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Effect of Central and Peripheral Injected Nesfatin-1 on Electrocardiography in Rats

Kubra CIFTCI[a], Gokcen GUVENC[a], Aysenur BAS[b], Murat YALCIN[a]*

a Department of Physiology, Faculty of Veterinary Medicine, Uludag University, Bursa, 16059, Turkey.
b Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Uludag University, Bursa, 16059, Turkey.

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Abstract

Nesfatin-1 is an anorexic nucleobindin-2 -derived peptide and it has directly and centrally effect on the heart. The current study was designed to determine the effect of centrally and peripherally administered nesfatin-1 on electrocardiography (ECG) of healthy both fasted rats for 12 h and satiated rats fed ad libitum. In order to record ECG, the electrodes were placed limbs of at lead II under ketamine (50 mg/kg; im) and xylazine (20 mg/kg; im) anesthesia mix.

Centrally administered different doses of nesfatin-1 (100 and 200 pmol; icv) resulted in dose- and time-dependently a statistically significant increase ($p < 0.05$) in T wave, Q-T interval, and R-R interval duration without changing in ECG waves' amplitude in both satiated and fasted rats. In a similar way, peripheral administration of nesfatin-1 (80 µg/kg; iv) in satiated rats prolonged statistically significant ($p < 0.05$) T wave, Q-T interval, and R-R interval without producing a change in ECG waves' amplitude. Moreover, icv administered nesfatin-1 in fasted and satiated rats, and iv injected nesfatin-1 in satiated rats induced a statistically significant decrease in heart rate ($p < 0.05$).

In conclusion, our findings suggest that centrally and peripherally administrated nesfatin-1 caused a delay in T wave, Q-T interval and two R-waves interval duration in ECG so that leading to a bradycardic effect in heart rate.

Keywords: Nesfatin-1, Electrocardiography, Intracerebroventricular, Intravenous, Heart rate.

Introduction

Nesfatin-1 is an 82 amino acid anorexigenic endogenous peptide produced by the proteolytic processing of nucleobindin-2 (NUCB-2).[1] Nesfatin-1 and its precursor molecule NUCB-2 show widespread distribution throughout the central nervous system[2,3] and the peripheral tissues such as gastric mucosa[4] and heart ventricles.[5] The central and peripheral nesfatin-1 expression is modulated by starvation and refeeding.[1,4] Experimental evidence supports the involvement of nesfatin-1 in the modulation of feeding, neuroendocrine functions, stress, metabolic responses and cardiovascular control.[1,6-9]

Indeed it was reported that central administration of nesfatin-1 increases blood pressure and renal sympathetic nerve activity following intracerebroventricular (icv) administration in conscious and urethane-anesthetized rats.[10,11] Moreover, nesfatin-1 modulates the excitability of nucleus tractus solitarius (NTS) neurons and produces hypertensive and tachycardic responses upon microinjection into the NTS.[12] When nesfatin-1 is injected intravenous (iv), the peptide also increases peripheral arterial resistance through a direct action arterioles.[13] Nesfatin-1 expression in the heart has been correlated with negative inotropism and protection against ischemia-reperfusion injury.[5] Recently it was reported that central administration of nesfatin-1 exerts pressor and bradycardic effects in normotensive animals[8,9] and produces pressor and tachy-

* Corresponding author: : Murat Yalcin, Uludag Universitesi Veteriner Fakultesi Fizyoloji Anabilim Dali Gorukle, 16059 Bursa Turkey Tel: + 90 224 294 1228 Fax: + 90 224 294 1202, E-mail: mrtylcn75@gmail.com , muraty@uludag.edu.tr

cardic effects under hypotensive conditions produced by severe hemorrhage.[8] It was also shown that icv administered nesfatin-1 increased the plasma catecholamines, vasopressin, and renin concentrations and those hormones contributed to the pressor effects of the peptide in both normotensive and hypotensive conditions.[8] Previously it was reported that nesfatin-1 expression in the heart has been correlated with negative inotropism and protection against ischemia-reperfusion injury.[4] Evidence on the effects of cardiovascular parameters following central administration of nesfatin-1 raises the possibility that it may modulate arterial pressure and heart function. However, there is no any report showing the effect of nesfatin-1 on electrical activity of the heart when it was administrated via icv or iv. Moreover, it was not known how central or peripheral injected nesfatin-1 was able to affect electrocardiography (ECG) waves in fasting or satiety condition.

