JBCR Journal of Biomedical & Clinical Research
Journal of Medical University of Pleven

Journal of Biomedical and Clinical Research 17(2): 133–142 (2024)
DOI: 10.3897/jbcr.e122925

Research Article

Effects of simultaneous pharmacological inhibition of cystathionine gamma-lyase and nitric oxide synthase on food and water intake, body mass gain, and body temperature in rats

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Summary

Various studies have emphasized the intricate relationship between hydrogen sulfide (H_2S) and nitric oxide (NO) in regulating diverse physiological processes. In this investigation, we aimed to elucidate the effects of simultaneous inhibition of the H_2S-producing enzyme cystathionine γ-lyase and the NO-producing enzyme nitric oxide synthase on food and water intake, body mass gain and body temperature in rats. Specifically, we explored the combined impact of dl-propargylglycine, an irreversible inhibitor of cystathionine γ-lyase, and Nω-Nitro-L-arginine methyl ester (l-NAME), a non-selective inhibitor of nitric oxide synthase, on these physiological parameters. Co-administration of dl-propargylglycine (50 mg/kg, i.p.) and l-NAME (50 mg/kg, i.p.) effectively suppressed food intake and body mass gain in fasted rats at 24 hours post-injection, accompanied by a notable decrease in water intake. Furthermore, this combined treatment induced a significant decline in body temperature at 90, 120, and 150 minutes post-injection compared to the control group, shedding light on the complex role of H_2S and NO systems in modulating body temperature regulation. These findings enhance our understanding of the potential physiological implications of targeting the cystathionine γ-lyase/H_2S and nitric oxide synthase/NO pathways.

Key words: dl-propargylglycine, enzyme inhibitor, gaseous mediator, hydrogen sulfide, l-NAME, nitric oxide

Academic editor: Galya Stavreva
Received: 12 March 2024
Accepted: 18 April 2024
Published: 20 June 2024

Citation: Hristov M, Andreeva-Gateva P (2024) Effects of simultaneous pharmacological inhibition of cystathionine gamma-lyase and nitric oxide synthase on food and water intake, body mass gain, and body temperature in rats. Journal of Biomedical and Clinical Research 17(2): 133–142. https://doi.org/10.3897/jbcr.e122925

Introduction

Hydrogen sulfide (H_2S) serves as a gaseous signaling molecule, synthesized by three key enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase. The physiological roles of H_2S are widely recognized, encompassing the regulation of gene transcription and translation, cellular bioenergetics, and metabolism. Additionally, it plays crucial roles in regulating vascular tone and immune function and modulating various functions in the central and peripheral nervous systems (Cirino et al. 2023). Numerous studies have underscored the significance of H_2S in regulating both food intake and body temperature. For example, H_2S plays a crucial role in the functionality of various feeding-related peptides

(Verbeure et al. 2021). H_2S has been proposed as a potent endogenous antipyretic agent, potentially operating via the suppression of prostaglandin E2 synthesis and/or the stimulation of cAMP production in the preoptic hypothalamus (Kwiatkoski et al. 2013). Moreover, H_2S serves as a cryogenic mediator of hypoxia-induced anapyrexia (Kwiatkoski et al. 2012). Recent research has revealed that CSE-induced H_2S synthesis permits brown adipose tissue activity in fever and during the maintenance of thermal homeostasis in cold environments (Soriano et al. 2018).

Nitric oxide (NO) is another gaseous signaling molecule that regulates various physiological functions, including endothelium-dependent vasodilation, neurotransmission, immunological responses, and secretion of hormones. It originates from the amino acid l-arginine through three isoforms of nitric oxide synthase (NOS): neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) (Branco et al. 2014). Like H2S, NO is a significant mediator in regulating body temperature and food intake. For instance, it has been proposed that iNOS and nNOS isoforms play a role in the induction of fever in response to lipopolysaccharide (Branco et al. 2014). NO is involved in the actions of both anorexigenic peptides (e.g., cholecystokinin, leptin) and orexigenic peptides (e.g., neuropeptide Y, ghrelin, orexin), thereby exerting a pivotal role in the control of satiety and hunger (Morley et al. 2011; Hristov et al. 2019).

