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ORIGINAL ARTICLE

SYSTEMIC NITRIC OXIDE SYNTHASE INHIBITION SUPPRESSES APELIN-INDUCED RISE IN BODY TEMPERATURE IN RATS

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Abstract: *Apelin is a peptide involved in the regulation of various physiological processes, including thermoregulation, metabolism, and energy balance. This study investigates the role of nitric oxide (NO) in mediating apelin's effects on body temperature, food intake, and body mass gain in rats. Using the non-selective nitric oxide synthase (NOS) inhibitor L-NAME and the selective inducible NOS inhibitor aminoguanidine (AG), we assessed how systemic inhibition of NO synthesis modulates apelin-induced responses. Male Wistar rats were administered intraperitoneal injections of [Pyr1]apelin-13 following pre-treatment with L-NAME or AG. Our results show that both L-NAME and AG suppressed the apelin-induced rise in body temperature, with L-NAME having a more pronounced effect. Additionally, L-NAME significantly reduced apelin-induced food intake and body mass gain, while AG had a lesser impact. These findings suggest that NO plays a key role in mediating the apelin's thermoregulatory and metabolic effects. The differential outcomes between L-NAME and AG highlight the potential involvement of multiple NOS isoforms in these processes. Further investigation into the distinct roles of NOS isoforms may provide deeper insights into NO-apelin interactions and their relevance to metabolic regulation, offering potential therapeutic targets for metabolic disorders.*

Key words: *apelin, aminoguanidine, L-NAME, metabolism, nitric oxide synthase, thermoregulation*

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INTRODUCTION

Apelin is a peptide that plays a crucial role in regulating various physiological processes, including energy balance, cardiovascular, gastrointestinal, neuroendocrine, and immune functions. It acts as an endogenous ligand for the G-protein-coupled receptor APJ. Apelin is initially synthesized as a 77-amino acid pre-protein, which is processed into several active forms, including apelin-13, -17, and -36. Among these, [Pyr^{1}]apelin-13, a pyroglutamated form of apelin-13, is particularly resistant to degradation and serves as a key physiological ligand for APJ [1, 2]. Previous research has indicated that apelin is involved in the regulation of body temperature and energy homeostasis. For example, intracerebroventricular (ICV) administration of apelin-13

in rats has been shown to elevate body temperature [3]. Additionally, chronic administration of apelin-13 into the third cerebral ventricle over a 10-day period increased food intake, body mass, locomotor activity, and body temperature in mice [4]. Recently, systemic intraperitoneal administration of [Pyr^1]apelin-13 was found to significantly increase body temperature and body mass in fasted rats [5].

Nitric oxide (NO) is a gaseous signaling molecule produced from the amino acid L-arginine through three isoforms of nitric oxide synthase (NOS): neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) [6]. The NOS/NO pathway plays a crucial role in regulating body temperature [6]. For instance, deficiencies in iNOS or nNOS have been shown to affect lipopolysaccharide-induced fever [7]. Additionally, pharmacological inhibition of NOS using the nonselective NOS inhibitor Nω-Nitro-L-arginine methyl ester (L-NAME) or the selective iNOS inhibitor aminoguanidine (AG) suppresses lipopolysaccharide-induced fever in rats [8, 9]. Systemic administration of AG significantly attenuates heatstroke-induced hyperthermia [10]. Furthermore, it has been demonstrated that inhibiting NOS with L-NAME, 7-nitroindazole, or AG effectively suppresses the leptin-induced febrile response in rats, highlighting the involvement of the NOS/NO pathway in mediating leptin-induced fever [11, 12].

Previous research has shown that ICV administration of L-NAME did not influence the increase in body temperature induced by apelin [3]. This indicates that central NO signaling may not play a significant role in these temperature changes. However, ICV administration primarily affects specific neuronal circuits involved in thermoregulation, such as those connected to the hypothalamus or brainstem, depending on the ventricle targeted [6]. To gain a more comprehensive understanding of NO's role in apelin-induced increases in body temperature, it is necessary to investigate systemic NO inhibition. Systemic administration of L-NAME or AG inhibits NO production both centrally and peripherally, offering a broader perspective on NO's involvement in thermoregulation [6, 8-10]. This approach allows us to determine whether peripheral NO pathways, which remain unaffected by ICV administration, contribute to the apelin-induced temperature response.

