Effect of Central and Peripheral Nesfatin-1 on Food Intake in Japanese Quail

Shousha S.¹, Kirat D.², Naso T.³

¹Department of Physiology, Faculty of Veterinary Medicine, Benha University, Moshtohor, Egypt
²Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt
³Department of Basic Veterinary Sciences, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

Email address
Physiology2009@yahoo.com (Shousha S.)

Citation

Abstract
Nesfatin-1 is an 82 amino acid polypeptide recently discovered in the brain which is derived from nucleobindin2 (NUCB2), a protein that is highly conserved across mammalian species. Intracerebroventricular (icv) injection of nesfatin-1 into rodents significantly reduced the food intake during the dark period, and increased oxygen consumption and body temperature suggested that nesfatin-1 is an anorectic and catabolic signaling molecule in mammals. In this study, we elucidated the influence of icv and peripheral (intraperitoneal, ip) injection of various rat nesfatin-1 doses on food intake and body temperature in avian species using the Japanese quail. Both ip and icv administration of rat nesfatin-1 resulted in a significant reduction in either dark phase or light phase food intake in a dose-dependent manner at all time-point measured. In concomitant with the reduction in food intake, there is a reduction in body weight gain when nesfatin-1 was injected either centrally or peripherally. Moreover, both ip and icv administration of nesfatin-1 caused a dose-related increase of body temperature but no significant alteration in gross locomotor activity in Japanese quails. These results suggest that nesfatin-1 plays an important role in the regulation of food intake and body temperature thus regulating energy expenditure in avian species.

1. Introduction

Nesfatin-1 was discovered in 2006 by Oh-I and colleagues as an 82 amino acid (aa) polypeptide derived from the calcium and DNA-binding protein, nucleobindin2 (NUCB2) (Oh et al., 2006). NUCB2/nesfatin is composed of 396 amino acids, preceded by a 24-amino acid signal peptide, with very high amino acid sequence homology among rat, mouse, and human species (87.4% homology to humans and 95.7% to mice). Structural analyses revealed the presence of several conserved cleavage recognition sites for prohormone convertases (PC) within rat NUCB2/nesfatin sequence, thus suggesting this to be a precursor that gives rise, by differential proteolytic processing, to several active peptides. The predicted (major) fragments of such processing were termed nesfatin-1 (spanning residues 1–82), nesfatin-2 (residues 85–163), and nesfatin-3 (residues 166–396) and conversion of nesfatin/NUCB2 to nesfatin-1 by PC should be necessary to suppress feeding behavior (Oh et al., 2006).

Identification of nesfatin-1 as a hypothalamic neuropeptide was immediately followed by the characterization of its pivotal role in regulating feeding by reducing feed intake (Oh et al., 2006; Brailoiu, et al., 2007; Maejima, et al., 2009). Thus, central (icv)
injection of nesfatin-1 was shown to decrease food intake (and consequently body weight) in rats in a dose-dependent manner. In contrast, neither nesfatin-2 nor nesfatin-3 evoked any anorectic responses (Oh et al., 2006). In good agreement, immunoneutralization of endogenous nesfatin-1, but not of nesfatin-3, significantly enhanced food intake. Likewise, knockdown of hypothalamic NUCB2/nesfatin content by means of a central infusion of an antisense morpholino oligonucleotide (as-MON) consistently increased food intake and body weight in adult rats (Oh et al., 2006). Altogether, these data evidenced that nesfatin-1 is an appetite suppressive molecule, a contention that has been further confirmed in the rat, where central injection of nesfatin-1 has been shown to decrease dark-phase food intake (Stengel et al., 2009a), and in the mouse, where recombinant human, rat, and mouse nesfatin-1 proteins were capable of acutely suppressing food intake (Shimizu et al., 2009b). The above observations have been recently by the identification of the active domain of nesfatin-1 molecule causing inhibition of food intake. Thus, based on structural analyses, three different fragments of the nesfatin-1 protein (N-terminal fragment (residues 1–23); mid-fragment (residues 24–53); and C-terminal fragment (residues 54–82); were tested for food intake suppression in mice. These analyses revealed that only the mid-fragment was capable to induce anorectic responses, thus unveiling the active core of nesfatin-1 molecule in terms of feeding control (Shimizu et al., 2009a,b).