Various studies have highlighted the intricate relationship between H_2S and NO in orchestrating diverse physiological processes. H_2S has been shown to enhance the release of glucagon-like peptide 1, subsequently promoting the production of both NO and H_2S (Verbeure et al. 2021). Additionally, research indicates that H_2S can inhibit the release of ghrelin, a known stimulator of NO production (Verbeure et al. 2021). It has been proposed that H_2S and NO collectively modulate the hypoxia-induced decrease in body temperature, with an intricate interplay observed within rats' anteroventral preoptic hypothalamus region (Kwiatkoski et al. 2012). Another study has revealed that inhibiting CSE with dl-propargylglycine and inhibiting NOS with 7-nitroindazole or aminoguanidine effectively suppresses the leptin-induced febrile response in rats, underscoring the involvement of the NOS/NO and CSE/H_2S systems in mediating leptin-induced fever (Hristov and Lazarov 2023). It has been found that CSE-induced H_2S production in response to a hypersodic diet plays an important proinflammatory role in the kidney, apparently counteracting NO actions in renal tissue (Moreira et al. 2021). In light of these insights, we opted to explore the ramifications of simultaneous inhibition of CSE and NOS on food and water intake, body mass gain, and body temperature. Previous studies have demonstrated that systemic administration of various NOS inhibitors suppresses food intake in rats (Squadrito et al. 1993; Kamerman et al. 2002; Hristov and Lazarov 2023). However, there is insufficient literature regarding the effects of CSE inhibition on food consumption. Therefore, we initially evaluated the effects of dl-propargylglycine (PAG), an irreversible inhibitor of CSE, on food and water intake in rats at different time points following systemic injection. Subsequently, we investigated the combined impact of PAG administration with the non-selective NOS inhibitor Nω-Nitro-L-arginine methyl ester (l-NAME) on food and water intake, body mass gain, and body temperature in rats.

Materials and methods

Drugs

DL-propargylglycine (PAG, a CSE inhibitor, P7888) and Nω-Nitro-L-arginine methyl ester (l-NAME, a non-selective NOS inhibitor, N5751) were sourced from Sigma-Aldrich, Schnelldorf, Germany. Both PAG and l-NAME were administered at a dose of 50 mg/kg, a dosage carefully selected based on previous investigations where no noticeable effects on core body temperature were observed following intraperitoneal administration in rats at subneutral ambient temperature (Kamerman et al. 2002; Hristov and Lazarov 2023). Before administration, both drugs were dissolved in a physiological saline solution (0.9% w/v NaCl) and delivered intraperitoneally (i.p.) at an injection volume of 0.2 ml per 100 g of body mass. Control animals received a physiological saline solution (0.9% w/v NaCl).

Animals

Male Wistar rats, aged 10–12 weeks, weighing 250±30 g, were procured from the Laboratory Animal Breeding Center of the Bulgarian Academy of Sciences in Slivnitsa, Bulgaria. They were housed in groups of six rats per cage in a temperature-regulated environment (20–22 °C) under a 12:12-hour light-dark cycle (07:00 to 19:00 h). The rats had ad libitum access to standard chow pellets and water. Each treatment group contained six animals. All procedures applied strictly adhered to the guidelines outlined in Directive 2010/63/EU of the European Parliament and the Council, dated 22 September 2010, regarding protecting animals used for scientific purposes. Approval for all experimental protocols was obtained from the Ethical Council of the Bulgarian Food Safety Agency (Approval Number: 375).