Considering the above data, the primary aim of the current study was to show the effect of centrally or peripherally administrated nesfatin-1 on ECG in fasted for 12h and in satiated fed ad libitum anesthetized rats.

Materials and Methods

Animals

The experiments were performed on 56 adults, male Sprague–Dawley rats (3 months old, 280–340 g). The rats were provided from Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey. The animals were accommodated as 4-5 rats in a cage and maintained on a 12 h light/dark cycle at 20–22 °C with 60–70% humidity. All experimental protocol was approved by The Animal Care and Use Committee of Uludag University (2018 – 01/07). Each group had 7 rats and each rat was used in a single experimental protocol.

Surgical procedures

The rats anesthetized with ketamine (50 mg/kg) and xylazine (20 mg/kg) mixture. Under anesthesia, rats were placed in a stereotaxic frame to insert the icv guide cannula. For this reason, a burr hole was drilled through the skull 1.5 mm lateral to midline and 1.0 mm posterior to bregma, which those coordinates were used from the atlas of Paxinos and Watson.[14] Then, the guide cannula, which was made 22-gauge stainless steel hypodermic tubing, was directed through the hole toward the lateral ventricle and lowered 4.5 mm below the surface of the skull and fixed to the skull with acrylic cement.

Experimental protocol

In the present study, first, the dose and time relation of ECG responses to icv injected nesfatin-1 were studied in fasted for 12 h or/and satiated fed ad libitum rats. Following baseline ECG measurements, nesfatin-1 (100 and 200 pmol) or saline (5 μL) was delivered i.c.v. and changes of ECG were recorded for the next 60 min. Hence to determine the effect of centrally injected nesfatin-1 on ECG, total 6 groups were constituted as so 3 groups from fasted for 12 h and as so 3 groups from satiated fed ad libitum rats: 1, control (saline 5 μL; icv); 2, nesfatin-1 (100 pmol; icv); and 3, nesfatin-1 (200 pmol; icv).

Secondly, to determine the effect of peripheral injected nesfatin-1 on ECG of satiated fed ad libitum rats, saline (1 mL/kg) or 80 μg/kg dose of nesfatin-1 were iv injected via tail vein. Before and after injections, changes of ECG were recorded for the next 60 min. So total 2 groups were formed from satiated fed ad libitum rats as 1, control (saline 1 mL/kg; iv); and 2, nesfatin-1 (80 μg/kg; iv) in terms of the effect of peripherally injected nesfatin-1 on ECG.

ECG recording

In order to record ECG, the electrodes (SS2L, BIOPAC Systems Inc. California, USA) were inserted limbs of at lead II. Findings were recorded and analyzed by using the MP36 system and AcqKnowledge software (BIOPAC Systems Inc.). The ECG was used to determine the P waves, P-R intervals, QRS complexes Q-T intervals, T waves, R-R intervals durations. Heart rate was calculated from R-R intervals according to the following formula: heart rate=60/(R-R intervals in seconds) and was expressed beats per minute (bpm).

Drug and icv injections

Nesfatin-1 (Sigma-Aldrich Co., Deisenhofen, Germany) solutions were prepared in saline on the day of the experiment. The dose of nesfatin-1 was chosen from the previous studies.[8,9]

Icv injections were made by using hand-made injection cannula (28 gauge stainless steel tubing). Injection cannula was connected to polyethylene tubing, which was filled with saline or saline solution of the studied agent in a 10 μL microsyringe. For the icv injection, 5 μL volume of the solution was infused within 60 s. During the injection, an air bubble moving in the polyethylene tubing was closely watched to ensure the drug was delivered in its entirety.