In this study, we tested the hypothesis that systemic inhibition of NO synthesis with L-NAME or AG could modulate apelin's effects on body temperature. Furthermore, we examined how NO synthase inhibition impacts the effects of apelin on food intake and body mass, providing a more holistic understanding of the interaction between NO signaling and apelin's physiological actions.

MATERIALS AND METHODS

Drugs

[Pyr^1]apelin-13 (SML2084), Nω-Nitro-L-arginine methyl ester (L-NAME, a non-selective NOS inhibitor, N5751), and aminoguanidine (AG, a selective iNOS inhibitor, 396494) were obtained from Sigma-Aldrich, Schnelldorf, Germany. L-NAME and AG were administered at a dosage of 50 mg/kg [11, 12], while [Pyr^1]apelin-13 was administered at a dosage of 0.5 mg/kg [5]. The selected doses of L-NAME and AG were based on previous investigations, where no significant effects on core body temperature were observed following intraperitoneal administration in rats [11, 12]. All drugs were dissolved in physiological saline (0.9% w/v NaCl) and administered intraperitoneally (IP) at a volume of 0.2 ml/100 g body mass. Control animals received only physiological saline (0.9% w/v NaCl).

Animals

Male Wistar rats (10-12 weeks old, 250±30 g) were obtained from the Laboratory Animal Breeding Center of the Bulgarian Academy of Sciences, Slivnitsa, Bulgaria. The rats were housed in groups of six per cage in a temperature-controlled environment (20–22 °C) under a 12-hour light/dark cycle (lights on from 07:00 to 19:00). They had ad libitum access to standard chow and water throughout the study. All animal experiments were conducted in full compliance with Directive 2010/63/EU of the European Parliament and Council (22 September 2010) on the protection of animals used for scientific purposes. The study protocols were approved by the Ethical Council of the Bulgarian Food Safety Agency.

Measurement of body temperature, food intake, and body mass gain

The experimental protocol was based on procedures described in previous studies [5, 11, 12]. Prior to the experiment, all rats underwent a 24-hour period of food deprivation with free access to water. Body mass was measured both before the injections and 24 hours afterward. To control for potential circadian effects, all experimental procedures were carried out between 10:30 AM and 11:30 AM. Rats were first IP injected with L-NAME, AG, or saline, followed 10 minutes later by an IP injection of [Pyr^1]apelin-13. The animals were divided into four treatment groups (n = 6 per group): saline+saline, saline+[Pyr^1]apelin-13, AG+[Pyr^1]apelin-13, and L-NAME+[Pyr^1]apelin-13. Each animal was used in only one experimental session. Core body temperature was monitored using thermocouple probes connected to an Iso-Thermex multi-channel thermometer (Columbus Instruments,

Columbus, Ohio, U.S.A.), with the probes lubricated with petroleum jelly and inserted at least 6 cm into the rectum for precise readings. The rats were mildly restrained during the temperature measurements. Baseline body temperature was recorded just before the first injection, showing no significant differences between the treatment groups. Following the second injection, body temperature was recorded at 30-minute intervals for 150 minutes. To assess the overall change in body temperature over time, the area under the curve (AUC) from -10 to 150 minutes was calculated using the "Area Below Curves" macro in SigmaPlot 12.5 (Systat Software GmbH, Erkrath, Germany). Following body temperature measurements, the rats were transferred to individual cages within 5 minutes. Pre-weighed chow pellets were provided, and food intake was measured 24 hours post-injection, with adjustments made for spillage. The experiment was conducted in a controlled room temperature environment (20-22 °C).

Statistical analysis

Statistical analysis was performed using SigmaPlot 12.5 software (Systat Software GmbH, Erkrath, Germany). The normality of the data was assessed using the Shapiro-Wilk test, confirming that the data followed a normal distribution. For comparisons between two treatment groups, a two-tailed Student's t-test was applied. For comparisons involving more than two groups, a one-way ANOVA was used, followed by the Student-Newman-Keuls multiple comparison test. Statistical significance was defined as a p-value less than 0.05. All results are expressed as mean ± standard error of the mean (SEM).