Nesfatin is expressed in the brain and peripheral tissues in rodents and humans. It is expressed in appetite-controlling hypothalamic nuclei, such as the arcuate nucleus, paraventricular nucleus, supraoptic nucleus, the lateral hypothalamic area (Oh et al., 2006), as well as, in the solitary tract (NTS) and dorsal nucleus of vagus (Brailoiu et al., 2007; Zhang et al., 2013). Several peripheral tissues express nesfatin mRNA, including adipose tissues (Oh et al., 2006; Hausman et al., 2012), serum, gastric mucosa (Stengel et al., 2009b) and pancreatic beta cells (Gonzalez et al., 2010). Such a wide pattern of distribution of NUCB2/nesfatin-1, with prominent expression not only in hypothalamic nuclei but also in diverse brainstem areas and autonomic centers, was already taken as an (indirect) index of its potential function as an integral regulator of energy homeostasis.

Both central and peripheral injections of nesfatin-1 cause a significant reduction of food intake in rodents (Oh et al., 2006; Shimizu et al., 2009a,b; Stengel et al., 2009a; Maejima et al., 2009; Su et al., 2010; Yosten and Samson, 2010; Atsushi et al., 2010; Goebel et al., 2011; Wernecke et al., 2014). Interestingly, central icv administration of nesfatin-1 resulted in a significant reduction in food intake and body weight as well as a decrease in se, mesenteric, and epididymal fat masses (Oh et al., 2006). These studies provided evidence for nesfatin-1 as a metabolic regulator with anorectic and weight reducing effects. Stengel et al (2009a) previously assessed nesfatin-1’s (5 pmol, icv) influence on behavior and nesfatin-1 did not induce alterations of locomotor activity or grooming (washing, licking, and/or scratching) behavior. Similarly, in a recent report a higher dose of nesfatin-1 (25 pmol, icv) did not alter locomotor activity when assessed automatically in rats, while food intake was decreased for a sustained period of time (Konczol et al., 2012). Also, it is reported that nesfatin-1 reduced duration of nocturnal food intake for 2 days independently of circadian time injected, and raised body temperature immediately, or with little delay depending on the dose and circadian time applied (Konczol et al., 2012; Stengel et al., 2012).

With exception that NUCB2 gene was identified from the sequenced chicken genome (Cisse 2012), thus far there are no research has been published on avian nesfatin and there are no published data regarding the peripheral or central effects of nesfatin in birds. Therefore, this study is the first about the effect of nesfatin-1 in birds.

In this study, the reason why we used Japanese quail instead of chickens was that the growth curve of young chicks is steep, so body weight gain and food intake vary widely from day to day, whereas adult Japanese quails have ceased growing, and therefore their body weight gain and food intake are not subject to such variability. Furthermore, it is technically possible to implant indwelling icv cannulae into adult Japanese quails. In the present study, therefore, we estimated the effects of peripherally (ip) and centrally administered rat nesfatin-1 on food intake, body temperature and gross locomotor activity in adult Japanese quails. Also, we used adult male quails to avoid the effect of female reproductive hormones on food intake and their interference with the injected peptides on food intake or other parameters. The purpose of this study was to elucidate the central and peripheral (intraperitoneal) effects of various doses of rat nesfatin-1 on food intake, body temperature and gross locomotor activity in the Japanese quail.

2. Materials and Methods

2.1. Animals

Adult male Japanese quail (Coturnix coturnix japonica) were housed in individual net cages (W: 14 x L: 26 x H: 17cm) in a room with a 12-h light (300 lx) /12-h dark (dim light, 25 lx) period (lights on at 07:00 h) and a temperature of 28±1 °C, and were given free access to food and water. Before the feeding experiment, the birds were weighed and assigned to an experimental group (six birds in each group) based on their body weight. The average body weight (110–120 g) in each group was kept as uniform as possible. To examine the orexigenic or anorexic effect of rat nesfatin-1 (Peptide Institute, Osaka, Japan) or 0.9% saline (vehicle control) was administered ip or icv at either 06:45 h (i.e., during the birds’ active phase) or 18:45 h (i.e., during the birds’ resting phase). The doses injected ip were 0.5, 1.0, and 3.0 nmol/200 µl saline while the doses injected icv were 0.05, 0.5 and 1.0 nmol/10 µl saline. The same animals were never used for more than one study. All procedures were performed...
in accordance with the Japanese Physiological Society’s guidelines for animal care.