Measurement of food and water intake and body mass gain

Before the experiment, all rats underwent a 24-hour food deprivation period while retaining access to water. Consistent conditions were maintained: all experimental procedures were conducted between 10:30 AM and 11:30 AM to minimize potential circadian rhythm-related effects. The body masses of the rats were recorded both before and 24 hours after the injections. The rats were individually placed in separate cages to measure food and water intake. Pre-weighed chow pellets were provided in each cage, and food intake measurements were collected at various intervals post-injection, with adjustments made for any spillage during measurement. Drinking water was accessible via graduated bottles, and water intake measurements were recorded at different time points following the injection.

Measurement of body temperature

Body temperature was carefully monitored using thermocouple probes connected to a computer-controlled multi-channel thermocouple thermometer Iso-Thermex (Columbus Instruments, Columbus, Ohio, U.S.A.). Before insertion, thermocouple probes were lubricated with petroleum jelly and inserted at least 6 cm into the rectum to ensure accurate core body temperature tracking. During temperature monitoring, the movements of the rats were gently restrained. Initial body temperature readings were obtained immediately before administering the injection, revealing

similar body temperatures across all treatment groups. Subsequently, body temperature was recorded at 30-minute intervals for 150 minutes post-injection. The entire experimental setup was maintained at a constant room temperature ranging from 20 °C to 22 °C to ensure consistency throughout the study.

Statistical analysis

Statistical analysis was performed using SigmaPlot 12.5 software (Systat Software GmbH, Erkrath, Germany). Normality testing was performed using the Shapiro-Wilk test, which confirmed that the data followed a normal distribution. A two-tailed Student's t-test was utilized to compare two treatment groups. When analyzing data from more than two treatment groups, a one-way analysis of variance (ANOVA) was conducted, followed by the Student-Newman-Keuls multiple comparison test for post hoc analysis. Statistical significance was set at a p-value less than 0.05. All values are presented as the mean ± standard error of the mean (SEM).

Results

Effects of systemic administration of PAG on cumulative food and water intake in rats

Systemic administration of PAG did not induce any significant change in food intake at 3 or 7 hours post-injection. However, at the 24-hour, the PAG-injected rats exhibited reduced food consumption compared to the control group (Fig. 1A; all $p > 0.192$, except t24: $p < 0.05$). Additionally, systemic administration of PAG did not elicit any alteration in water intake at 3, 7, or 24 hours post-injection (Fig. 1B; all $p > 0.99$).