RESULTS

Effects of combined administration of NOS inhibitors and apelin on body temperature

Systemic administration of the non-selective NOS inhibitor L-NAME significantly inhibited the apelin-induced increase in body temperature at the 60th, 90th, and 120th minutes after injection (Fig. 1A). Throughout the 150-minute recording period, the combined administration of the selective iNOS inhibitor AG and [Pyr^1] apelin-13 significantly reduced the apelin-induced rise in body temperature at the 60th and 90th minutes (Fig. 1B). As shown in Figure 2, there were no statistically significant differences in temperature responses between the L-NAME+[Pyr^1]apelin-13, AG+[Pyr^1]apelin-13, and control (saline+saline) groups.

Analysis of the AUC indicated that the differences in mean values among the treatment groups were greater than would be expected by chance (Fig. 3A, $F_{2,15}$ = 4.425, p = 0.031; Fig. 3B, $F_{2,15}$ = 4.389, p = 0.032). A post hoc test of the mean AUC values revealed a significant difference in the L-NAME+[Pyr^1]apelin-13 group (Fig. 3A) and the AG+[Pyr^1]apelin-13 group (Fig. 3B) compared to the saline+[Pyr^1]apelin-13 group. However, no significant differences were observed when comparing the L-NAME+[Pyr^1]apelin-13 group and the AG+[Pyr^1] apelin-13 group to the control (saline+saline) group.

Effects of combined administration of NOS inhibitors and apelin on food intake and body mass gain

The combined administration of L-NAME and [Pyr^1] apelin-13 significantly affected food intake (Fig. 4A, $F_{2,15}$ = 3.815, p = 0.046) and body mass change

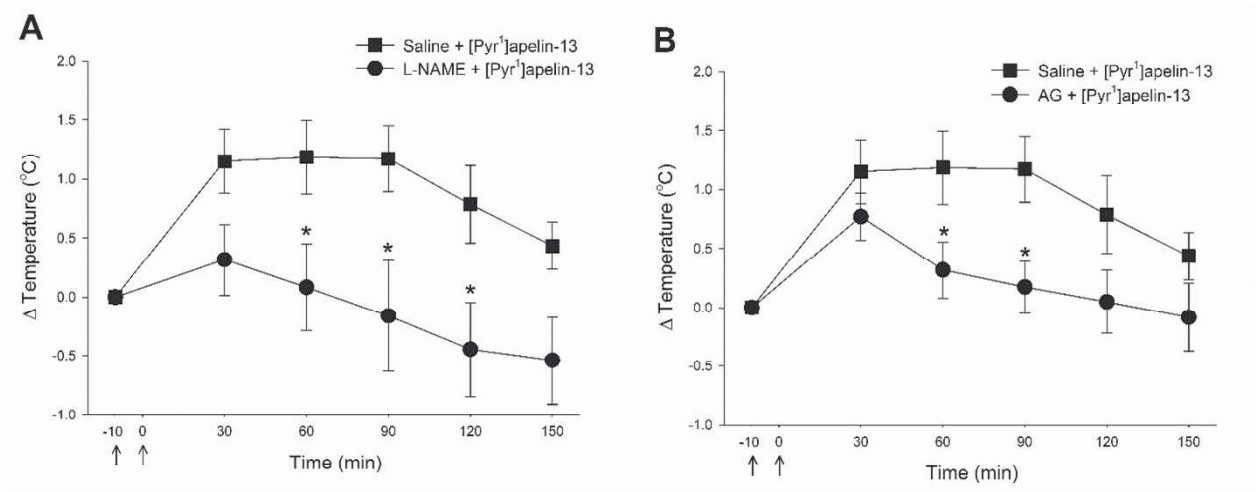

Fig. 1. Effects of systemic NOS inhibition on apelin-induced increase in body temperature in 24-hour-fasted rats. The first arrow indicates the time of the initial injection (saline, L-NAME, or AG), and the second arrow indicates the time of the subsequent injection (saline or [Pyr^1]apelin-13). In panel (A), intraperitoneal (IP) injection of the non-selective NOS inhibitor L-NAME suppressed the apelin-induced increase in body temperature at the 60th, 90th, and 120th minutes post-injection. In panel (B), administration of the selective iNOS inhibitor AG significantly reduced the apelin-induced rise in body temperature at the 60th and 90th minutes. ΔTemperature represents the change in body temperature from baseline (time -10). Data are presented as mean ± SEM, n = 6 rats per group. *p < 0.05 vs. saline + [Pyr^1]apelin-13 group.

