Committee of Kazan State Medical University of the Ministry of Healthcare of the Russian Federation (Minutes № 6 of 20 June 2023).

The object of the study

The object of the study was metformin (OzonFarm, Russia), administered intragastrically at doses of 35 mg/kg and 70 mg/kg. Intragastric administration of the studied drug was performed using a special atraumatic stainless steel probe for feeding rats (the probe length was 55 mm). The average effective dose in rats in the experiment is 300 mg/kg in a model of type 2 diabetes mellitus (Zhou et al. 2024). The choice of the frequency of metformin administration is determined by the recommendations of the Guidelines for Conducting Preclinical Studies of Medicines (Mironov et al. 2012) and the analysis of literature data (Buckner 2013; Arezoumand et al. 2024; Hafizova et al. 2025).

The effect of a 30-day intragastric metformin administration on behavioral characteristics (anxiety and spatial memory) and expression of the *Bdnf* gene in old rats was studied. For this purpose, the animals were divided into groups by gender, which were administered metformin at a dose of 35 mg/kg (♂ *n=8*, ♀ *n=8*) and 70 mg/kg (♂ *n=8*, ♀ *n=8*). Control rats were injected with saline solution (♂ *n=8*, ♀ *n=8*).

Behavioral testing

The effect of metformin on the anxiety level of rats was assessed using the Elevated Plus maze (EPM) test (OpenScience, Russia). It is believed that an increase in the time spent in open, brightly lit maze arms is associated with a decrease in anxiety levels (Mironov et al 2012; Semina et al. 2023). The duration (s) of the animals' stay in the open "arms" (OA) and closed "arms" (CA) of the maze was estimated.

The spatial memory of rats was studied in the Water Maze test (WM) (OpenScience, Russia) (Fan et al. 2019). The duration (s) of swimming of rats in the area of the platform, where the rescue platform was located during the training period, was recorded. An increase in this indicator may indicate an improvement in the cognitive functions of animals.

On the 36th day of the experiment, the rats were decapitated using a guillotine (OpenScience, Russia).

Genetic analysis

The isolation of RNA (ExtractRNA (Eurogen, Russia)) and subsequent reverse transcription (Biolabmix, Russia) were performed according to the manufacturers' instructions. Real-time quantitative PCR was performed on a CFX96 amplifier (BioRad, USA) using a reaction mixture of HS-qPCR SYBR Blue (2×) biomasters (Biolabmix, Russia), primers for *Bdnf* and the *Gapdh* reference gene, and water. The relative expression level of the *Bdnf* gene was calculated using the formula $2^{-\Delta\Delta Cq}$ (Livak and Schmittgen 2001; Li et al. 2022).

Statistical processing

The registration and analysis of behavioral reactions of animals was carried out using the computer program Ethovision^{XT} (Noldus, the Netherlands) with an automatic track analysis method. Statistical analysis was performed using Student's t-test (GraphPad Prism, version 8.0.1, USA). The experimental results of the relative level of gene expression are presented as Relative Quantity (RQ, Lower–Upper Limit). The correlation analysis was carried out using the calculation of the Pearson coefficient. The differences were considered significant at $p<0.05$.

Results and Discussion

Metformin as a potential geroprotector was experimentally studied at low doses of 35 mg/kg and 70 mg/kg, which are 1/8 and 1/4 of the average effective dose. Literature data were considered when choosing doses: in animal experiments, the anxiolytic and mnemotropic effects of metformin were found in the dose range of 75–100 mg/kg (Kight and McCarthy 2017; Semina et al. 2023), which was the basis for the study of metformin in even lower doses to minimize side effects.

