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Acute Effect of Centrally Injected Nesfatin-1 on Some Blood Electrolytes and Metabolites in Rats

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Abstract

Nesfatin-1 is a newly found food and water intake regulatory neuropeptide. Because it can regulate nutrition and thirst, nesfatin-1 may also have the potential to affect levels of blood electrolytes and metabolites. The current study was intended to resolve the acute influence of intracerebroventricularly injected nesfatin-1 on the levels of some blood electrolytes and metabolites in rats.

The experiments were conducted on Sprague Dawley male rats. Nesfatin-1 (200 pmol) or saline (5 µL) was given the rats intracerebroventricularly. Central nesfatin-1 treatment caused increases in the concentrations of blood glucose, lactate, hematocrit, and hemoglobin without changing the blood pH, creatine, Na, K, Ca, Cl, and HCO3 levels.

In conclusion, our findings show that the central nesfatin-1 could affect the concentrations of blood glucose, lactate, hematocrit, and hemoglobin without altering the blood electrolytes. This could be interpreted as the secondary effect of nesfatin-1 as a consequence of centrally injected nesfatin-1-evoked activation of sympathetic nerves.

Keywords: Nesfatin-1, Intracerebroventricular, Blood electrolytes, Blood metabolites, Hematocrit, Hemoglobin

Introduction

Nesfatin-1 is a polypeptide with originated from nucleobindin 2 (NUCB2), and it has 82 amino acids.[1] Two more peptides, called nesfatin-2 and -3, are generated from NUCB2 but their functions have been unknown yet.[1] Nesfatin-1 was early described as regulating nutrition intake regulatory neuropeptide synthesized in the hypothalamus[2], and subsequent studies showed its autonomic effects on cardiovascular,[3-5] respiratory[6], and reproductive[7] systems. Further studies revealed that nesfatin-1 has also been found in the organs belonged the gastrointestinal,[8] adipose,[9] endocrine[10-12], reproductive[11-13], and cardiovascular[14] systems. In the breakthrough study,[2] nesfatin-1 was explained as anorexigenic polypeptide because it induced a diminution in nutrition desire and bodyweight loss. Intracerebroventricularly (ICV) injection of the neuropeptide had an inhibitory action on antral and duodenal motility[15], and also a deceleration effect of gastric emptying.[16] As well as the anorexigenic effect, centrally injected nesfatin-1 also caused to reduce thirst.[17] It can be responsible in the control of blood electrolytes and metabolites levels because of its regulatory effects on food and water intake but there has been no report showing these effects, yet.

It was also reported nesfatin-1 could move up body temperature in rats after central injection[18] and modulate energy consumption, dry heat, growing of brown adipose tissue, and body temperature.[19] Our recent findings also

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explained that nesfatin-1 had an hyperventilating effect on the respiratory system, as well.[6] Those reports clearly explain that nesfatin-1 might affect energy metabolism. Nesfatin-1-evoked change in energy metabolism could cause an alteration in blood electrolytes and metabolites levels but this action of nesfatin-1 has not been identified, yet. Considering these findings, the current report intended to define the acute action of ICV administration of nesfatin-1 on the concentration of some blood electrolytes and metabolites in rats.

Materials and Methods

For the experiment, twelve, three months old, male Sprague–Dawley rats (290–320 g) were provided from Experimental Animals Breeding and Research Center, Bursa Uludag University, Bursa, Turkey. They are housed as three rats in an individual cage with free access to food and water. The temperature, the humidity, and the lighting of the room, which put the rats, were set as 20–22 0C, 60–70%, and 12 h dark/light cycle, respectively. All experimental procedures were approved by The Animal Care and Use Committee of Bursa Uludag University (2020-05/15).

