Neural circuitry underlying the central hypertensive action of nesfatin-1: melanocortins, corticotropin-releasing hormone, and oxytocin

Gina L. C. Yosten and Willis K. Samson
Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, Saint Louis, Missouri

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Yosten GL, Samson WK. Neural circuitry underlying the central hypertensive action of nesfatin-1: melanocortins, corticotropin-releasing hormone, and oxytocin. Am J Physiol Regul Integr Comp Physiol 306: R722–R727, 2014. First published March 5, 2014; doi:10.1152/ajpregu.00396.2013.—Nesfatin-1 is produced in the periphery and in the brain where it has been demonstrated to regulate appetite, stress hormone secretion, and cardiovascular function. The anorexigenic action of central nesfatin-1 requires recruitment of neurons producing the melanocortins and centrally projecting oxytocin (OT) and corticotropin-releasing hormone (CRH) neurons. We previously have shown that two components of this pathway, the central melanocortin and oxytocin systems, contribute to the hypertensive action of nesfatin-1 as well. We hypothesized that the cardiovascular effect of nesfatin-1 also was dependent on activation of neurons expressing CRH receptors, and that the order of activation of the melanocortin-CRH-oxytocin circuit was preserved for both the anorexigenic and hypertensive actions of the peptide. Pretreatment of male rats with the CRH-2 receptor antagonist astressin2B abrogated nesfatin-1-induced increases in mean arterial pressure (MAP). Furthermore, the hypertensive action of CRH was blocked by pretreatment with an oxytocin receptor antagonist ornithine vasotocin (OVT), indicating that the hypertensive effect of nesfatin-1 may require activation of oxytocinergic (OTergic) neurons in addition to recruitment of CRH neurons. Interestingly, we found that the hypertensive effect of α-melanocyte stimulating hormone (α-MSH) itself was not blocked by either astressin2B or OVT. These data suggest that while α-MSH-producing neurons are part of a core melanocortin-CRH-oxytocin circuit regulating food intake, and a subpopulation of melanocortin neurons activated by nesfatin-1 do mediate the hypertensive action of the peptide, α-MSH can signal independently from this circuit to increase MAP.

nesfatin-1; melanocortin; central control of blood pressure; oxytocin; CRH

HYPERTENSION, a major comorbidity of obesity, is present in over 50% of overweight and obese patients (19). While the etiology of obesity-associated hypertension is not fully understood, many obese patients exhibit an elevation in sympathetic nervous system (SNS) activity, as measured by microneurography (38) or elevated serum catecholamines (39). It is unclear whether the relationship between obesity and sympathoactivation is causal or correlative; however, several potential mechanisms have been proposed for their co-occurrence. One such hypothesis centers on the role of the adipocyte-derived hormone leptin, which acts centrally to inhibit food intake and increase SNS activity and subsequently mean arterial pressure (MAP). It has been suggested that in the setting of obesity, a state of leptin hypersecretion (32), individuals develop selective resistance to leptin, wherein they are incapable of responding to the anorexigenic effect of the peptide, yet continue to exhibit leptin-induced sympathoactivation (26, 27).

In addition to leptin, adipocytes produce a multitude of paracrine and endocrine factors called adipokines, including the peptide hormone nesfatin-1. Like leptin, nesfatin-1 is found in high circulating levels in obese patients (35, 46) and potently inhibits food intake (20, 43). Nesfatin-1 has been shown to exert its anorexigenic action independently from leptin, since nesfatin-1 inhibited food intake in Zucker fatty rats (20), and nesfatin-1 midssegment significantly reduced food intake in the setting of leptin resistance (31). Single nucleotide polymorphisms in the nesfatin-1 gene have been linked to human obesity (47), and, in addition, nesfatin-1 was shown to elevate MAP (43) and renal sympathetic nerve activity (36). These data indicate that nesfatin-1 may play a role in the development of obesity-associated hypertension.

Interestingly, both leptin and nesfatin-1 must interact with the central melanocortin system to exert their anorexigenic and hypertensive actions (4, 20, 43). The central melanocortin system is an essential neural circuit that integrates nutritional status information from both central and peripheral sources and interacts with downstream effector systems to initiate appropriate behavioral responses (5). Recent evidence suggests that the central melanocortin system contributes to the control of cardiovascular function (5), and hyperstimulation of this system may underlie the development of hypertension in several animal models (7, 30). Thus the selective resistance (anorexigenic but not autonomic) of obese individuals to adipokines, such as leptin and nesfatin-1, may reflect changes at the level of melanocortin neurons, particularly those responsible for regulating appetite, become resistant to peripherally derived energy signals.