Considering that increased anxiety is described as one of the main behavioral symptoms of the aging process (Gudasheva et al. 2017; Hafizova et al. 2025), we evaluated the effect of metformin on the anxiety level of rats in the EPM. The anxiolytic activity of metformin was revealed after its repeated administration to old males at a dose of 70 mg/kg. There was a 3.5–fold increase in the time spent in the labyrinth area (p=0.0443), mean±SEM (36.1±13.85) and a 1.5–fold decrease in the duration of stay in the labyrinth area (p=0.0161), mean±SEM (183.05±62.41) compared with such in the control group (Fig. 1 A–B). When applied at a dose of 35 mg/kg, only a tendency in the development of an anti-anxiety effect was noted. As for the females, we did not find statistically significant differences in any of the studied groups (Fig. 1 C–D).

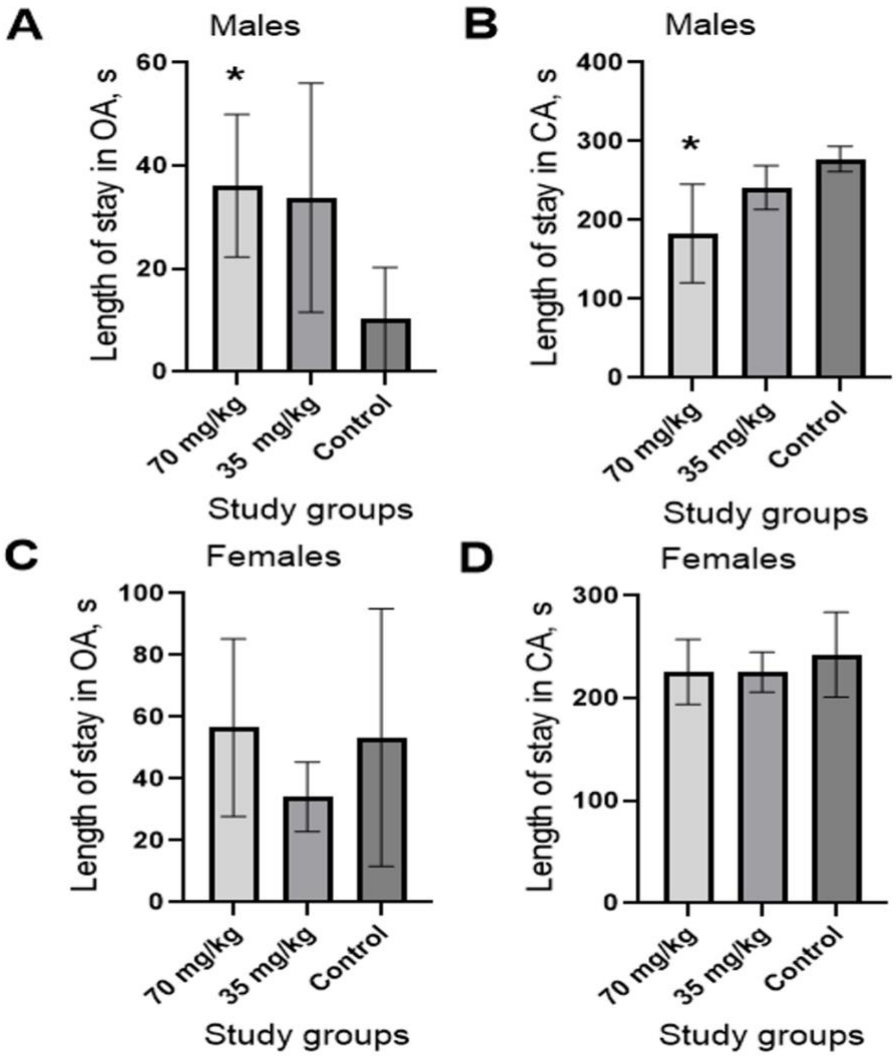


Figure 1. Effect of 30-day intragastric administration of metformin on the anxiety state of old male rats (**A, B**) and old female rats (**C, D**) (age – 24 months) in the Elevated plus maze test. *Note:* the ordinate axis shows the duration of stay in the open "arms" (**A, C**) and in the closed "arms" (**B, D**) in relation to the control; the abscissa axis shows the study groups. *$p < 0.05$ – statistically significant differences in relation to the control group.