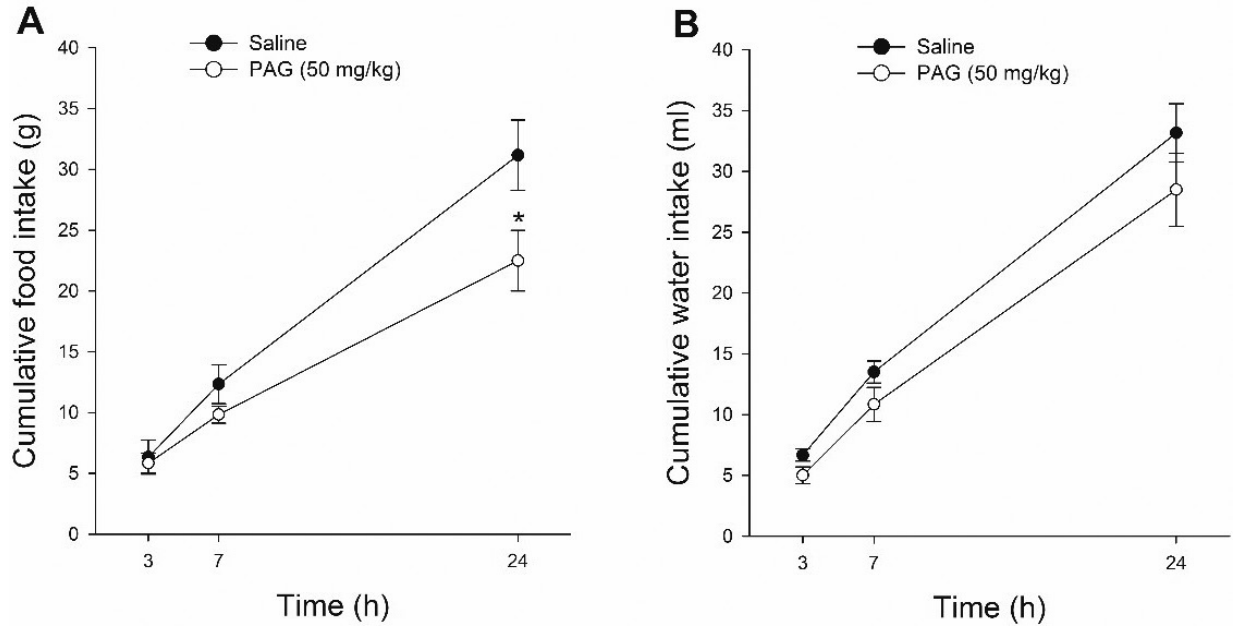

Figure 1. Effects of CSE inhibition on food and water intake in rats: Systemic administration of PAG showed no significant effect on food (panel **A**) and water (panel **B**) intake at 3 and 7 hours after injection but resulted in reduced food consumption compared to controls after 24 hours, while water intake remained unaffected. The results are presented as the mean ± SEM. n = 6 rats per group. *, $p < 0.05$ vs. control group (Saline).

Effects of co-administration of PAG and l-NAME on food and water intake and body mass change in rats

Systemic administration of PAG and l-NAME, both individually and in combination, significantly influenced food intake in rats at 24 hours post-injection (Fig. 2A, $F_{3,20} = 21.953$, $p < 0.001$). Animals treated with either PAG or l-NAME consumed significantly less food compared to those treated with saline (PAG vs. saline: $p = 0.011$; l-NAME vs. saline: $p = 0.008$, Fig. 2A). There was no statistically significant difference in food intake between PAG-treated and l-NAME-treated rats (PAG vs. l-NAME: $p = 0.55$). Notably, animals administered with the combination of PAG and l-NAME exhibited a substantially reduced food intake compared to the other treatment groups (all $p < 0.001$, Fig. 2A).

Intraperitoneal delivery of PAG and l-NAME significantly impacted water intake in rats 24 hours post-injection (Fig. 2B, $F_{3,20} = 29.948$, $p < 0.001$). Post hoc analysis revealed that only the rats treated with the combination of PAG and l-NAME exhibited a significant reduction in water intake compared to the other treatment groups (all $p < 0.001$, Fig. 2B) (Saline vs. PAG vs. l-NAME: all $p > 0.127$, Fig. 2B).

Systemic administration of PAG and l-NAME, alone and in combination, significantly influenced rat body mass gain at 24 hours post-injection (Fig. 2C, $F_{3,20} = 22.781$, $p < 0.001$). Animals treated with either PAG or l-NAME experienced a reduction in body mass gain compared to those treated with saline (PAG vs. saline: $p = 0.003$; l-NAME vs. saline: $p = 0.002$, Fig. 2C). There were no statistically significant differences in body mass gain between PAG-treated and