### 2.2. Surgical Procedures

For the implantation of the icv cannula, each bird was anesthetized with 5% sodium pentobarbital (1.4µl/g body weight) and placed in a stereotaxic frame. A stainless steel guide cannula (outer diameter 550 µm, length 14 mm) was stereotaxically implanted into the third cerebral ventricle using a modification of a previously reported method (Bayle et al., 1974). The coordinates were 5 mm anterior to the interaural axis and 6.5 mm below the dura at the midline. One stainless steel anchoring screw was fixed to the skull, and the guide cannula was secured in place with acrylic dental cement. The birds were returned to their individual cages and allowed to recover for at least 4 days. They were acclimatized to handling every day before the start of the experiments. The icv injections were administered through the implanted guide cannulae without anesthesia or restraining of the birds.

At the end of the experiments, proper placement of the cannulae was verified by administering Evans Blue dye (10 µl), followed by sacrifice and brain sectioning (20 µm intervals). Data for birds lacking dye in the third ventricle were excluded from the analysis.

### 2.3. Measurement of Food Intake

Food consumption was determined 2, 4, and 12 h after administration of nesfatin-1 or saline by measuring the disappearance of food from the pre-weighed feeder. Any spillage was also collected and weighed.

### 2.4. Measurement of Body Temperature

The quails’ body temperature was measured 0 (before injection), 5, 10, 20, 40, 60 min, and 2, 3, 4, 5, and 6 h after administering nesfatin-1 or saline at the doses stated above \((n = 6\) in each group) at 10:00 h, using a previously reported method (Bayle et al., 1974). Briefly, the temperature was measured electronically with a small sensor (measurable range: 25–50 °C; measurement error: 0.05 °C) connected to a line (outer diameter 0.7 mm; length 45 cm) which is connected to the monitor body. The sensor tip was inserted into the cloaca, and part of the line was fixed to the bird’s body, with the digital signal transferred to the monitor body.

### 2.5. Measurement of Gross Locomotor Activity

Locomotor activity was measured in each bird under LD condition for 1 week, and thereafter under constant dim light at an intensity of about 30 lux, with a rat locomotor activity recording system (Muromachi, Tokyo, Japan) comprising infrared sensors, an interface, and a computer (Marumato et al., 1996). The infrared sensors were placed above the cages and measured all locomotions such as eating and movement around within the cage. Each cage with its infrared sensor was placed in an isolated chamber box with a controlled light/dark cycle. The data were collected at 15-min intervals and analyzed by CompactACT AMS software (Muromachi). The doses of rat nesfatin-1 injected ip were 1.0 and 3.0 nmol/200 µl saline while the doses injected icv were 0.5 and 1.0 nmol/10 µl saline. All groups were administered at 07:30 \((n = 6\) per group). The birds were immediately returned to the individual cages. Locomotor activity counts were made every 15min and summed for 2 h after administration.

### 2.6. Statistical Analysis

All results are expressed as means ± SEM. Data were analyzed by the two-way analysis of variance (ANOVA) and the post hoc Fisher’s test.

### 3. Results

#### 3.1. Food Intake

In the ip administration group, 0.5, 1.0 and 3.0 nmol nesfatin-1 significantly \((P<0.05\) decreased food intake in a dose-dependent manner during both the light (Fig. 1A) and dark periods (Fig. 1B) compared with the saline-control group. This anorectic action of various doses of nesfatin-1 was more evident during the dark period (Fig. 1B) than the light period (Fig. 1A). During the first 2 h after the ip injection, the 1.0 nmol dose of nesfatin-1 inhibited food intake more potently during the dark period (Fig. 1B) whereas the 3.0 nmol dose inhibited feeding more markedly during the light period (Fig. 1A). The significant \((P<0.05\) decrease in food intake was observed after icv injections of nesfatin-1 (Figs. 2A and B). Indeed, 0.5 and 1.0 nmol nesfatin-1 inhibited food intake during the both the light and dark periods (Fig. 2A and B). No significant difference was observed between the saline group and the 0.05 nmol nesfatin-1-treated group (Fig. 2A and B). Additionally, the reduction in food intake was apparent at 4 h after injection and continued for 12 h after either ip injection of 3.0 nmol or icv injection of 1.0 nmol nesfatin-1. This effect was observed the following day (data not shown). Also, body weight gain decreased significantly \((P<0.05\) vs saline) after both ip and icv of nesfatin-1 (data not shown).