All surgical process was performed under sevoflurane (2–4%/100% O2) anesthesia. PE 50 tubing was inserted in the rats' left carotid artery to collect the blood sample. Before the cannulation, all tubings were filled with heparin (50 U/ml). Following cannulation, the guide canula for ICV treatment was placed to the rats by using a stereotaxic frame. A bore was drilled through the skull of rats consistent with coordinates obtained the atlas of Paxinos and Watson.[20] Sterilized 22-gauge stainless steel guide canula was inserted in the 3rd ventricle by being let down 4.5 mm below the skull by through the bore and immobilized to the skull by using acrylic cement. The rats were observed as recovered from the anesthesia effect in their separate cages 4-5 h after the surgical process.

To define the effect of centrally injected nesfatin-1 on the blood electrolytes and metabolites including pH, glucose, lactate, creatine, hematocrit (Hct), hemoglobin (Hgb), Na, K, Ca, Cl, and HCO3 levels, the rats divided 2 groups as saline (5 µL; n=6) and nesfatin-1 (200 pmol; n=6) groups. Immediately before (0 min) and at the 20 minutes after (20 min) the injections, approximately 200 µL of blood samples from each animal were collected in cold EDTA tubes by using the arterial catheter. The EPOC® Analysis System Reader together with the EPOC BGEM™ test card (Epocal Inc., Ottawa, Canada) was used in the measurements. Briefly, the collected blood sample (100 µl) was injected into the test card and then the test card was inserted into the reader for measurements. The measurement lasted almost 30 sec. During the experiments, nesftain-1 solution, which was bought from Sigma-Aldrich Co., Deisenhofen, Germany, was freshly prepared. The dose of the drug was taken from our previous studies3-7. The central injection of drug solution or saline was performed by using a 10-µl microsyringe at a 5 µl volume in 60 sec.

By using Sigma Stat 3.5 software (CA, USA), statistical evaluation was performed with repeated-measures analysis of variance subsequently Bonferroni test.

Results

Centrally injected saline did not change the blood electrolytes and metabolites levels compare to the levels obtained before the injection (Table 1). Before the treatment, nesfatin-1-treated rats had blood electrolytes and metabolites levels similar to those of saline-treated animals (Table 1). While ICV injected nesfatin-1 did not produce alteration in blood pH, creatine, Na, K, Ca, Cl, and HCO3 levels, it caused to increase in blood glucose, lactate, Hct, and Hgb level.

Discussion and Conclusion

Table1. The effect of central injected nesfatin-1 on some blood parameters.

	Saline (0 min)	Saline (20 min)	Nesfatin-1 (0 min)	Nesfatin-1 (20 min)
pH	7.39 ± 0.01	7.40 ± 0.05	7.39 ± 0.01	7.40 ± 0.01
Ca (mmol/L)	1.29 ± 0.01	1.30 ± 0.01	1.30 ± 0.02	1.29 ± 0.01
Na (mmol/L)	142.3 ± 0.5	142.5 ± 0.7	143.2 ± 0.3	143.0 ± 0.3
K (mmol/L)	3.48 ± 0.06	3.47 ± 0.05	3.47 ± 0.04	3.48 ± 0.05
Cl (mmol/L)	100.8 ± 0.8	101.0 ± 0.7	100.1 ± 0.8	100.5 ± 0.3
HCO_3 (mmol/L)	24.47 ± 0.91	24.62 ± 1.13	24.85 ± 0.56	24.82 ± 0.83
Glucose (mg/dL)	135.8 ± 8.2	135.3 ± 9.3	135.5 ± 4.9	145.5 ± 4.8*
Lactate (mmol/L)	0.65 ± 0.12	0.64 ± 0.05	0.65 ± 0.16	0.74 ± 0.24*
Creatine (mg/dL)	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.33 ± 0.01
Hct (%)	39.1 ± 0.3	39.2 ± 0.7	39.0 ± 0.4	44.3 ± 1.0*
Hgb (g/dL)	13.38 ± 0.17	13.30 ± 0.22	13.25 ± 0.14	15.07 ± 0.33*

Saline (5 µl; ICV) or nesfatin-1 (200 pmol; ICV) was injected to the rats. Before (0 min) and 20 minutes after (20 min) the injections, 200 µl of blood samples from each animal were collected in tubes containing EDTA by using the arterial catheter. The parameters were measured by using the EPOC® Analysis System and the EPOC BGEM™ test card. Data was given as mean ± SEM of six rats. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *p<0.05, significantly different from the values of the "Saline (0 min)", "Saline (20 min)" and "Nesfatin-1 (0 min)".