Data and statistical analysis

All values are given as mean ± standard error of the mean (SEM) with $p<0.05$ considered as the level of significance. Statistical evaluation was performed by analysis of variance (RM-ANOVA; two-way) and the post-ANOVA test of Bonferroni by using Sigma Stat 3.5 software (CA, USA).

Results

Effects of central or peripheral injected nesfatin-1 on ECG

There was no difference in basal value of the P waves (Table 1), P-R intervals (Table 2), QRS complexes (Table 3), Q-T intervals (Table 4), T waves (Table 5), R-R intervals (Table 6) durations and heart rate of both fasted (Fig 1A) and satiated (Fig 1B) rats. Icv injected nesfatin-1 led to dose- and time-dependently statistically significant increase ($p < 0.05$) in Q-T interval (Table 4), T wave (Table 5) and R-R interval (Table 6) duration without changing in P wave (Table 1), P-R interval (Table 2) and QRS complex (Table 3) duration in both fasted and satiated rats. Also centrally injected nesfatin-1 induced dose- and time-dependently statistically significant ($p < 0.05$) decrease in heart rate of both fasted (Fig 1A) and satiated (Fig 1B) rats.

Table 1. The effect of central and peripheral injected nesfatin-1 on P wave duration.

P wave duration (second)								
Time	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.022±0.001	0.022±0.002	0.022±0.001	0.023±0.001	0.023±0.003	0.023±0.002	0.022±0.002	0.023±0.001
1	0.022±0.003	0.022±0.003	0.023±0.002	0.023±0.002	0.022±0.003	0.023±0.002	0.022±0.002	0.023±0.002
3	0.022±0.001	0.022±0.001	0.023±0.003	0.023±0.002	0.021±0.002	0.022±0.001	0.022±0.001	0.023±0.002
5	0.023±0.002	0.022±0.002	0.022±0.003	0.024±0.002	0.023±0.003	0.020±0.002	0.022±0.003	0.024±0.001
10	0.023±0.001	0.022±0.004	0.021±0.002	0.023±0.001	0.022±0.003	0.021±0.001	0.023±0.003	0.024±0.003
20	0.024±0,004	0.021±0.002	0.023±0.002	0.023±0.003	0.022±0.004	0.022±0.002	0.023±0.002	0.024±0.002
30	0.023±0.003	0.022±0.001	0.023±0.002	0.023±0.004	0.023±0.001	0.022±0.001	0.023±0.002	0.025±0.003
60	0.024±0.001	0.022±0.002	0.024±0.002	0.023±0.002	0.024±0.002	0.023±0.003	0.023±0.002	0.023±0.001

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven P wave duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test.

Table 2. The effect of central and peripheral injected nesfatin-1 on P-R interval duration.

P-R interval duration (second)								
Ti me	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.051±0.002	0.052±0.002	0.052±0.001	0.052±0.001	0.051±0.003	0.051±0.007	0.053±0.003	0.051±0.005
1	0.051±0.003	0.053±0.004	0.052±0.003	0.051±0.003	0.052±0.003	0.052±0.007	0.055±0.002	0.051±0.006
3	0.051±0.006	0.053±0.004	0.054±0.003	0.053±0.003	0.052±0.004	0.053±0.004	0.052±0.004	0.050±0.005
5	0.053±0.003	0.054±0.003	0.052±0.003	0.053±0.001	0.053±0.002	0.054±0.005	0.050±0.006	0.052±0.003
10	0.054±0.002	0.054±0.007	0.055±0.002	0.052±0.003	0.051±0.003	0.053±0.006	0.051±0.008	0.052±0.005
20	0.053±0.004	0.055±0.005	0.054±0.003	0.052±0.002	0.053±0.004	0.053±0.007	0.052±0.006	0.053±0.004
30	0.053±0.006	0.054±0.005	0.055±0.002	0.053±0.001	0.051±0.006	0.052±0.006	0.052±0.005	0.053±0.005
60	0.054±0.002	0.056±0.006	0.056±0.002	0.050±0.002	0.052±0.004	0.051±0.008	0.053±0.003	0.050±0.001

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven P-R interval duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test.

