



## The role of centrally injected nesfatin-1 on cardiovascular regulation in normotensive and hypotensive rats



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### ABSTRACT

This study investigated the cardiovascular effects of nesfatin-1 in normotensive rats and animals subjected to hypotensive hemorrhage.

Hemorrhagic hypotension was induced by withdrawal 2 mL blood/100 g body weight over a period of 10 min. Acute hemorrhage led to a severe and long-lasting decrease in mean arterial pressure (MAP) and heart rate (HR). Intracerebroventricularly (i.c.v.) administered nesfatin-1 (100 pmol) increased MAP in both normotensive and hemorrhaged rats. Nesfatin-1 also caused bradycardia in normotensive and tachycardia in hemorrhaged rats. Centrally injected nesfatin-1 (100 pmol, i.c.v.) also increased plasma catecholamine, vasopressin and renin concentrations in control animals and potentiated the rise in all three cardiovascular mediators produced by hemorrhage.

These findings indicate that centrally administered nesfatin-1 causes a pressor response in conscious normotensive and hemorrhaged rats and suggest that enhanced sympathetic activity and elevated vasopressin and renin concentrations mediate the cardiovascular effects of the peptide.

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### 1. Introduction

Nesfatin-1, an 82 amino acid cleavage product of nucleobindin-2 protein, was initially identified as a central and peripheral satiety molecule that induces acute and chronic anorexigenic effects (Maejima et al., 2009; Oh-I et al., 2006). Its widespread distribution throughout the central nervous system (Brailoiu et al., 2007; Goebel et al., 2009) and the periphery (Pałasz et al., 2012) suggests that nesfatin-1 may regulate other functions in addition to feeding. Experimental evidence supports the involvement of nesfatin-1 in the modulation of neuroendocrine functions, stress, and metabolic responses (García-Galiano et al., 2010; Stengel and Taché, 2011).

Nesfatin-1 also elevates blood pressure and renal sympathetic nerve activity following intracerebroventricular (i.c.v.) administration in conscious and urethane-anesthetized rats (Tanida and Mori, 2011; Yosten and Samson, 2009). Furthermore, nesfatin-1 modulates the

excitability of nucleus of the solitary tract (NTS) neurons and produces hypertensive and tachycardic responses upon microinjection into the NTS (Mimee et al., 2012). The peptide also increases peripheral arterial resistance after intravenous administration, evidently, through direct action arterioles (Yamawaki et al., 2012). Nesfatin-1 expression in the heart has been correlated with negative inotropism and protection against ischemia–reperfusion injury (Angelone et al., 2013). Nesfatin-1 immunoreactivity has been detected in nucleus ambiguus in rodents (Goebel et al., 2009; Goebel-Stengel et al., 2011) and may thus influence premotor cardiac vagal neurons (Mendelowitz, 1999).

Evidence that nesfatin-1 raises arterial pressure and increases sympathoadrenal outflow following central administration raises the possibility that it may modulate cardiovascular function, not only under normotensive conditions, but during hemorrhagic hypotension, as well. Furthermore, the effect of nesfatin-1 on hormonal mediators of cardiovascular function, including vasopressin, renin and catecholamines, has not been fully elaborated. Therefore, the present study investigated the effect of intracerebroventricularly injected nesfatin-1 on arterial pressure and heart rate and plasma concentrations of catecholamines, vasopressin and renin in normotensive control animals and rats subjected to hypotensive hemorrhage.

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## 2. Methods

### 2.1. Animals

A total of 56 adult, male Sprague–Dawley rats (280–340 g) (Experimental Animals Breeding and Research Center, Uludag University, Bursa, Turkey) were used for these experiments. Four or five rats were housed per cage under controlled conditions of temperature (20–22 °C), humidity (60–70%) and lighting (12 h light/dark cycle) and were provided with food and water *ad libitum*. The Animal Care and Use Committee of Uludag University, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, approved all experimental procedures.

Each animal was studied separately, in a single experiment, and each experimental group consisted of seven rats.

### 2.2. Surgical procedures

Under sevoflurane (2–4%/100% O<sub>2</sub>) anesthesia, the left femoral artery was cannulated with PE 50 tubing filled with heparinized saline (100 U/mL) to measure mean arterial pressure (MAP) and heart rate (HR) or to collect blood samples for measurement of the plasma vasopressin, catecholamine and renin levels. The tip of tubing was covered and exteriorized at the neck of the rat. For intracerebroventricular (i.c.v.) treatment, a burr hole was drilled through the skull 1.5 mm lateral to the midline and 1.0 mm posterior to bregma. A 22-gauge stainless steel cannula was lowered 4.5 mm below the surface of the skull and fixed to the skull with acrylic cement. After surgery, the rats were placed in individual cages and allowed to recover from anesthesia for 4–5 h.