The current findings clarify that ICV administration of nesfatin-1 caused to enhance the levels of blood glucose, lactate, Hct, and Hgb. Moreover, central treatment with nesfatin-1 did not alter the concentrations of blood pH, creatine, Na, K, Ca, Cl, and HCO3.

Nesfatin-1, having many autonomic effects, is expressed in both the brain areas and peripheral tissues.[21] The neuropeptide, bidirectionally being able to pass between blood and brain,[22] plays a role as a neuroregulator showing autonomic homeostatic effects. Nesfatin-1 was first detected as an anorexic neuropeptide[2], and subsequent studies extended its role on water intake[17]. Although, as a food and water intake regulatory neuropeptide, nesfatin-1 seems to have the potential to affect the blood electrolytes, in the present findings shows that there is no influence of central administration of nesfatin-1 on blood pH and concentrations of blood Na, K, Ca, Cl, and HCO3. Reported that hypothalamic NUCB2 mRNA expression[2] and the gastric mucosal tissue8 was down-regulated after 24h fasting in rats. However, refeeding after a 48h fast increased in activated nesfatin-1 immunoreactive hypothalamic neurons.[23] These reports clearly explain that the anorexinergic effect of nesfatin-1 takes more time. In the current study, we tested the acute role of nesfatin-1 on the blood electrolytes. Therefore, we might not have been able to detect the influence of nesfatin-1 on the blood electrolytes.

Evidenced that nesfatin-1 may play an important role in the control of glucose homeostasis and central glucose sensing.[24] Reported that ICV infusion of nesfatin-1 decreased blood glucose concentration by causing the increase in muscle glucose consumption, intensified insulin receptor signaling, reducing gluconeogenesis, and decreasing hepatic mRNA and protein expression of phosphoenolpyruvate carboxykinase as well as its activity.[25] It was also demonstrated that in the same paper that those effects of ICV infused nesfatin-1 resulting in the reduction in blood glucose concentration was detected 1 to 3h after the starting of the infusion.[25] The current finding clarified that central injection of nesfatin-1 could increase the values of blood glucose, lactate, Hct, and Hgb. We believe that increases in the concentrations of blood glucose, lactate, Hct, and Hgb is an acute secondary effect of the centrally injected nesfatin-1. It is well documented that centrally injected nesfatin-1 activated the sympathetic nervous system.[26-28] We previously showed that ICV injected nesfatin-1 also elevated plasma catecholamines.[3] These papers explained that adrenergic actions of nesfatin-1 were observed just after injection.[3,26-28] It is well known that the sympathetic nervous activity caused the increase in blood glucose[29] and lactate[30] levels and also passing the depo erythrocyte in the spleen to circulation by resulting in the contraction of smooth muscle within the splenic capsule.[31]

In conclusion, current findings indicate that central injection nesfatin-1 did not have any effect on blood pH and blood electrolytes, at least acutely. Moreover, nesfatin-1 increased blood Hct, Hgb, lactate, and glucose level within 20 minutes after the ICV injection due to nesfatin-1-evoked stimulation of sympathetic nerves. In the present study, we aimed to test the acute effect of the drug on some blood electrolytes and metabolites in rats. To show the acute effect of nesfatin-1 on those parameters, we collected the blood samples 20 min after centrally drug administration. Because we reported that nesfatin-1 exhibited the most potent cardiovascular,[3-5] respiratory,[6] and neuroendocrine[3,7] effects on the 20th min of the central injection.

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