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Modulation of nesfatin-1-induced cardiovascular effects by the central cholinergic system

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ABSTRACT

Nesfatin-1, a peptide whose receptor is yet to be identified, has been shown to be involved in the modulation of feeding, stress, and metabolic responses. Recently, increasing evidence has supported a modulatory role of nesfatin-1 in cardiovascular activity. We have previously reported that nesfatin-1 causes an increase in blood pressure in normotensive and hypotensive rats by increasing plasma catecholamine, vasopressin, and renin levels. Recent reports suggest that nesfatin-1 may activate the central cholinergic system. However, there is no evidence showing an interaction between central nesfatin-1 and the cholinergic system. Therefore, this study aimed to determine whether the central cholinergic system may have a functional role in the nesfatin-1-induced cardiovascular effect observed in normotensive rats.

Intracerebroventricular injection of nesfatin-1 caused short-term increases in mean arterial pressure and heart rate responses including bradycardic/tachycardic phases in normotensive animals. Central injection of nesfatin-1 increased the acetylcholine and choline levels in the posterior hypothalamus, as shown in microdialysis studies. Central pretreatment with the cholinergic muscarinic receptor antagonist atropine and/or nicotinic receptor antagonist mecamylamine blocked nesfatin-1-induced cardiovascular effects. In conclusion, the results show that centrally administered nesfatin-1 produces a pressor effect on blood pressure and heart rate responses including bradycardic/tachycardic phases in normotensive rats. Moreover, according to our findings, the central cholinergic system can modulate nesfatin-1-evoked cardiovascular activity.

1. Introduction

Nesfatin-1 is an 82-amino acid neuropeptide produced by the proteolytic processing of nucleobindin-2 (NUCB2) (Oh-I et al. 2006). NUCB2/nesfatin-1 is widely distributed throughout the central nervous system including the cardiovascular regulatory areas such as hypothalamus, dorsal vagal complex (DVC), the nucleus of the solitary tract (NTS), and the dorsal motor nucleus of the vagus (DMNX) (Goebel-Stengel et al. 2011; Oh-I et al. 2006). Experimental evidence suggests that NUCB2/nesfatin-1 is involved in the control of the cardiovascular system in addition to multiple central functions including modulation of feeding behavior and neuroendocrine control of the reproductive axis (Garcia-Galiano et al. 2010a; 2010b; Oh-I et al. 2006; Stengel et al. 2010). For instance, central administration of nesfatin-1 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 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 through a direct action on arterioles (Yamawaki et al. 2012). Nesfatin-1 expression in the heart has been correlated with negative inotropic effects and protection against ischemia-reperfusion injury (Angelone et al. 2013). Recently, we reported that central administration of nesfatin-1 exerts pressor and bradycardic effects in normotensive animals and produces pressor and tachycardic effects under hypotensive conditions produced by severe hemorrhage (Yilmaz et al. 2015). In that study, i.c.v. injection of nesfatin-1 increased plasma catecholamine, vasopressin, and renin concentrations, and those hormones contributed to the pressor effects of the peptide in both conditions (Yilmaz et al. 2015).

The central cholinergic system has been repeatedly shown to play an important role in the regulation of the cardiovascular system. Centrally acting cholinergic agonists and indirectly acting cholinergic drugs have been demonstrated to increase arterial blood pressure (Buccafusco 1996). Previously, choline (Arslan et al. 1991) and CDP-

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choline (Savci et al. 2002, 2003), a choline donor, have been shown to produce pressor and bradycardic responses by activating central cholinergic transmission. Central cholinergic neurons also mediate pressor cardiovascular effects induced by other neurotransmitters and/or neuromodulators including prostaglandins (Yalcin et al. 2005, 2006; Yalcin and Erturk, 2007) and histamine (Altinbas et al. 2015, 2016; Yalcin et al. 2009).

Evidence that nesfatin-1 raises arterial pressure following central administration raises the possibility that nesfatin-1 may modulate cardiovascular function. The central melanocortin and oxytocin systems, as well as central corticotropin-releasing hormone (CRH), are known to contribute to the hypertensive action of nesfatin-1 in normotensive animals (Tanida and Mori 2011; Yosten and Samson 2009, 2010, 2014). Additionally, nesfatin-1 immunoreactivity has been detected in the nucleus ambiguus (NAmb) (Goebel et al. 2009; Goebel-Stengel et al. 2011) and thus may influence premotor cardiac vagal neurons (Mendelowitz 1999). Microinjection of nesfatin-1 into the NAmb has been reported to produce bradycardia without changing blood pressure in conscious rats (Brailoiu et al. 2013). On the other hand, NUCB2/nesfatin-1 has been shown to be expressed in DMNX cholinergic neurons that project to the stomach (Bonnet et al. 2013). Activation of sympathetic (Tanida and Mori 2011; Yosten and Samson 2009) and parasympathetic cardiac tones (Brailoiu et al. 2013) with central injection of nesfatin-1 has been observed. Those reports suggested that nesfatinergic and cholinergic neurons might be interacting to effect cardiovascular control. Neuroanatomically, brain regions that express NUCB2/nesfatin-1, such as the NAmb, DMNX and hypothalamus, include cholinergic neurons and are involved in cardiovascular regulation (Brailoiu et al. 2007). Therefore, in the current study, experiments were done to determine if the central cholinergic system mediates the nesfatin-1-induced cardiovascular effect in normotensive rats.