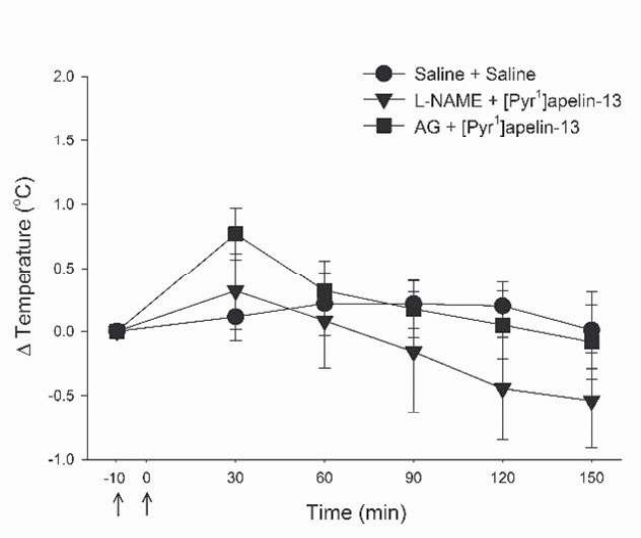

Fig. 2. Combined administration of NOS inhibitors and [Pyr[1]]apelin-13 shows temperature response similar to control group. ΔTemperature indicates the change in body temperature from baseline (time -10). Data are presented as mean ± SEM; n = 6 rats per group

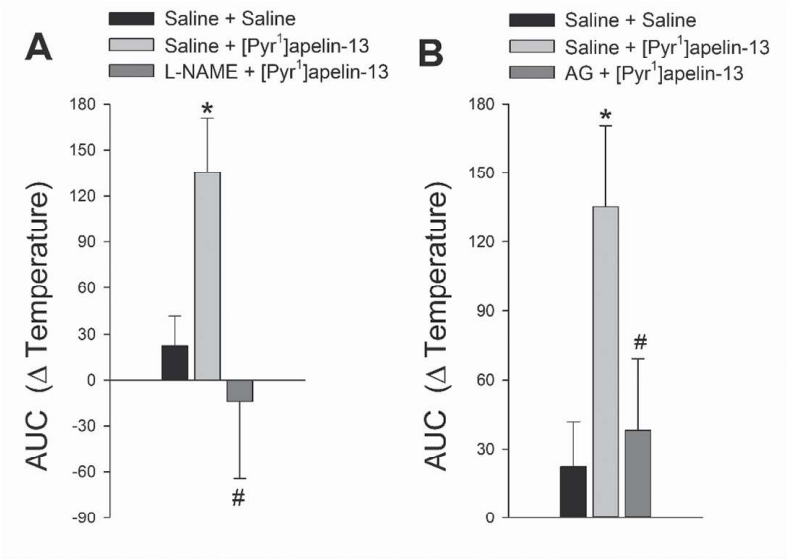

Fig. 3. Area under the curve (AUC) analysis of the overall change in body temperature from -10 to 150 minutes post-injection. Panel (A) compares the AUC values for the control group, the group treated with [Pyr[1]]apelin-13 alone, and the group treated with L-NAME and [Pyr[1]]apelin-13. Panel (B) compares the AUC values for the control group, the group treated with [Pyr[1]]apelin-13 alone, and the group treated with AG and [Pyr[1]]apelin-13. Data are presented as mean ± SEM, n = 6 rats per group. *p < 0.05 vs. saline + saline group; #p < 0.05 vs. saline + [Pyr[1]]apelin-13 group

(Fig. 4B, $F_{2,15}$ = 7.002, p = 0.007) in rats. Animals treated with L-NAME and [Pyr[1]] apelin-13 consumed less food compared to those receiving [Pyr[1]]apelin-13 alone, but no significant difference in food intake was observed compared to control animals (Fig. 4A). Systemic administration of L-NAME also suppressed the apelin-induced increase in body mass (Fig. 4B).