The anorexigenic action of nesfatin-1 has been shown to be dependent on central corticotrophin-releasing hormone (CRH) receptors (33) and central oxytocin (OT) receptors (16, 44), in addition to the central melanocortin system (20, 43). We previously have shown that the hypertensive action of nesfatin-1 was also dependent on the central OT system (44), and we sought to determine whether activation of central CRH receptors was essential for this effect of nesfatin-1 as well. In addition, both CRH and OT have been shown to act as downstream mediators of melanocortin action, therefore, we sought to determine whether these systems formed a circuit with the central melanocortin system to regulate acute changes in MAP. Surprisingly, we uncovered two parallel circuits that lie downstream of the central melanocortin system, and differential activation of those circuits may underlie selective resistance to adipokines, such as leptin and nesfatin-1.
MATERIALS AND METHODS

Animals

All procedures and protocols have been approved by the Saint Louis University Animal Care and Use Committee (protocol no. 2041). Male Sprague-Dawley rats (Harlan, Indianapolis, IN) (230–250 g, 7–8 wk of age) were anesthetized with a mixture of ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA)-xylazine (TranquiVed, Vedco, St. Joseph, MO) intraperitoneally (60 mg ketamin-8 mg Xylazine per ml, 0.1 ml/100 g body wt), and a stainless steel cannula (23 gauge, 17 mm) was inserted into the right lateral cerebroventricle (intracerebroventricular) using a stereotaxic device (stereotaxic coordinates: A, +6.2; H, +7.4; L, −0.9; relative to the interaural line) (23), as previously described (43). A separate group of animals was implanted with a stainless steel cannula into the fourth ventricle (4V, stereotaxic coordinates: A, −2.7; H, +2.5; L, 0.0; relative to the interaural line) (23). Buprenorphine (0.05 mg/kg) was administered subcutaneously on the day of surgery for postoperative pain, and 10 ml sterile saline (0.9% NaCl) was injected subcutaneously to compensate for anticipated postsurgical fluid loss. After a minimum of 5 days of recovery, rats again were anesthetized and an additional cannula (PE-50) was implanted into the left carotid artery (43). Experiments were conducted the day following carotid cannulation.

Blood Pressure Monitoring

One day after implantation of the carotid cannula, rats were habituated to a quiet testing room for at least 2 h. The carotid catheter was connected to a pressure transducer (Digi-Med BPA, Micro-Med, Louisville, KY), and the catheter was flushed with heparinized saline (200 U/ml in sterile, 0.9% NaCl). Baseline MAP was recorded at 1-min intervals for at least 30 min.

Lateral ventricle injections. Rats bearing intracerebroventricular cannulas were pretreated with either saline vehicle (2 μl), or vehicle containing the OT receptor antagonist ornithine vasotocin (OVT) (10 μg) (44), or the CRH type 2 receptor antagonist astressin2B (30 μg) (33). Ten minutes later, rats received an intracerebroventricular injection of either saline vehicle (2 μl), or vehicle containing 180 pmole nesfatin-1 (43), 0.1, 0.5, or 1 nmole α-melanocyte stimulating hormone (α-MSH) (44), or 20 pg CRH (dose determined in preliminary experiments).

Fourth ventricular injections. Rats bearing fourth ventricular (4V) cannulas were pretreated with either saline or vehicle containing the melanocortin 3/4 receptor antagonist SHU9119 (300 pmole) (39), 10 min before with either saline vehicle or 30 ng OT (44). MAP was recorded for at least 60 min at 1-min intervals.

Peptides and Antagonists

Nesfatin-1, α-MSH, OT, CRH, OVT, and SHU9119 were purchased from Phoenix Pharmaceuticals (Burlingame, CA). Astressin2B was purchased from Tocris Biosciences (Bristol, UK).

Data Analysis and Statistics

Data are presented as change from preinjection baseline, calculated as the average MAP or heart rate for 5 min before intracerebroventricular injection, to account for the natural variation in resting cardiovascular parameters between animals. Changes in MAP are shown as area under the curve. Data were analyzed using a nonparametric test (Mann-Whitney U) because data were transformed (45).

RESULTS

Hypertensive Action of Nesfatin-1 (Intracerebroventricular) is Reversed by Pretreatment With a CRH Receptor Antagonist

As demonstrated previously (43), treatment with nesfatin-1 elicited a significant increase in MAP (Fig. 1) in animals pretreated with saline vehicle. Although astressin2B did not alter basal MAP, pretreatment with the antagonist completely abrogated the hypertensive action of nesfatin-1 when observed...
as area under the curve (Fig. 1A) or minute-to-minute traces (Fig. 1B).

**CRH-Induced Increase in MAP is Blocked by Pretreatment With OVT When Injected Intracerebroventricularly**

Central injection of CRH resulted in a significant increase in MAP (Fig. 2). Administration of OVT by itself did not significantly alter MAP; however, the central hypertensive effect of CRH was completely abolished by pretreatment with OVT (Fig. 2).