2. Materials and methods

2.1. Animals

A total of 70 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 temperature (20–22 °C), humidity (60–70%) and lighting (12-h light/dark cycle) conditions 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_2) anesthesia, the left femoral artery was cannulated with PE 50 tubing filled with heparinized saline (100 U/ml) to measure the mean arterial pressure and heart rate. The tip of the tubing was covered and exteriorized at the neck of the rat. For i.c.v. treatment, a burr hole was drilled through the skull 1.5 mm lateral (left) 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. For the microdialysis study for the detection of acetylcholine and choline in the extracellular posterior hypothalamus, rats were anesthetized with sevoflurane (2–4%/100% O_2), and catheterized rats were placed on a stereotaxic frame. The skull was exposed and drilled over the posterior hypothalamus (coordinates: 3.6 mm posterior to bregma, 0.5 mm lateral (right) to the midline and 9.0 mm ventral to the skull) (Paxinos and Watson 2005). Handmade microdialysis probes with dialysis membrane with a 18-kDa molecular weight cut-off and a 0.3-mm diameter and 2.0-mm-long dialysis area (by Burcin Altinbas) were used for the microdialysis study. Probes were implanted and then fixed with acrylic cement to the skull. After surgery, the rats were placed in individual cages and allowed to recover from anesthesia for 4–5 h.

2.3. Measurement of cardiovascular parameters

The arterial cannula was connected to a volumetric pressure transducer (BPT 300, BIOPAC Systems Inc., California, USA) attached to an MP36 system (BIOPAC Systems Inc.) to measure the cardiovascular parameters of rats. Rats were allowed to stabilize for 30 min before experiments, and baseline mean arterial pressure and heart rate measurements were recorded within this time period. The blood pressure was reported as the mean arterial pressure (mmHg), and heart rate was expressed as beats per minute (bpm).

2.4. Microdialysis study and measurement of acetylcholine and choline levels

The microdialysis probe was attached to the perfusion pump. The probe was perfused with artificial cerebrospinal fluid (pH 7.4) of the following composition: 120 mmol/l NaCl, 1.3 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l NaH_2PO_4, 3.5 mmol/l KCl, 25 mmol/l $NaHCO_3$ and 10 mmol/l glucose. The 10 mmol/l glucose level in the artificial cerebrospinal fluid is critical to the maintenance of the osmolality of the microdialysis fluid. An acetylcholinesterase inhibitor, neostigmine, was added to the artificial cerebrospinal fluid at 20 μM to prevent the degradation of acetylcholine to choline. The perfusion rate was 2 μl/min. The dialysis probe was perfused for the first 30 min of the stabilization period, and samples were collected at 10-min intervals. After this period, 3 consecutive samples were collected, and these samples were considered as basal acetylcholine and choline levels. Next, an i.c.v. injection of the drug or vehicle was given, and samples were collected over the next 60 min. At the end of the experiments, the rats were sacrificed by using an i.v. injection of an overdose of pentobarbital sodium, and the brains were removed and fixed in 10% formalin. Serial coronal sections were sliced at 40 μm and used to verify the location of the tip of the dialysis probe.

Equipment consisted of an on-line membrane degasser, isocratic pump, and pulse dampener (Hitachi L2130, Japan). Injections (10 μl) were made through a Rheodyne 7725i valve fitted with a 20 μl loop. Online electrochemical detection was performed with an Amor detector fitted with a platinum electrode (Antec-Leyden Ltd., NL). The columns and detector cell were kept at 24 °C in a column oven. Chromatograms were collected and analyzed using EZchrom Elite software. The results were calculated from peak area readings. Levels of neurotransmitter (pmol) were analyzed for PH.

Acetylcholine and choline are electrically inert and therefore need to be converted to H_2O_2 prior to detection. To do this, we used a dedicated column arrangement consisting of guard, separation, and immobilized-enzyme reactor (IMER) columns containing acetylcholinesterase and choline oxidase (from Bioanalytical Systems, BASi, West Lafayette, IN, USA). The flow rate through the system was 1 ml/min. The applied potential at the platinum working electrode was +500 mV set against an Ag/AgCl reference electrode, which was found to be the optimum potential for these conditions. The mobile phase was a phosphate buffer (consisting of 0.05 M Na_2HPO_4, pH 8.5) with the addition of Kathon as a bacteriostatic agent. Stock solutions of 10 μM acetylcholine and 10 μM choline standards (Sigma-Aldrich Co. Deisenhofen, Germany) were prepared in 50 mM acetic acid (pH 5) and diluted with mobile phase prior to injection to give standards containing either 1462.0 pg (10 pmol) acetylcholine or 1041.7 pg (10 pmol) choline per 10 μl injected. Standards were run before, during, and after each set of 11 sample measurements from one animal; each sample was measured twice.