### 2.3. Experimental protocols

To measure MAP and HR the arterial cannula was connected to a volumetric pressure transducer (BPT 300, BIOPAC Systems Inc., CA, U.S.A.) attached to MP36 system (BIOPAC Systems Inc., CA, U.S.A.) and baseline MAP and HR were recorded and allowed to stabilize for 30 min before each experiment. For experiments with normotensive animals, nesfatin-1 (100 pmol) or saline (5 µL) was then injected i.c.v. and MAP and HR were recorded at 1 min intervals for 120 min. For experiments with hemorrhaged animals, a total volume of 2.0 mL of blood/100 g body weight was withdrawn through the arterial catheter at a constant rate over a period of 10 min. The arterial catheter was then flushed with 0.1 mL of heparinized saline (50 U/mL) and reconnected to the pressure transducer. After a 10 min stabilization period, nesfatin-1 (100 pmol) or saline (0.5 µL) was injected i.c.v., and MAP and HR were recorded at 1 min intervals for 120 min.

The effect of nesfatin-1 on plasma vasopressin, catecholamine and renin levels in normotensive and hemorrhaged rats was investigated in a separate experiment. In normotensive rats, 250 µL blood samples were taken via the arterial catheter immediately before and 30, 60 and 120 min after i.c.v. nesfatin-1 (100 pmol) or saline (5 µL) administration. In hemorrhaged rats, 250 µL blood samples were withdrawn through the arterial catheter before and immediately after the hemorrhage procedure and, 30, 60 and 120 min after i.c.v. nesfatin-1 (100 pmol) or saline (5 µL) injection. Ice-cold polypropylene tubes containing EDTA were used for blood sample collection. Blood samples were immediately placed on ice. After centrifugation at +4 °C, 1800 r.p.m. for 20 min, plasma was separated and stored at –80 °C.

### 2.4. Determination of plasma vasopressin, catecholamine and renin levels

In the present study, plasma samples from rats were analyzed for concentrations of vasopressin, catecholamine, and renin with double-antibody microplate enzyme-linked immunoassay (ELISA) as described in the manufacturer's instructions (Shanghai Sunred

Biological Technology Co. Ltd. Shanghai P.R.C.). Briefly, 40 µL plasma samples were added to micro-plate wells pre-coated with a rat catecholamine, vasopressin, or renin antibody. After incubation and washing, rat catecholamine, vasopressin, or renin antibodies were conjugated with biotin. After incubation and washing, streptavidin-HRP was added to the wells to form an immune complex, followed by incubation and washing to remove the uncomplexed enzymes. Then a chromogenic HRP enzyme substrate solution was added to the wells and the color of the liquid in the wells changed to blue. To terminate the HRP enzyme reaction, stop solution was added to the wells. The plates were read at 450 nm with a plate reader (Bio-Tek Inc., VT, U.S.A.).

### 2.5. Drug and i.c.v. 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 previous study (Könczöl et al., 2012).

Intracerebroventricular injections were made by using an injection cannula composed of a 28 gauge stainless steel tubing connected to a 10 µL microsyringe with polyethylene tubing. Nesfatin-1 or saline was injected i.c.v. using an injection volume of 5 µL infused over a 60 s time period. During the injection, an air bubble moving in the polyethylene tubing was closely watched to ensure the drug was delivered in its entirety.

### 2.6. Data and statistical analysis

All values are reported as mean ± standard error of mean (S.E.M.) with  $p < 0.05$  considered as the level of significance. Statistical evaluation was performed by repeated-measures two way analysis of variance (RM-ANOVA; two-way) and the post-ANOVA test of Bonferroni.

## 3. Results

### 3.1. The effects of centrally injected nesfatin-1 on arterial pressure and heart rate in conscious normotensive and hemorrhaged rats

Centrally injected nesfatin-1 elevated MAP in normotensive animals significantly (Fig. 1A). The maximum increase in MAP, observed 30 min after nesfatin-1 administration, was  $10 \pm 1$  mm Hg. The pressor response lasted up to 120 min after nesfatin-1 administration (Fig. 1A). Nesfatin-1 also lowered HR of rats significantly (Fig. 2A). The effect reached its maximum level 15 min after nesfatin-1 injection and lasted for 120 min (Fig. 2A).

Hemorrhage produced severe and long-lasting hypotension in rats (Fig. 1B). Mean arterial pressure decreased by  $75 \pm 2$  mm Hg ( $n = 14$ ) by the end of the 10 min hemorrhage period. Subsequent i.c.v. injection of nesfatin-1 (100 pmol) rapidly increased MAP (Fig. 1B) to a maximum of  $38 \pm 3$  mm Hg 30 min after injection and it was significantly higher than those observed in the saline-injected group at around 120 min (Fig. 1B). Nesfatin-1 also caused tachycardia in hemorrhaged rats (Fig. 2B). The maximum increase in HR,  $41 \pm 3$  bpm, was observed within the first min of nesfatin-1 injection (Fig. 2B). The tachycardic effect persisted for approximately 90 min after nesfatin-1 administration (Fig. 2B).

### 3.2. Effect of centrally administered nesfatin-1 on plasma catecholamine, vasopressin and renin

The basal levels of plasma catecholamines in normotensive and hemorrhaged rats were  $461.5 \pm 2.7$  and  $470.5 \pm 3.1$  ng/L, respectively. Intracerebroventricular injection of nesfatin-1 (100 pmol) increased the concentration of plasma catecholamines significantly, further elevating catecholamine levels by approximately 34.2% (Fig. 3A). Hemorrhage independently increased plasma catecholamine levels by approximately 19.2% compared to baseline levels (Fig. 3B). Nesfatin-1 potentiated the

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