Table 3. The effect of central and peripheral injected nesfatin-1 on QRS complex duration.

QRS complex duration (second)								
Time	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.026±0.002	0.026±0.001	0.026±0.001	0.025±0.001	0.025±0.001	0.025±0.002	0.023±0.001	0.023±0.002
1	0.026±0.002	0.026±0.001	0.026±0.002	0.024±0.001	0.025±0.001	0.025±0.001	0.023±0.002	0.023±0.001
3	0.025±0.001	0.025±0.002	0.027±0.003	0.024±0.001	0.026±0.001	0.025±0.001	0.024±0.001	0.025±0.002
5	0.025±0.001	0.026±0.002	0.027±0.003	0.022±0.001	0.026±0.002	0.026±0.003	0.025±0.001	0.025±0.003
10	0.025±0.001	0.026±0.001	0.026±0.003	0.024±0.002	0.026±0.001	0.027±0.002	0.023±0.001	0.025±0.002
20	0.025±0.001	0.026±0.002	0.026±0.003	0.023±0.001	0.025±0.001	0.025±0.001	0.023±0.001	0.025±0.002
30	0.025±0.003	0.026±0.002	0.027±0.003	0.023±0.001	0.026±0.002	0.025±0.002	0.023±0.002	0.024±0.001
60	0.026±0.002	0.027±0.001	0.026±0.001	0.025±0.002	0.025±0.003	0.0245±0.002	0.023±0.002	0.024±0.001

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven QRS complex duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test.

Table 4. The effect of central and peripheral injected nesfatin-1 on Q-T interval duration.

Q-T interval duration (second)								
Time	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.066±0.002	0.066±0.002	0.065±0.003	0.067±0.004	0.067±0.002	0.067±0.001	0.065±0.002	0.065±0.001
1	0.065±0.002	0.064±0.003	0.065±0.002	0.066±0.002	0.066±0.004	0.067±0.001	0.064±0.005	0.064±0.004
3	0.065±0.004	0.065±0.002	0066±0.004	0.067±0.002	0.067±0.005	0.067±0.002	0.063±0.005	0.065±0.005
5	0.066±0.001	0.066±0.001	0.069±0.001*	0.066±0.001	0.069±0.001*	0.069±0.001*	0.064±0.001	0.067±0.001*
10	0.064±0.001	0.067±0.001*	0.071±0.001*	0.065±0.001	0.069±0.001*	0.070±0.001*	0.065±0.001	0.069±0.001*
20	0.065±0.001	0.070±0.001*	0.072±0.001*	0.066±0.001	0.070±0.001*	0.072±0.001*	0.066±0.001	0.070±0.001*
30	0.064±0.001	0.069±0.001*	0.070±0.001*	0.065±0.001	0.065±0.001	0.070±0.001*	0.064±0.001	0.068±0.001*
60	0.062±0.001	0.070±0.001*	0.069±0.001*	0.065±0.003	0.065±0.004	0.063±0.003	0.064±0.001	0.065±0.003

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven Q-T interval duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *$p<0.05$, significantly different from the value of the saline-treated group.

Iv injected nesfatin-1 had a similar effect on the ECG parameters with icv injected nesfatin-1. Iv injected nesfatin-1 also prolonged Q-T interval (Table 4), T wave (Table 5) and R-R interval (Table 6) duration but no effect on P wave (Table 1), P-R interval (Table 2) and QRS complex (Table 3) duration in satiated rats. Moreover, iv injected nesfatin-1 caused the bradycardia in satiated rats (Fig 1C).

Both central and peripheral injection of nesfatin-1 did not cause any change in amplitude and shape of the ECG waves and intervals (Fig 2).

Table 5. The effect of central and peripheral injected nesfatin-1 on T wave duration.