The effects of smaller doses of nesfatin-1 (0.005 nmol icv and 0.01 nmol ip) were examined, but these doses effected no significant change in food intake \((n = 6\); data not shown).
Fig. 1. Effect of intraperitoneal (ip) administration of nesfatin-1 on food intake in the Japanese quail. Saline (vehicle control), 0.5, 1.0 or 3.0 nmol nesfatin-1 were injected ip at 07:00 h (A) or 19:00 h (B). Each bar and vertical line represents the mean ± SEM (n = 12). *Significantly different from the saline-treated group; P < 0.05.

Fig. 2. Effect of intracerebroventricular (icv) administration of nesfatin-1 on food intake in the Japanese quail. Saline (vehicle control), 0.05, 0.5 or 1.0 nmol nesfatin-1 were injected icv at 07:00 h (A) or 19:00 h (B). Each bar and vertical line represents the mean ± SEM (n = 12). *Significantly different from the saline-treated group; P < 0.05.

3.2. Body Temperature

Fig. 3. Effect of ip administration of nesfatin-1 on body temperature in the Japanese quail. Saline (vehicle control), 0.5, 1.0 or 3.0 nmol of nesfatin-1 were injected ip and saline (vehicle control) at 10:00 h. Body temperature after immediate administration for 60 min (A). Body temperature till 6 h after administration (B). Each bar and vertical line represents the mean ± SEM (n = 6). *Significantly different from the saline-treated group; P < 0.05.

Fig. 4. Effect of icv administration of nesfatin-1 on body temperature in the Japanese quail. Saline (vehicle control), 0.05, 0.5 or 1.0 nmol nesfatin-1 were injected icv at 10:00 h. Body temperature after immediate administration for 60 min (A). Body temperature till 6 h after administration (B). Each bar and vertical line represents the mean ± SEM (n = 6). *Significantly different from the saline-treated group; P < 0.05.
Both ip and icv injections of nesfatin-1 increased body temperature in a dose-dependent manner (Fig. 3, 4). Lower dose of nesfatin-1 injected either peripherally (0.5 nmol) or centrally (0.05 nmol) caused only a transient elevation in body temperature within the first 3 h (Fig. 3, 4). At 1.0 and 3.0 nmol ip, nesfatin-1 significantly (P<0.05) increased body temperature 10, 20, 40, 60 min, and 2, 3, 4, 5, and 6 h after treatment. However, no such change was observed with 0.5 nmol ip after 3 h (Fig. 3A, B). Similarly, 0.5 and 1.0 nmol nesfatin-1 icv produced a significant (P<0.05) increased body temperature 10, 20, 40, 60 min, and 2, 3, 4, 5, and 6 h after treatment (Fig. 4A, B). Although 0.05 nmol nesfatin-1 also caused an increase in body temperature till 3 h post-injection, this increase was not observed after 3 h compared to that seen with saline alone (i.e., control birds).

3.3. Gross Locomotor Activity

Fig. 5. Effect of ip or icv administration of nesfatin-1 on gross locomotor activity in the Japanese quail. Saline (vehicle control), 1.0 or 3.0 nmol of nesfatin-1 were injected ip (A), and saline (vehicle control), 0.5 or 1.0 nmol nesfatin-1 were injected icv (B) at 10:00 h. Each bar and vertical line represents the mean ± SEM (n = 6).

To elucidate whether changes in the locomotion may be responsible for the alterations in the body temperature, gross locomotor activity data were evaluated through the 2 h immediately after the injections of rat nesfatin-1 in the Japanese quail. In the quail, ip administration of nesfatin-1 resulted in non-significant increase in gross locomotor activity in a dose-dependent manner (average of increasing rate, 1.0 nmol; 7.63 %, 3.0 nmol; 23.32%/2 h after injection vs control) (Fig. 5A). Moreover, icv administration of nesfatin-1 resulted in non-significant increase in gross locomotor activity (average of increasing rate, 0.5 nmol; 15.23%, 1.0 nmol; 25.70%/2 h vs control) (Fig. 5B).