The combined administration of AG and [Pyr[1]]apelin-13 did not significantly affect food intake (Fig. 4C, $F_{2,15}$ = 0.400, p = 0.677), but it did significantly influence changes in body mass (Fig. 4D, $F_{2,15}$ = 3.867, p = 0.044). Post hoc analysis revealed no significant difference in body mass gain between the control group and the AG+[Pyr[1]]apelin-13group, indicating that AG suppresses the apelin-induced increase in body mass (Fig. 4D).

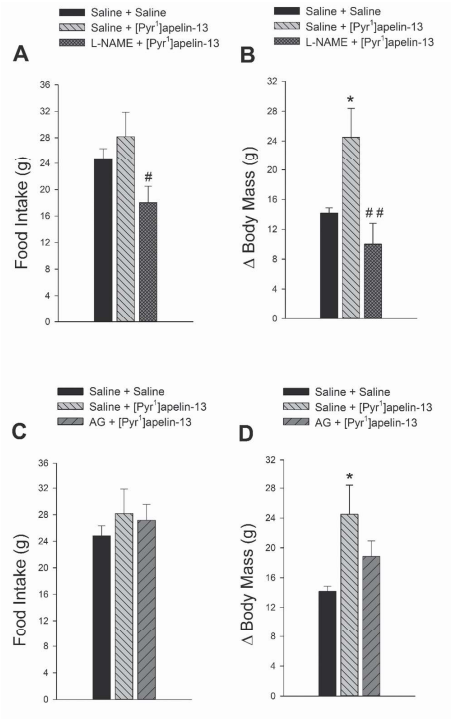

Fig. 4. Effects of combined administration of NOS inhibitors and [Pyr[1]]apelin-13 on food intake (A, C) and body mass change (B, D) in 24-hour fasted rats. Results are presented as mean ± SEM, with * p < 0.05 vs. saline+saline group; #p < 0.05, ##p < 0.01 vs. saline+[Pyr[1]]apelin-13 group. n = 6 rats per group

DISCUSSION

In our study, we demonstrated that systemic NOS inhibition with L-NAME or AG suppresses the apelin-induced increase in body temperature, suggesting that NO plays a key role in mediating the apelin's effects on thermoregulation. These findings align with previous studies that have highlighted NO's involvement in apelin signaling. For instance, apelin has been shown to directly activate the vascular L-arginine/NOS/NO pathway, which plays a key role in regulating vascular function [13, 14]. Additionally, post-infarct treatment with [Pyr^{1}]apelin-13 reduces myocardial damage by decreasing oxidative injury and enhancing NO levels in a rat model of myocardial infarction [15]. Another study demonstrated that apelin improves sensory-motor balance defects by reducing neuronal death and restoring serum NO levels [16]. Apelin-13 also alleviates diabetic nephropathy by enhancing NO production and suppressing kidney tissue fibrosis [17]. Further evidence supporting NO's role comes from research showing that ICV injection of apelin in fed mice improves glucose control via NO-dependent mechanisms. These effects were confirmed using transgenic models (eNOS knockout mice), pharmacological interventions (L-NMMA-treated mice), and real-time NO release measurements via amperometric probes. Notably, high-fat diet-fed mice exhibited a blunted response to apelin, associated with a lack of NO response in the hypothalamus [18].

Previous research has emphasized the apelin's crucial role in thermogenesis, particularly in brown adipose tissue (BAT). Apelin treatment has been shown to raise body temperature, increase oxygen consumption, and lower the respiratory quotient, indicating enhanced metabolic activity. Additionally, apelin increased the expression of uncoupling protein 1, a key marker of energy expenditure, in BAT and uncoupling protein 3, a regulator of fatty acid export, in skeletal muscle [19]. Moreover, apelin-APJ signaling promotes brown adipocyte differentiation by upregulating thermogenic and adipogenic transcription factors. It also enhances the basal activity of brown adipocytes by stimulating mitochondrial biogenesis and increasing oxygen consumption, further reinforcing its role in thermogenesis [20]. Previous studies suggest that NO plays a pivotal role in enhancing BAT thermogenic functions. Sympathetic stimulation, both in vivo (e.g., cold exposure or β3-adrenergic agonist treatment) and in vitro (e.g., noradrenaline treatment of cultured cells), significantly increases the expression and activity of both cytosolic and nuclear eNOS and iNOS in rat brown adipocytes [21]. Additionally, NO has been shown to support thermogenesis-related processes such as mitochondriogenesis, angiogenesis, and tissue hyperplasia by influencing their molecular basis. These findings indicate that NO is a key regulator of BAT thermogenic programming, affecting gene expression, protein levels, and tissue structure [22]. Thus, we speculate that inhibiting NOS may suppress the apelin's thermogenic effects on BAT.