T wave duration (second)								
Time	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.048±0.002	0.048±0.002	0.048±0.003	0.049±0.003	0.048±0.002	0.049±0.002	0.048±0.002	0.048 ±0.003
1	0.048±0.002	0.047±0.002	0.048±0.003	0.048±0.001	0.048±0.003	0.048±0.002	0.047±0.003	0.048±0.003
3	0.049±0.003	0.047±0.002	0.048±0.004	0.046±0.002	0.047±0.002	0.047±0.001	0.047±0.003	0.048±0.004
5	0.047±0.002	0.049±0.002	0.047±0.003	0.047±0.001	0.048±0.003	0.049±0.001	0.047±0.005	0.049±0.005
10	0.046±0.001	0.049±0.001*	0.049±0.001*	0.046±0.001	0.049±0.001*	0.051±0.001*	0.046±0.001	0.049±0.001*
20	0.047±0.001	0.051±0.001*	0.049±0.001*	0.046±0.001	0.050±0.001*	0.051±0.001*	0.046±0.001	0.050±0.001*
30	0.047±0.001	0.051±0.001*	0.049±0.001*	0.046±0.001	0.049±0.001*	0.050±0.001*	0.047±0.003	0.049±0.003
60	0.048±0.004	0.048±0.004	0.049±0.003	0.047±0.004	0.048±0.003	0.049±0.001	0.048±0.001	0.049±0.004

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven T wave duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *$p<0.05$, significantly different from the value of the saline-treated group.

Table 6. The effect of central and peripheral injected nesfatin-1 on R-R interval duration.

R-R interval duration (second)								
Time	Fasted (icv)			Satiated (icv)			Satiated (iv)	
	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 5 µl	Nesfatin-1 100 pmol	Nesfatin-1 200 pmol	Saline 1 ml/kg	Nesfatin-1 80 µg/kg
0	0.264±0.02	0.262±0.03	0.269±0.02	0.259±0.01	0.253±0.05	0.258 ±0.05	0.251±0.04	0.250±0.01
1	0.269±0.01	0.274±0.02*	0.271±0.02	0.255±0.01	0.261±0.02*	0.263±0.02*	0.260±0.03	0.259±0.02
3	0.254±0.01	0.274±0.01*	0.277±0.02*	0.252±0.02	0.265±0.04*	0.271±0.05*	0.263±0.02	0.265±0.01
5	0.242±0.01	0.271±0.02*	0.278±0.03*	0.240±0.05	0.267±0.04*	0.273±0.06*	0.260±0.01	0.265±0.01*
10	0.241±0.03	0.271±0.06*	0.278±0.04*	0.231±0.05	0.269±0.04*	0.276±0.05*	0.260±0.01	0.269±0.03*
20	0.243±0.02	0.269±0.06*	0.276±0.03*	0.242±0.05	0.269±0.09*	0.279±0.04*	0.261±0.01	0.269±0.03*
30	0.245±0.01	0.253±0.02*	0.271±0.04*	0.235±0.04	0.256±0.03*	0.281±0.06*	0.268±0.01	0.271±0.03
60	0.247±0.01	0.248±0.03	0.263±0.05*	0.239±0.04	0.247±0.04*	0.286±0.05*	0.268±0.01	0.265±0.03

For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted or the satiated rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven R-R interval duration measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *$p<0.05$, significantly different from the value of the saline-treated group.