4. Discussion

Energy homeostasis results from a balance of food intake and energy expenditure, accomplished by the interaction of peripheral and central nervous signals (Wernecke et al., 2014). This is supported by the fact that many anorexigenic and orexigenic neuropeptides are also involved in the central control of thermogenesis, like corticotropin-releasing hormone, thyrotropin-releasing hormone (TRH) and oxytocin in the PVN, proopiomelanocortin and cocaine- and amphetamine-regulated transcript in the arcuate nucleus (ARC), prolactin-releasing peptide in the NTS and in the caudal ventrolateral medulla and melanin concentrating hormone in the tuberohypothalamic area (Konczol et al., 2010; Kohno et al., 2008; Brailoiu et al., 2007; Fort et al., 2008; Pereira-da-Silva et al., 2003). Nesfatin-1 is co-expressed with all of the above-mentioned neuropeptides suggesting a possible association with thermoregulation that had been recently investigated (Konczol et al., 2012). The recently discovered nesfatin-1 is involved in the central control of food intake. The effects of feeding-related peptides on food intake in birds have thus far been examined by icv injections into 1-day-old chicks (Ando et al., 2000; Furuse et al., 2000; Kawakami et al., 2000). It has yet to be established whether 1-day-old chicks can control the central regulation of various physiological functions, such as feeding, body temperature, and the sleep–wake cycle. In the study presented here, therefore, we used adult Japanese quail, which have an almost constant daily food intake and body temperature. Although there is enough data to support the anorexigenic action of nesfatin-1 in rodents, there is no any data about the effect of nesfatin-1 on feeding behavior and energy expenditure in any of avian species.

Our results revealed that both icv and ip injections of rat nesfatin-1 decreased food intake and increased body temperature in Japanese quail. These results are consistent with those obtained in rats and mice following injections of rat nesfatin-1 (Oh et al., 2006; Shimizu et al., 2009a; Stengel et al., 2009a; Maejima et al., 2009; Su et al., 2010; Yosten and Samson, 2009, 2010; Atsuchi et al., 2010; Goebel et al., 2011). Therefore, these results indicate that nesfatin-1 acts both anorectically and catabolically in avian species as well as in mammals. The icv or ip administration of rat nesfatin-1 resulted in a significant decrease in food intake and body weight gain, concomitant with an increase in body temperature in Japanese quail, suggesting that rat nesfatin-1 acts both anorectically and catabolically in Japanese quail. These effects in Japanese quail indicate that rat nesfatin-1 increases energy expenditure in this bird. The fact that the change in body weight gain was more prominent than the change in food intake during the 12 h following the ip
administration of rat nesfatin-1 (data not shown) may be due to both the anorectic and catabolic actions of rat nesfatin-1.

Neural mechanisms involved in nesfatin-1’s anorexigenic effect encompass the recruitment of several hypothalamic and medulary anorexigenic pathways (Oh et al., 2006; Maejima et al., 2009; Stengel et al., 2009a; Yosten and Samson, 2009). Our results revealed that icv administration of nesfatin-1 decreased food intake and body weight gain in Japanese quail. Nesfatin is expressed in appetite-controlling hypothalamic nuclei, such as the arcuate nucleus, paraventricular nucleus, supraoptic nucleus, lateral hypothalamic area (Oh et al., 2006), suggesting a physiological function of hypothalamic NUCB2/nesfatin-1 as an anorexigenic modulator of food intake. A physiological role of central NUCB2/nesfatin-1 in food intake regulation is further supported by changes in the expression of NUCB2 depending upon the metabolic status of the animals. Fasting for 24 h decreased NUCB2 mRNA expression selectively in the PVN which translated into reduced NUCB2 protein content in this nucleus (Oh et al., 2006). Likewise, fasting decreased NUCB2 mRNA expression in the supraoptic nucleus, which was recently implicated in the regulation of food intake (Johnstone et al., 2006), whereas re-feeding restored basal levels (Kohno et al., 2008). Conversely, re-feeding after a 24 h fast activated NUCB2/nesfatin-1 immunoreactive neurons in the supraoptic nucleus as assessed by Fos immunoreactivity (Stengel et al., 2012). Other researchers reported that nesfatin inhibits food intake by modulating the glucose sensing neurons controlling food intake in the hypothalamus (Chen et al., 2012). Nesfatin reduces body weight gain in rodents (Stengel et al., 2011) and its levels are elevated in obese subjects (Tan et al., 2011). While icv injection of nesfatin decreases food intake in rats, providing antibodies that bind nesfatin stimulates feeding and increases body weight (Oh et al., 2006) suggesting its role as a central anorexigenic factor and modulator of energy balance. The results of Oh et al., (2006) and Shimizu et al., (2009a) indicated that hypothalamic leptin signaling pathway does not exist at the downstream of the pathway by which nesfatin-1 causes the anorexia and therefore nesfatin-1 was identified as anorexigenic signal, acting in a leptin-independent manner. Moreover, central injection of alpha-melanocyte-stimulating hormone (α-MSH) elevated nesfatin/NUCB2 gene expression in the PVN, and the reduction of food intake by nesfatin-1 was abolished by administration of the melanocortin-3/4 receptor antagonist, SHU9119 (Yosten and Samson, 2009). However, icv administration of nesfatin-1 failed to show a significant change in POMC gene expression in the ARC. From those observations, it is supposed that nesfatin-1 signaling pathway might be associated with melanocortin signaling pathway in the hypothalamus.