In this study, we also demonstrated that systemic inhibition of NOS with L-NAME significantly suppressed the apelin-induced effects on food intake and body mass gain in rats. The observed suppression of the apelin's effects by L-NAME suggests a broad involvement of NO in the regulation of both energy intake and metabolic processes. In contrast, the selective inhibition of iNOS with AG was less effective in modulating these apelin-induced responses, as indicated by the lack of significant effects on food intake and only partial suppression of the apelin-induced increase in body mass. The distinct outcomes between L-NAME and AG administration suggest that different NOS isoforms may play unique roles in mediating the apelin's effects on metabolism. L-NAME is a non-selective NOS inhibitor, blocking both eNOS and nNOS in addition to iNOS, which may account for its broader impact on apelin-induced changes. Interestingly, while previous studies have shown that the IP administration of L-NAME or AG at the same dosage (50 mg/kg) suppressed food intake and body mass gain 24 hours after injection [11, 12, 23, 24], these effects were not replicated when NOS inhibitors were co-administered with apelin in our study. This discrepancy could be due to the complex interplay between apelin and NO pathways. Apelin's known effects on thermogenesis and metabolic regulation could interact with NO in a manner that alters the usual impact of NOS inhibition on these variables.

Further investigation is required to fully elucidate the mechanisms underlying these findings. The distinct roles of various NOS isoforms in apelin-mediated metabolic regulation remain unclear, making it essential to explore their specific contributions. Gaining a deeper understanding of these complex interactions between NO and apelin could provide valuable insights into their roles in thermogenesis and metabolism, potentially leading to new therapeutic targets for metabolic disorders.

CONCLUSION

Our study demonstrates that systemic inhibition of NOS using L-NAME or AG suppresses the apelin-induced increase in body temperature, highlighting the role of nitric oxide in mediating the apelin's thermoregulatory effects. Additionally, L-NAME effectively

suppressed the apelin's impact on food intake and body mass gain, while selective inhibition of inducible NOS with AG was less effective. These findings suggest that different NOS isoforms may have distinct roles in modulating the apelin's effects on metabolism. Further investigation into the specific contributions of these isoforms may provide deeper insights into the mechanisms governing apelin's influence on thermogenesis and energy homeostasis, potentially leading to novel therapeutic targets for metabolic disorders.

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Ethical statement: *All animal experiments were conducted in full compliance with Directive 2010/63/EU of the European Parliament and Council (22 September 2010) on the protection of animals used for scientific purposes. The study protocols were approved by the Ethical Council of the Bulgarian Food Safety Agency.*