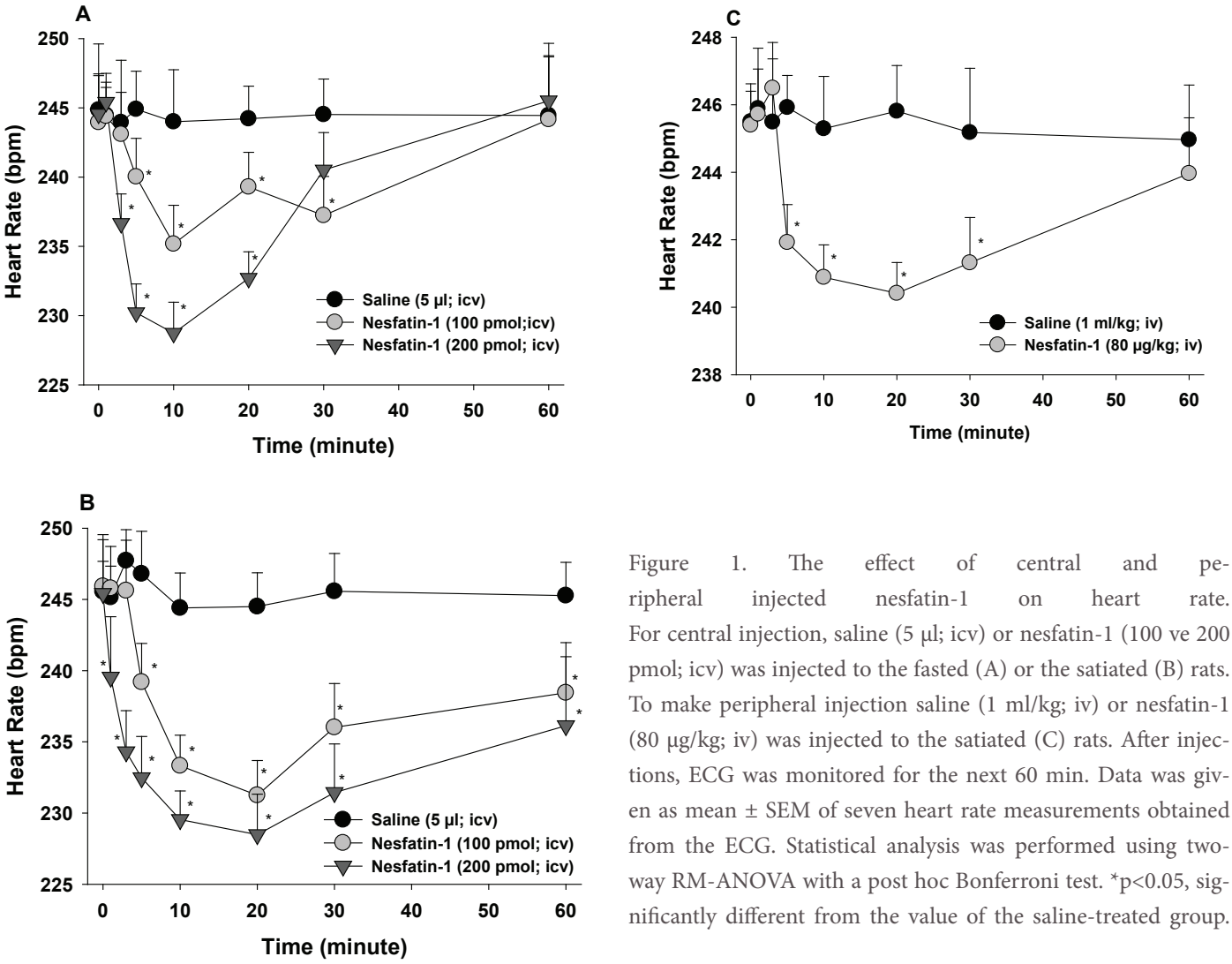

Figure 1. The effect of central and peripheral injected nesfatin-1 on heart rate. For central injection, saline (5 µl; icv) or nesfatin-1 (100 ve 200 pmol; icv) was injected to the fasted (A) or the satiated (B) rats. To make peripheral injection saline (1 ml/kg; iv) or nesfatin-1 (80 µg/kg; iv) was injected to the satiated (C) rats. After injections, ECG was monitored for the next 60 min. Data was given as mean ± SEM of seven heart rate measurements obtained from the ECG. Statistical analysis was performed using two-way RM-ANOVA with a post hoc Bonferroni test. *p<0.05, significantly different from the value of the saline-treated group.