Aging is characterized by a decrease in cognitive functions (Markowicz-Piasecka et al. 2017; Fan et al. 2019; Hafizova et al. 2025), including a deterioration in memory processes, including the ability to learn spatially (Fan et al. 2019; Hafizova et al. 2025). A statistically significant effect in WM was detected only in males: when using metformin at a dose of 70

mg/kg, the duration of their swimming in the platform area was lasted 1.7 times longer than such in control rats (p=0.03), mean±SEM (40.0±1.15) and 1.5 times longer at a dose of 35 mg/kg (p=0.04), and mean±SEM (36.0±3.21) (Fig. 2). There was no effect of metformin on cognitive functions in females. The data obtained are consistent with the results obtained by other researchers in higher doses (AboTaleb et al. 2024; Arezoumand et al. 2024).

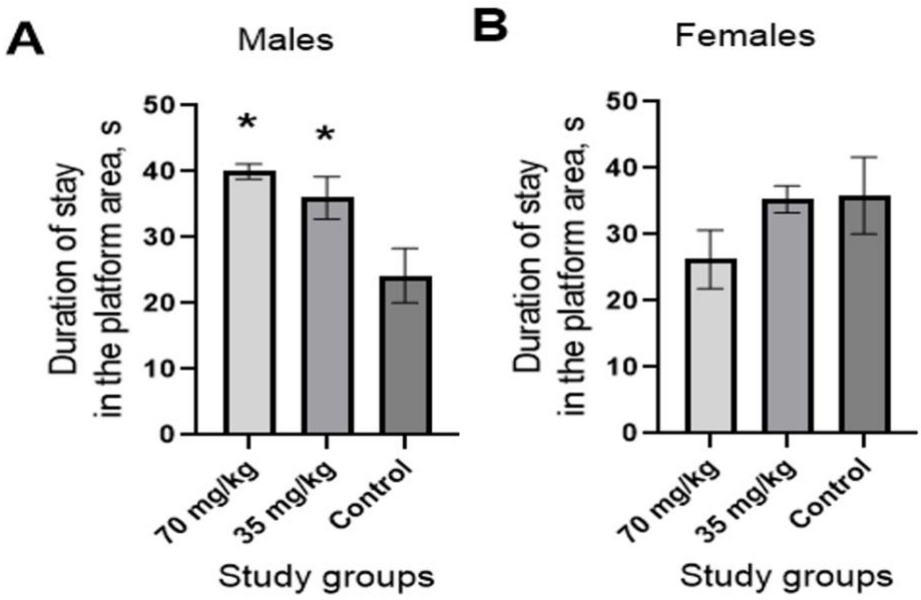


Figure 2. Effect of 30-day intragastric administration of metformin on spatial memory of old male rats (**A**) and old female rats (**B**) (age – 24 months) in the Water Maze test. ***Note:*** the ordinate axis shows the duration of stay in the platform zone in relation to the control; the abscissa axis shows the study groups. *$p < 0.05$ – statistically significant differences in relation to the control group.