REFERENCES

1. Hu G, Wang Z, Zhang R et al. The Role of Apelin/Apelin Receptor in Energy Metabolism and Water Homeostasis: A Comprehensive Narrative Review. Front Physiol., 2021,12:632886
2. Knauf C, Drougard A, Fournel A, et al. Hypothalamic actions of apelin on energy metabolism: new insight on glucose homeostasis and metabolic disorders. Horm Metab Res., 2013,45(13):928-934.
3. Jászberényi M, Bujdosó E, Telegdy G. Behavioral, neuroendocrine and thermoregulatory actions of apelin-13. Neuroscience., 2004,129(3):811-816.
4. Valle A, Hoggard N, Adams AC et al. Chronic central administration of apelin-13 over 10 days increases food intake, body weight, locomotor activity and body temperature in C57BL/6 mice. J Neuroendocrinol., 2008,20(1):79-84.
5. Hristov M, Andreeva-Gateva P. Effects of [Pyr1]apelin-13 on body temperature, food intake, and body mass in fasted rats: role of cystathionine gamma-lyase. Current Topics in Peptide and Protein Research., 2023,24:77-83.
6. Branco LG, Soriano RN, Steiner AA. Gaseous mediators in temperature regulation. Compr Physiol, 2014,4(4):1301-1338.
7. Kozak W, Kozak A. Genetic Models in Applied Physiology. Differential role of nitric oxide synthase isoforms in fever of different etiologies: studies using Nos gene-deficient mice. J Appl Physiol (1985), 2003, 94(6):2534-2544.
8. Soszynski D, Chelminiak M. Intracerebroventricular injection of neuronal and inducible nitric oxide synthase inhibitors attenuates fever due to LPS in rats. J Physiol Pharmacol, 2007, 8(3):551-561.
9. Steiner AA, Rudaya AY, Ivanov AI et al. Febrigenic signaling to the brain does not involve nitric oxide. Br J Pharmacol, 2004, 141(7):1204-1213.
10. Chang CP, Lee CC, Chen SH et al. Aminoguanidine protects against intracranial hypertension and cerebral ischemic injury in experimental heatstroke. J Pharmacol Sci, 2004, 95(1):56-64.
11. Hristov M, Lazarov L. Inhibition of nitric oxide synthase or cystathionine gamma-lyase abolishes leptin-induced fever in male rats. J Therm Biol, 2023, 112:103443.
12. Hristov M. Enhanced food intake suppression and body mass reduction with combined l-name and leptin administration in fasted rats. Trakia journal of sciences, 2024, 22(3):7.
13. Tatemoto K, Takayama K, Zou MX, et al. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. Regul Pept, 2001, 99(2-3):87-92.
14. Jia YX, Lu ZF, Zhang J, et al. Apelin activates L-arginine/nitric oxide synthase/nitric oxide pathway in rat aortas. Peptides, 2007, 28(10):2023-2029.
15. Azizi Y, Faghihi M, Imani A, et al. Post-infarct treatment with [Pyr1]-apelin-13 reduces myocardial damage through reduction of oxidative injury and nitric oxide enhancement in the rat model of myocardial infarction. Peptides, 2013, 46:76-82.
16. Gholamzadeh R, Aboutaleb N, Nazarinia D. Intravenous injection of apelin-13 improves sensory-motor balance deficits caused by cerebral ischemic reperfusion injury in male wistar rats via restoration of nitric oxide. J Chem Neuroanat, 2021, 112:101886.
17. Gao Z, Zhong X, Tan YX, et al. Apelin-13 alleviates diabetic nephropathy by enhancing nitric oxide production and suppressing kidney tissue fibrosis. Int J Mol Med, 2021, 48(3):175.
18. Duparc T, Colom A, Cani PD, et al. Central apelin controls glucose homeostasis via a nitric oxide-dependent pathway in mice. Antioxid Redox Signal, 2011, 15(6):1477-1496.
19. Higuchi K, Masaki T, Gotoh K, et al. Apelin, an APJ receptor ligand, regulates body adiposity and favors the messenger ribonucleic acid expression of uncoupling proteins in mice. Endocrinology, 2007, 148(6):2690-2697.
20. Than A, He HL, Chua SH, et al. Apelin Enhances Brown Adipogenesis and Browning of White Adipocytes. J Biol Chem, 2015, 290(23):14679-14691.
21. Giordano A, Tonello C, Bulbarelli A, et al. Evidence for a functional nitric oxide synthase system in brown adipocyte nucleus. FEBS Lett, 2002, 514(2-3):135-140.
22. Petrović V, Buzadzić B, Korać A, et al. NO modulates the molecular basis of rat interscapular brown adipose tissue thermogenesis. Comp Biochem Physiol C Toxicol Pharmacol, 2010, 152(2):147-159.
23. Hristov M, Andreeva-Gateva P. 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, 2024,17:133–42.
24. Kamerman P, Mitchell D, Laburn H. Circadian variation in the effects of nitric oxide synthase inhibitors on body temperature, feeding and activity in rats. Pflugers Arch, 2002, 443(4):609-616