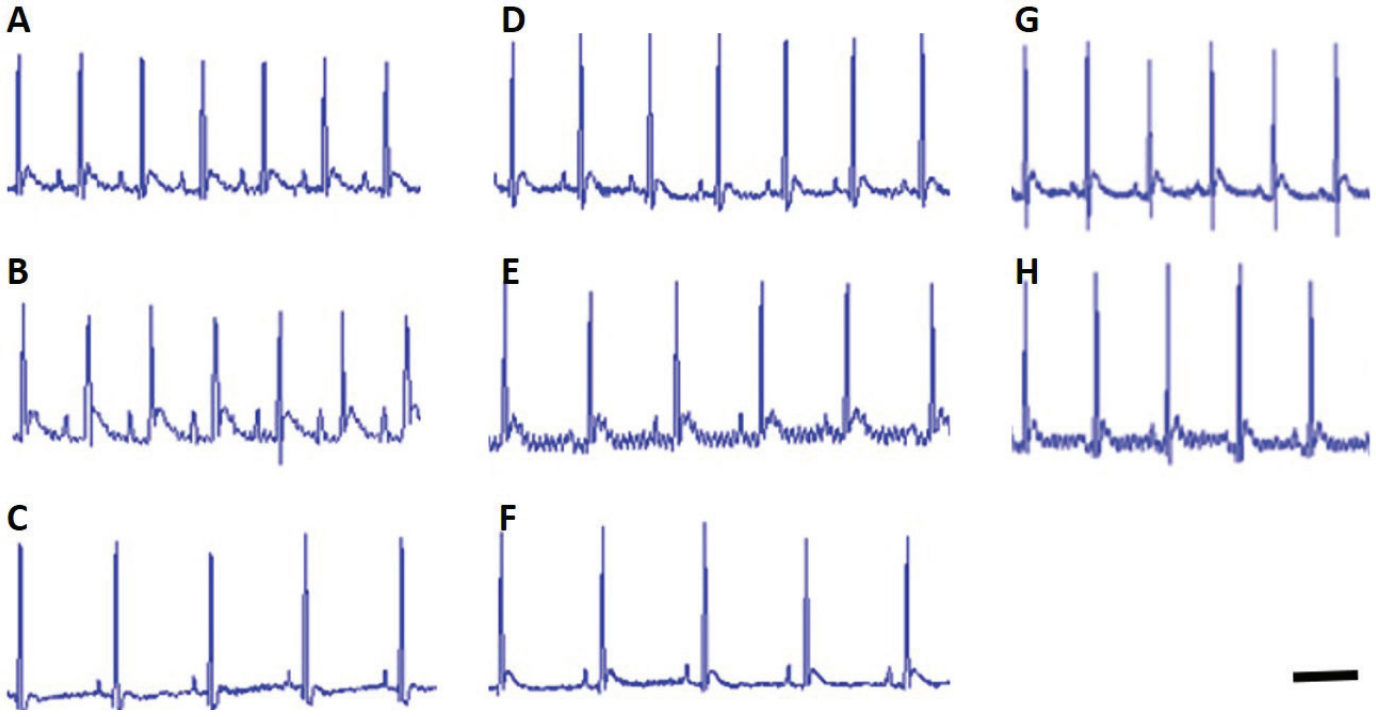

Figure 2. The effects of central and peripheral injected nesfatin-1 on ECG waves and intervals. The fasted rats were treated with saline (5 µl; icv) (A), nesfatin-1 (100 pmol; icv) (B) or nesfatin-1 (200 pmol; icv) (C); the satiated rats were treated with saline (5 µl; icv) (D), nesfatin-1 (100 pmol; icv) (E) or nesfatin-1 (200 pmol; icv) (F); the satiated rats were treated with saline (1 ml/kg; iv) (G) or nesfatin-1 (80 µg/kg; iv) (H). The bar shows 0.3 second.

Discussion and Conclusion

These data demonstrate that icv administered nesfatin-1 dose- and time-dependently increases T wave, Q-T and R-R intervals durations but not any changes in P wave, P-R intervals, and QRS complex durations in both fasted and satiated rats. Furthermore, central injection of nesfatin-1 causes dose- and time-dependently bradycardic responses in fasted and satiated rats. When nesfatin-1 injected iv, it produces a similar effect with its central administration and causes the delay in T wave, Q-T and R-R intervals along with bradycardia. Central and peripheral injection of nesfatin-1 does not alter the ECG waves' amplitude of the rats.