A number of peptides acting in the brain to influence food intake also regulate digestive functions which may also impact on satiety signaling (Gardiner et al., 2008). It has been recently reported that nesfatin-1 injected icv at a dose effective to suppress food intake, dose-dependently suppresses gastric emptying in fasted rats (Stengel et al., 2009a). However, this effect was independent of the activation of CRF2 signaling (Stengel et al., 2009a) indicating different mechanisms involved in the suppression of gastric transit and food intake. Additional mechanisms of central nesfatin-1’s action may involve a direct inhibitory effect on the activity of neurons in the arcuate nucleus containing the orexigenic peptide, NPY as suggested by the electrophysiological demonstration that nesfatin-1 hyperpolarizes NPY positive arcuate neurons in vitro (Price et al., 2008). These observations suggest that the anorexetic effect of centrally administered nesfatin-1 is mediated by the hypothalamus, especially the PVN. Many reports have investigated the involvement of the hypothalamus in feeding regulation in chickens (Sugahara et al., 1999). It is possible that the avian PVN mediates the anorexetic effect of nesfatin-1, in a manner similar to that in rats since this brain region plays an important role in feeding regulation in avian species. These facts observed in mammals, together with results of our study suggest that central nesfatin-1 participates in feeding regulation in adult avian species, as well as in mammals. Further studies will be beneficial to investigate the exact mechanism of action of central nesfatin-1 in avian species.

How the peripheral nesfatin-1 induce anorexigenic effect in avian species remains to be elucidated. The actions of nesfatin-1 injected peripherally in rodents have been less well explored than its central effects. In broiler chickens, the mRNA for NUCB2 was detected by RT-PCR in many peripheral tissues such as spleen, gizzard, duodenum, breast (pectoralis major) and leg muscles, with spleen expressing the greatest amount and muscles the least (Cisse 2012). These results are almost identical to those gleaned from rats (Oh et al., 2006; Brailoiu et al., 2007; Foo et al., 2008; Kohno et al., 2008; Goebel et al., 2009; Ramanjaneya et al., 2010; Stengel et al., 2010; Zhang et al., 2010).These NUCB2 mRNA expression in the peripheral tissues and organs suggest a regulatory anorexigenic role of peripheral nesfatin-1/NUCB2 in energy homeostasis in either mammals or avian species. Earlier studies demonstrated a reduction of the dark phase food intake upon intraperitoneal nesfatin-1 in ad libitum fed mice (Shimizu et al., 2009a). The inhibitory effect was retained under leptin resistant conditions such as high fat diet-induced obesity or in db/db mice bearing a mutation in the leptin receptor gene (Shimizu et al., 2009a) pointing towards a leptin-independent action as observed upon brain injection (Oh et al., 2006). In addition, recent studies indicated that nesfatin-1 can cross the blood-brain barrier in both directions in a non-saturable manner (Pan et al., 2007; Price et al., 2007). Moreover, it is investigated whether there is a peripheral source of nesfatin-1 and identified NUCB2 mRNA in the rat stomach (Stengel et al., 2009b). Expression levels were 20-fold higher in the gastric oxyntic mucosa than those in the brain and 12-fold compared to other viscera such as the heart (Stengel et al., 2009b) as assessed by microarray analysis and confirmed by RT-qPCR. The broad distributions of nesfatin-1/NUCB2 in the digestive system suggest a possible regulatory role of peripheral...
nesfatin-1/NUCB2 in carbohydrate metabolism, gastrointestinal function and nutrition absorption. These data, along with mapping of medullary Fos positive neurons in response to intraperitoneal injection of nesfatin-1, support the assumption that peripheral nesfatin-1 can influence the activity of vagal afferents and possibly suppress feeding by activating POMC and CART neurons in the NTS (Shimizu et al., 2009a). Therefore, data so far support a peripheral nesfatin-1 signaling pathway that may involve the vagus nerve consistent with the prominent expression of NUCB2/nesfatin-1 in the stomach and the vagal mediation commonly established for other gut satiety peptides (Stengel and Taché, 2009). To understand the site and mechanism of action for the anorectic signal mediated by peripheral nesfatin-1, further studies are required to ascertain the nesfatin-1 sequence, nesfatin-1 binding sites, receptors structure and its tissue distribution in Japanese quail.