Interesting results were obtained by studying the level of *Bdnf* gene expression. It is known that metformin rapidly penetrates the blood-brain barrier and accumulates in various areas of the brain (Markowicz–Piasecka et al. 2017; Li et al. 2022; Hafizova et al. 2025), especially in the hippocampus and prefrontal cortex. We showed that in male rats, metformin (35 mg/kg) increased *Bdnf* gene expression in the prefrontal cortex (RQ 3.27 (2.57–4.16) compared with the control (RQ 1 (0.65–1.53), p=0.047,), while no changes were detected in females (p>0.05) (Fig. 3). The results obtained demonstrate sex differences in the regulation of *Bdnf* gene expression under the action of metformin and are also consistent with the results of behavioral testing at WM. An increase in the level of *Bdnf gene* expression in the prefrontal cortex in males may indicate a different sensitivity of neurotrophic mechanisms to metformin in males and females, which is consistent with data on differences in neuroplasticity considering sexual dimorphism and is due to the nature of sex hormones (Kight and McCarthy 2017; Wang et al. 2018). The ability of metformin to increase *Bdnf* gene expression in the prefrontal cortex in male rats can also be explained by its anxiolytic effect in the EPM test due to the possible enhancement of neuroplasticity, modulation of monoaminergic systems and suppression of hyperactivity of stress response pathways (Kondaurova et al. 2020), as well as due to the ability to alter the activity of dopaminergic transmission, affecting GAB_A-mediated regulation of dopamine (Grigoryan 2020). It is these systems that play a significant role in the development of anxiety, and the degree of their involvement may explain the sex differences in the anxiolytic effect of metformin.

As for the cerebellum, despite the absence of differences between the groups in terms of *Bdnf* expression, further correlation analysis combining data from three groups (control, metformin 35 mg/kg and 70 mg/kg) revealed a positive correlation in females between the level of *Bdnf* expression in the cerebellum and the time they spent in the area where they should be platforms (r=0.47, p=0.039) in WM. This is consistent with modern ideas about the role of the cerebellum in cognitive processes (Buckner 2013). It is noteworthy that males, unlike females, did not show such a correlation, which may be due to the presence of estrogens in female rats (Kight and McCarthy 2017).

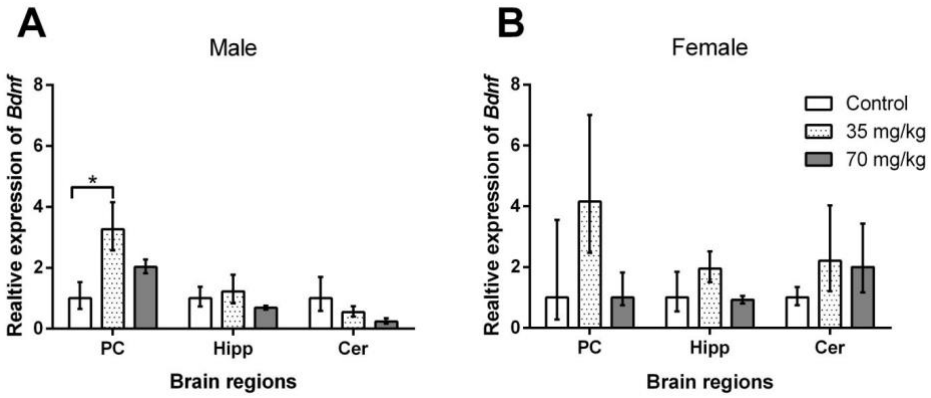


Figure 3. Relative expression level of the *Bdnf* gene in the brain structures of male (**A**) and female rats (**B**). ***Note*:** **p*=0.047 – statistically significant differences in relation to the control group. PC – prefrontal cortex; Hipp – hippocampus; Cer – cerebellum.

Conclusion

The study demonstrates that repeated 30-day intragastric administration of metformin (35 mg/kg and 70 mg/kg) to old male rats improved spatial memory in the Water Maze test and reduced anxiety levels in the Elevated Plus-Maze test (70 mg/kg). Under the influence of metformin, males showed an increase in *Bdnf* expression in the prefrontal cortex, while females showed a positive correlation in the cerebellum between *Bdnf* expression in the cerebellum and spatial memory. The corrective behavioral effects of metformin when administered to elderly rats in small doses differed depending on gender.

The results obtained confirm that the mechanisms of the geroprotective effects of metformin differ significantly in male and female rats, which underscores the need for further research based on gender.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

Funding

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Ethics statement

Experimental studies were carried out with the approval by the Local Ethics Committee of Kazan State Medical University of the Ministry of Health of the Russian Federation (Minutes № 6 of 20 June 2023).

Data availability

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

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