ECG in rats is a widely applied experimental method in basic cardiovascular research. It has become one of the most widespread diagnostic tools in clinical medicine. ECG recording reflects the electrical activity of the heart and may provide important insights into the functional and structural characteristics of the myocardium. The physiological and pathological criteria of ECG recordings have been thoroughly described in multiple handbooks and research papers.[15,16] An action potential in the heart is generated in the sinoatrial node and subsequently conducted through the atrioventricular node, His bundle, His bundle branches, and Purkinje fibers, finally reaching ventricular cardiomyocytes. A typical ECG tracing mirrors the repeating cycle of three major electrical events, including atrial depolarization (P wave), ventricular depolarization (QRS complex) and ventricular repolarization (T wave). In the current study, central or peripheral injection of the nesfatin-1 prolonged the T wave and Q-T intervals without changing other waves and intervals durations. It could be considered that nesfatin-1 has a direct effect on heart tissue. It was reported that heart, particularly ventricles, had the presence of both nesfatin-1 protein and NUCB-2 mRNA in rat cardiac extracts according to western blotting and QT-PCR analyses.[5] On isolated and Langendorff-perfused rat heart preparations, it was found that exogenous nesfatin-1 depressed contractility and relaxation without affecting coronary motility by inducing dose-dependent negative inotropism.[5] Our results are consistent with this report. Because the iv injected nesfatin-1 had to have affected the ventricles of the heart so that there was a delay in T wave and Q-T interval. Also, it could be considered that nesfatin-1 has indirectly effect heart tissue via sympathetic or parasympathetic neurons. Principally, parasympathetic stimulation causes a marked decrease in heart rate (negative chronotropic effect) and a slight decrease in heart muscle contractility (negative inotropic effect). Activation of sympathetic[10,11] and parasympathetic cardiac tones[17] with the central injection of nesfatin-1 has been observed. It appears that injected nesfatin-1 has a potency of a parasympathetic effect on the heart. Recently it was reported that the central cholinergic system modulated the centrally injected nesfatin-1 induced cardiovascular responses.[9] Because icv injected nesfatin-1 increased the level of the hypothalamic acetylcholine and choline, and also central muscarinic and nicotinic receptors mediated pressor and bradycardic responses to centrally injected nesfatin-1.[9] Activation of the central cholinergic parasympathetic pathway with the icv injection of nesfatin-1 might lead to a decrease in T wave and Q-T interval in ECG.

Heart rate represents the number of heart contractions in 1 min. There is a correlation between R-R interval duration and heart rate. In the present study, both central and peripheral injection of the nesaftin-1 caused the bradycardia. Central administration of nesfatin-1-evoked bradycardic effect in normotensive animals was demonstrated.[8,9] It was also reported that nesfatin-1 decreases heart rate when injected intracerebroventricularly.[10,11,18,19] Moreover, microinjection of nesfatin-1 into the NAmb generated a decrease in heart rate in conscious rats.[17] Those reports and our current data clearly explain that nesfatin-1 might have a bradycardic effect.

The first report determined the anorexigenic effects of nesfatin-1 and its precursor NUCB-2.[1] In this first report, it was shown that NUCB-2 mRNA expression in the hypothalamus was significantly down-regulated after 24-h fasting in rats.[1] On the other hand refeeding after a 48-h fast resulted in an increase of activated nesfatin-1 immunoreactive neurons in the hypothalamus.[20] It was also reported that gastric mucosa abundantly expressed NUCB-2 mRNA.[4] Again NUCB-2 mRNA expression in gastric mucosal tissue was significantly down-regulated after 24-h fasting in rats. Those reports evidently indicate that the fasting suppresses the production of nesfatin-1 in central and peripheral. Considering the change in the amount of nesfatin-1 in both periphery and central according to the condition of fasting and satiety, we performed nesfatin-1 application to both fasted and satiated animals, but we did not obtain any significant differences between the two conditions.

In summary, the present results suggest that icv or iv administration of nesfatin-1 causes an increase in T wave, Q-T and R-R intervals durations in ECG without affecting fasting or satiety conditions. Moreover, central or peripheral injected nesfatin-1 produces the bradycardic response by directly affecting the heart or indirectly activating the central nervous system.

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