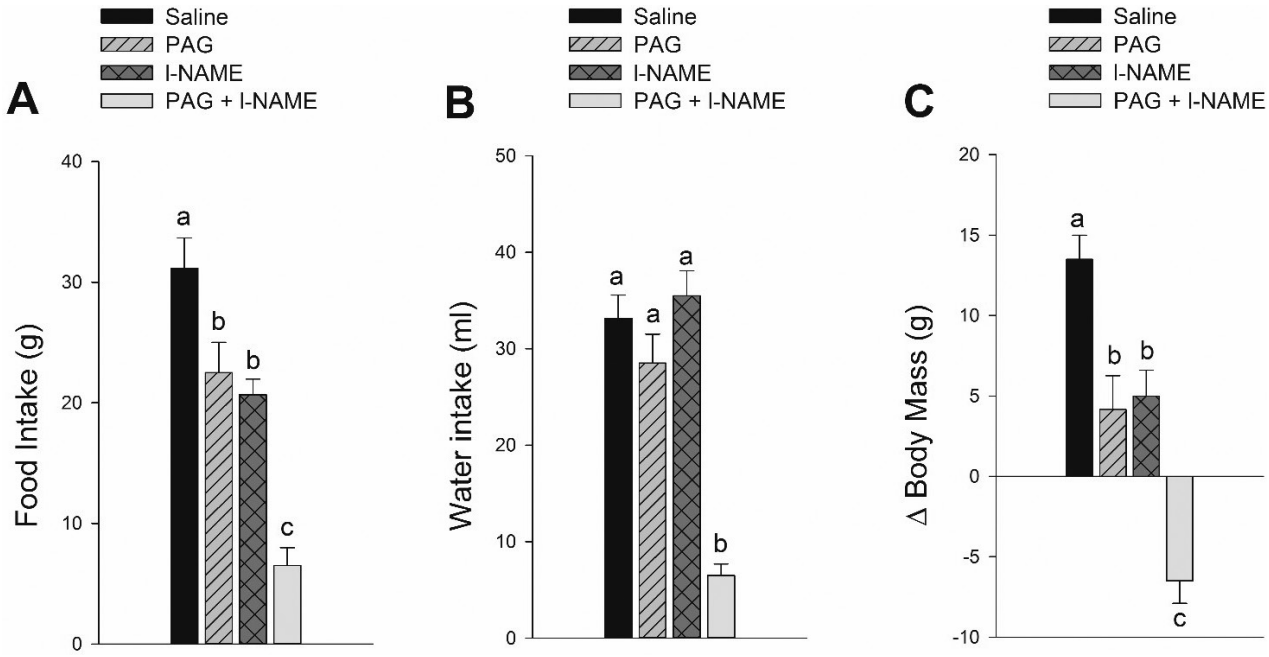

Figure 2. Effects of simultaneous inhibition of CSE and NOS on food and water intake and body mass gain in rats: Systemic administration of PAG and l-NAME, either alone or in combination, significantly reduced food intake (panel **A**) and body mass gain (panel **C**) 24 hours post-injection, with the combined treatment showing the most pronounced effects on these parameters compared to individual treatments and saline control. Notably, water intake (panel **B**) was reduced only after co-administration of PAG and l-NAME. Δ Body mass represents the change in body mass. The results represent the mean ± SEM. n = 6 rats per group. Details of statistical tests are given in the text; letters indicate significant differences according to the post hoc test.

l-NAME-treated rats (PAG vs. l-NAME: p = 0.735). Notably, animals administered with the combination of PAG and l-NAME exhibited a substantial decrease in body mass gain compared to the other treatment groups (all p < 0.001, Fig. 2C).

Effects of co-administration of PAG and l-NAME on body temperature in rats

The combined intraperitoneal administration of PAG and l-NAME did not elicit significant changes in body temperature at 30 and 60 minutes post-injection. However, at the 90th, 120th, and 150th minutes, this combination led to a statistically significant decrease in body temperature compared to the control group (Fig. 3; all p < 0.049, except t30 and t60: all p > 0.15).