**Hypertensive Action of Intracerebroventricular α-MSH is not Reversed by Astressin2B or OVT**

Central administration of 0.5 or 1 n mole, but not 0.1 n mole, α-MSH resulted in significant increases in MAP (Figs. 3 and 4) compared with vehicle-pretreated, vehicle-treated control animals. This effect was not blocked by pretreatment with either astressin2B (Fig. 3) or OVT (Fig. 4). As in the previous experiments, neither astressin2B nor OVT altered basal MAP.

**SHU9119 Does Not Inhibit Hypertensive Effect of OT (4V)**

The results described above suggested that while the ability of nesfatin-1 to increase MAP depends on activation of a melanocortin-CRH-OT pathway, α-MSH itself can affect autonomic function independent of that circuit. Indeed, it is possible that the circuit activated by nesfatin-1 in hypothalamus communicates via caudally projecting OT neurons to a second population of melanocortin neurons in medulla (12). Third ventricular administration of OT has been shown to inhibit food intake, and this effect was reversed by pretreatment with the melanocortin 3/4 receptor antagonist SHU9119 (16). However, the ability of OT to elevate MAP when administered into the 4V was not prevented in the current experiment by pretreatment with SHU9119 (Fig. 5).
Central administration of nesfatin-1 also activates stress hormone secretion (13, 41), and the anorexigenic action of nesfatin-1 can be blocked by CRH antagonist pretreatment (33). The melanocortin agonist melanotan II (MT II) activated the hypothalamic-pituitary-adrenal axis, and pretreatment with a CRF2 receptor antagonist blocked that activation as well (15), suggesting that the action of nesfatin-1 on stress hormone secretion and food intake might be relayed from POMC neurons to CRH neurons in the PVN, which those authors demonstrated to express melanocortin receptors.

Pretreatment with an OT antagonist also prevented the anorexigenic and autonomic actions of nesfatin-1 (44). A link between POMC and OT neurons in hypothalamus was provided by Sabatier and colleagues (29), and the obese phenotype of Sim-1-deficient mice has been hypothesized to be causally linked to the significant decreases of PVN OT and melanocortin 4 receptor mRNAs in those animals (14, 37). CRH receptors are expressed in OT neurons in the PVN (1, 6). Furthermore, the anorexigenic action of CRH can be blocked by antagonism of central OT receptors (21). Additionally, as noted above, CRH neurons are innervated by POMC neurons, CRH neurons express MC4R receptors (15), and CRH appears to mediate the anorexigenic action of α-MSH (15).

Thus we hypothesized that, like the anorexigenic actions of the peptide, nesfatin-1 exerts its central hypertensive action either by crossing from the circulation into the brain at the level of the arculate nucleus or via a neural pathway beginning with its action in the arcuate nucleus on POMC neurons (Fig. 6, step 1). Those activated POMC neurons project to and activate CRH neurons in the hypothalamic PVN (Fig. 6, step 2), that relay that message to OT neurons in the same nucleus (Fig. 6, step 3), particularly those that project to brain stem cardiovascular centers (Fig. 6, step 4). If that were the case then antagonism of any step along the circuit should abrogate the hypertensive action of nesfatin-1.

As mentioned above, the OT receptor antagonist OVT prevented the ability of nesfatin-1 given into the lateral cerebroventricle to increase MAP in conscious male rats (44). We then moved one step back in the proposed circuit and demonstrated

Figure 5. Hypertensive action of oxytocin (OT) injected into the fourth ventricle (4V) does not depend on central melanocortin receptors. Rats bearing 4V and carotid cannulas were pretreated via the 4V cannula with either vehicle or the melanocortin 3/4 receptor antagonist SHU9119 (300 pmole) and then injected with vehicle or vehicle containing a hypertensive dose (30 ng) of OT. OT induced a significant increase in mean arterial pressure that was not affected by blockade of central melanocortin receptors by SHU9119. Data are presented as changes in mean arterial pressure compared with preinjection baseline and area under the curve. *P < 0.05, ***P < 0.001 vs. Saline/Saline-injected control animals. No significant difference was observed between Saline/OT- and SHU9119/OT-treated rats.