Regarding whether nesfatin-1 plays a role in energy expenditure, we investigated the effect of nesfatin-1 on gross locomotor activity and body temperature in free-feeding Japanese quails. Both central and peripheral injection of nesfatin-1 increase body temperature (increase thermogenesis) without affecting gross locomotor activity. These results are in agreement with results reported earlier in rats (Konczol et al., 2012). These results suggest that nesfatin-1 stimulates energy expenditure without affecting locomotor activity. It seems that nesfatin-1 decreased weight gain by decreasing feeding intake and stimulating lipolysis (Oh et al., 2006) the same as reported in case of neuropeptide W (Skrzypski et al., 2012), while has thermogenic effect, that is, maybe chemical thermogenesis due to absence of the effect of nesfatin-1 on locomotor activity.

Regulation of heat loss and thermogenesis are the two main effectors to maintain body temperature (Morrison and Nakamura, 2011). To reveal whether nesfatin-1 may alter the body temperature in Japanese quail, we injected it during the light phase (birds’ active phase) when the body temperature of the diurnal animals is naturally higher. Lower dose of nesfatin-1 injected either peripherally (0.1 nmol) or centrally (0.05 nmol) caused only a transient elevation in body temperature within the first 3 h. In contrast, nesfatin-1 elevated body temperature immediately for 1 h, indicating that there may be a potentiating action on the increased sympathetic activity characteristic the avian species at this time of the day (birds’ active phase). There is a general consensus that nesfatin-1 itself is able to increase sympathetic activity, therefore such interaction is not surprising (Yosten and Samson, 2009; Tanida and Mori, 2011). The mechanism through nesfatin-1 is able to modify the core temperature of the animals is not yet clear. Participation of nesfatin-1/NUCB2 in several autonomic functions is proposed, as nesfatin-1/NUCB2 neurons are present in high number in the hypothalamus and the lower brainstem autonomic centers (Brailoiu et al., 2007; Foo et al., 2008). Many of these neurons were activated by cold, suggesting that nesfatin-1/NUCB2 may mediate thermoregulatory, as well as other responses to cold in rodents as well as avian species.

We do not know why the effect of nesfatin-1 on body temperature is shorter (less than 12 h and not observed on the following day) than its effect on food intake (more than 24 h and observed on the following day). The effect of nesfatin-1 on body temperature in Japanese quail is not so long as reported in rats by Konczol et al., (2012). After either ip or icv administration of nesfatin-1, changes in feeding may produce a change in body temperature. The mechanism of this thermal change is still unclear, but may be related to the changes in metabolism observed after nesfatin-1 administration, because the regulatory center of body temperature is located close to feeding center in same hypothalamus. Two-hour food restriction every day in rats induced anticipatory increase of body temperature (Boulos and Terman, 1980) suggesting that both regulatory mechanisms for temperature and feeding may link each other, but we have no data concerning the shorter effect on body temperature than the effect on food intake in avian species.

In conclusion, nesfatin-1 has a remarkably prolonged effect on food intake and body temperature and these results suggest that nesfatin-1 plays an important role in the regulation of food intake and body temperature, thus confirming its involvement in regulation of energy expenditure in avian species. Although there is an increase in the amount of literatures on the biological actions of administered nesfatin-1 and expression of endogenous NUCB2/nesfatin-1 in tissues in rodents, still nothing is known about the receptor(s) mediating those effects, their characterization, localization in the brain as well as periphery, e.g. in the stomach, pancreas and on vagal afferents, along with their regulation will be key components required for the understanding of the NUCB2/nesfatin-1 signaling system either in mammals or avian species.

References