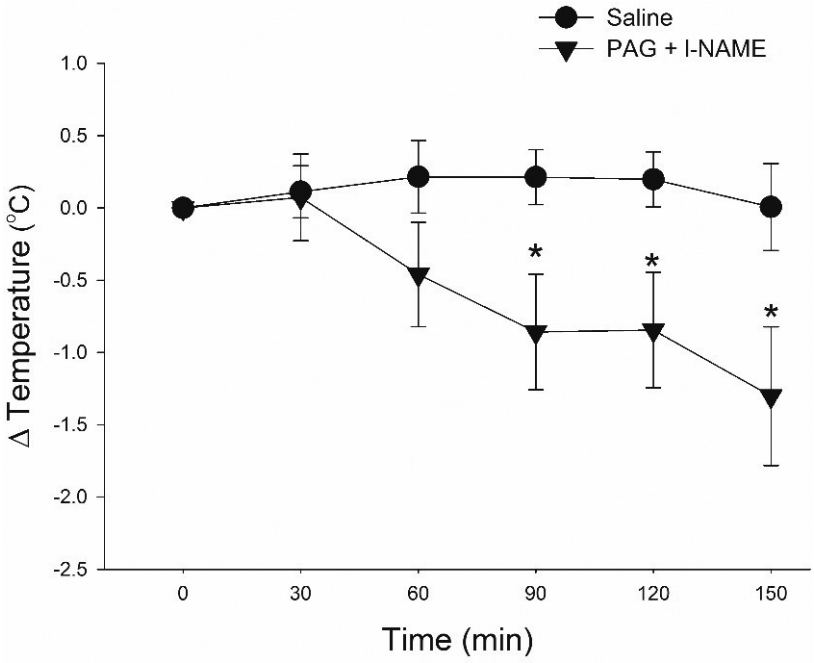

Figure 3. Effects of simultaneous inhibition of CSE and NOS on core body temperature in rats: The combined intraperitoneal administration of PAG and l-NAME did not significantly alter body temperature at 30 and 60 minutes after injection but resulted in a statistically significant decrease in body temperature at 90, 120, and 150 minutes post-injection compared to the control group. ΔTemperature represents the change in body temperature from the baseline (time 0). All results are presented as the mean ± SEM. n = 6 rats per group. *, p < 0.05 vs. control group (Saline).

Discussion

In our current study, we have demonstrated that the administration of the irreversible CSE inhibitor PAG effectively reduces food intake and mitigates body mass gain in fasted rats 24 hours after injection, aligning with previous findings on the intraperitoneal administration of PAG in rats (Hristov and Lazarov 2023). Notably, co-administration of PAG with the non-selective NOS inhibitor l-NAME resulted in a marked suppression of food intake and body mass gain in fasted rats 24 hours post-injection. We postulate that this combined effect may arise from suppressing gastric accommodation, supported by previous

research indicating that NOS inhibitors can impede gastric accommodation in rats (Verschueren et al. 2014) and dogs (Meulemans et al. 1995). Additionally, non-selective NOS inhibition in humans has been linked to inhibited gastric accommodation during meal intake, increased satiety scores, and significantly reduced calorie consumption at maximum satiety (Tack et al. 2002). Studies have shown the expression of CSE and CSB in the stomachs of rodents (Martin et al. 2010). Notably, the H_2S donors NaHS and L-cysteine induced relaxation in either the gastric fundus or body in mice, while aminooxyacetic acid, a CBS inhibitor, inhibited gastric compliance and reduced food intake and body mass in mice. However, PAG has been shown to exhibit no discernible effect on gastric compliance in mice (Xiao et al. 2015). It is important to note the discrepancy in the effect of PAG between mice and rats, where PAG inhibits H_2S synthesis in the rat stomach but not in mice (Martin et al. 2010). Consequently, we hypothesize that simultaneous inhibition of NOS and CSE could significantly suppress gastric accommodation, reducing food intake and body mass gain in rats.