DISCUSSION

Nesfatin-1 is produced in the brain (3, 10, 11, 20), in particular in the hypothalamus, and nesfatin-1 secreted by peripheral tissues, including stomach, pancreas, and adipose tissue (9, 11, 28, 34), can cross from the circulation into the central nervous system (22, 25). Thus in addition to the well-characterized actions of the peptide on feeding behavior, other potential actions of endogenous nesfatin-1 have been proposed (13, 17, 41). We have demonstrated previously that the action of nesfatin-1 in the brain to elevate MAP was blocked by pretreatment with the melanocortin antagonist SHU9119 (43). Likewise, the anorexigenic action of nesfatin-1 can be blocked by SHU9119 (20, 43). Because a unique receptor for nesfatin-1 has not been identified, we can only infer from our electrophysiology studies (24) that the peptide acts directly to depolarize pro-opiomelanocortin (POMC) neurons in the arcuate nucleus. In those studies we did observe direct, hyperpolarizing actions of nesfatin-1 on neuropeptide Y/agouti-related protein (NPY/AgRP) neurons in the arcuate nucleus, which may have been the basis for the depolarizing actions of the peptide on the neighboring POMC neurons (24). Alternatively, nesfatin-1 could exert direct effects on neurons in the paraventricular or lateral hypothalamic nuclei, since microinjection studies have suggested that nesfatin-1 exerts an anorexigenic effect when injected directly into these sites (16). Recent electrophysiology studies also have identified a potential site of the cardiovascular actions of nesfatin-1 to be the nucleus tractus solitarius (18), where a second population of POMC neurons, that may be responsive to OT, is located (5, 16).

Fig. 6. Proposed model of the circuit underlying the hypertensive action of nesfatin-1 (NES). NES activates α-MSH-producing proopiomelanocortin (POMC) neurons in the arcuate (ARC) nucleus (1), which project to and stimulate CRH neurons in the hypothalamic paraventricular nucleus (PVN) (2). CRH neurons activate OT neurons in the same nucleus (3), which project to brain stem autonomic centers (4). Alternatively, POMC neurons may directly project to brain stem autonomic centers (4). 3V, third ventricle.
that antagonism of the CRH2 receptors, with astressin2B, also prevented the action of nesfatin-1 to increase MAP. But does that mean that OT neurons necessarily transmit the information conveyed to the CRH neurons by the action of nesfatin-1? We propose that to be the case since antagonism of central OT receptors blocked the increase in MAP observed following CRH alone. Thus we provide here evidence for a neural circuit activated by nesfatin-1 that is initiated by an action on POMC producing neurons in the arcuate nucleus, going through a population of CRH neurons to caudally projecting OT neurons involved in the central control of autonomic function (12). Indeed, injection of OT into the fourth cerebroventricle directly adjacent to those brain stem cardiovascular centers, where OT receptors have been identified (40, 42), resulted in increased MAP.

The anorexigenic action of OT administered into the third cerebroventricle has been reported to be blocked by local SHU9119 administration (16). Could it then be that the proposed circuit that began with the action of nesfatin-1 on melanocortin neurons in arcuate nucleus was finally expressed by an OT-dependent relay to the other population of POMC neurons, those located in the nucleus tractus solitarii? This may not to be the case, since 4V administration of the melanocortin antagonist SHU9119 did not block the hypertensive action of OT administered into the same fluid cavity.

Our experiments also have uncovered evidence for at least two separate neural circuits through which α-MSH can increase MAP. One clearly responds to nesfatin-1 administration recruiting CRH- and OT-producing neurons in the hypothalamus. There must be, however, a second melanocortin pathway that activates autonomic function because the ability of centrally administered α-MSH, unlike that released endogenously in response to nesfatin-1, could not be blocked by antagonism of either CRH2 or OT receptors. Those pathways may originate in POMC-producing neurons in the arcuate nucleus independent of those activated by nesfatin-1 (Fig. 6) or in the second population of central nervous system neurons that produce POMC, in the nucleus tractus solitarius. Regardless, aberrations in melanocortin signaling in the setting of obesity have been demonstrated (8) and thus may contribute to the comorbidity of obesity.

**Perspectives and Significance**

While it is unclear if that second population of POMC-producing neurons also might be responsive to the major adipokine circulating in plasma, leptin, we would suggest that the melanocortin neurons that mediate the anorexigenic action of leptin, and perhaps nesfatin-1, and which develop resistance to chronically elevated levels of these adipokines, are not the same neurons that mediate the hypertensive actions of leptin and nesfatin-1. Furthermore, we suggest that this is the reason for the maintained sympathostimulatory actions of at least leptin in the obese state (26, 27). Future studies should focus on the identification of those two potentially distinct populations of POMC-producing neurons, whether they are located within the same nucleus as those responsible for the anorexigenic actions of the adipokines or those located caudally that may be more related to central cardiovascular control. Finally our proposed circuit does not identify the downstream target of those OT neurons or even their location. Our intent is to examine both the medullary cardiovascular centers where OT receptors are expressed and temporal lobe structures where OT binding is prominent (40). Additionally, we intend to examine the possible requirement for OT activation of brain-derived neurotrophic factor neurons in the proposed circuit since brain-derived neurotrophic factor has been suggested to be a downstream target of melanocortins in other neural systems (2).

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**REFERENCES**