The suppression of the orexigenic peptide neuropeptide Y may represent another potential mechanism leading to significant reductions in food intake and body mass gain following the combined administration of PAG and l-NAME in fasted rats. Neuropeptide Y expression typically rises during fasting but returns to normal levels upon refeeding, indicating its physiological role in stimulating food intake in response to food deprivation (Gruenewald et al. 1996). Studies suggest that NO may act as a central mediator of neuropeptide Y's effect on feeding behavior. For example, unlike their wild-type counterparts, homozygous NOS KO mice failed to exhibit increased food intake after neuropeptide Y administration (Morley et al. 2011). Moreover, it has been demonstrated that l-NAME inhibits food consumption induced by fasting and neuropeptide Y administration (Morley and Flood 1992). Research has revealed that GYY4137, a water-soluble and slow-releasing H_2S donor, augments food intake in mice and stimulates the production of neuropeptide Y in the arcuate nucleus of the hypothalamus, suggesting that H_2S amplifies appetite via its interaction with neuropeptide Y (Zhou et al. 2018). We propose that intraperitoneal injection of PAG may attenuate H_2S production in the hypothalamus, as prior studies have demonstrated the efficacy of systemic administration of PAG in inhibiting CSE in the rodent brain (Kodama et al. 1985; Diwakar and Ravindranath 2007). However, it is important to note that CBS is the brain's primary enzyme accountable for H_2S synthesis (Martin et al. 2010). Hence, further investigation is warranted to elucidate the hypothesis that systemic administration of PAG could potentially impede H_2S synthesis in the hypothalamus, consequently dampening neuropeptide Y activity.

The findings of this study revealed that systemic administration of either PAG or l-NAME in fasted rats did not induce a change in water intake 24 hours post-injection. However, intriguingly, the concurrent administration of PAG and l-NAME led to a significant suppression of water intake 24 hours after injection. Previous studies have demonstrated that intracerebroventricular injection of sodium sulfide (Na_2S), an inorganic donor of H_2S, resulted in reduced water intake in water-deprived rats after 5 minutes. However, by the 15-minute mark, there was no discernible difference in water intake between Na_2S-treated and vehicle-treated groups. Furthermore, intracerebroventricular injection of the NOS inhibitor Nω-methyl-l-arginine (l-NMMA) decreased water intake at 5 minutes. Interestingly, concurrent administration of l-NMMA and Na_2S amplified

the reduction in water intake induced by l-NMMA (Coletti et al. 2015). However, it is important to note that a direct comparison between our study and this one is not feasible due to differences in experimental design, hydration status of the animals, and routes of substance administration.

In the present study, we administered combined PAG and l-NAME at doses that do not alter body temperature following intraperitoneal injection in rats at subneutral ambient temperature (Kamerman et al. 2002; Soriano et al. 2018; Hristov and Lazarov 2023). Our findings revealed that systemic administration of PAG and l-NAME led to reduced body temperature at 90, 120, and 150 minutes post-injection. It has been reported that inhibiting peripheral NO production reduces thermogenic mechanisms. However, this effect is consistent and relevant only when animals are subjected to subneutral ambient temperatures, as in our study conditions (Branco et al. 2014). Recent evidence has shown that CSE-induced H_2S production does not influence plasma NO concentrations during fever; instead, it permits increased brown adipose tissue thermogenesis to uphold thermal homeostasis in cold environments and during fever response (Soriano et al. 2018). Consequently, the decline in body temperature observed following simultaneous inhibition of CSE and NOS in our study likely stems from the suppression of thermogenic mechanisms under conditions of subneutral ambient temperature.

Conclusion

In conclusion, our study sheds light on the intricate interplay between H_2S and NO in regulating physiological processes. Co-administration of the CSE inhibitor PAG and the NOS inhibitor l-NAME effectively suppressed food intake and body mass gain in fasted rats 24 hours post-injection, along with a notable decrease in water intake. Moreover, this combined treatment induced a significant decline in body temperature at 90, 120, and 150 minutes post-injection compared to the control group, highlighting the complex role of H_2S and NO systems in modulating body temperature regulation. These findings contribute to our understanding of the potential physiological implications of targeting both CSE/H_2S and NOS/NO pathways.

Conflict of interest statement

The authors have no competing interests to declare that are relevant to the content of this article.

Acknowledgments

This work was supported by the Council of Medical Science, Medical University of Sofia (Grant 2023, Contract № Д-172/03.08.2023).

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