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Nesfatin-1: Localization and expression in avian gonads and its modulation by temporal phase relation of neural oscillations in female Japanese quail, *Coturnix coturnix japonica*

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ABSTRACT

In a search for new appetite-controlling signals, the peptide nesfatin-1, expressed in the brain and peripheral tissues of rodents and humans has been reported to regulate feeding by reducing food intake. Recently it has also been reported that nesfatin-1 might be involved in regulating the reproductive axis in fishes and mammals, but its expression and physiological role if any, is not yet known in birds. In the present study, localization and expression of nesfatin-1 was observed in the testis, ovary and shell gland of poultry species Japanese quail, *Coturnix coturnix japonica*.

Our earlier studies have reported that serotonin precursor 5-HTP and dopamine precursor L-DOPA given 8 h apart induces gonadal suppression, when given 12 h apart leads to gonadal stimulation while other relationships were found ineffective. In the present study intense *ir*-nesfatin-1 was observed in the regressed ovary (stromal cells) and shell gland (endometrium) of 8-h Japanese quail while in 12-h quail, weak and scarce immunostaining for nesfatin-1 was detected in the hyperactive ovary and shell gland compared to control.

These findings led us to conclude that, an inverse relationship exists between ovarian activity (both in the control and simulated conditions) and nesfatin-1 expression. Present avian study, first of its kind, also suggests the role of nesfatin-1 in reproductive regulation possibly via appetite control and energy balance in female Japanese quail and needs to be investigated further in relation to food intake.

1. Introduction

Nesfatin-1, identified as a novel molecule, serves in appetite regulation as well as energy metabolism (Oh-I et al., 2006). In rat, it is expressed in appetite-controlling hypothalamic nuclei, such as the arcuate nucleus, paraventricular nucleus, supraoptic nucleus, the lateral hypothalamic area (Oh-I et al., 2006), as well as in the solitary tract (NTS) and dorsal nucleus of vagus (Brailoiu et al., 2007). Several peripheral tissues express nesfatin-1, including adipose tissue (Oh-I et al., 2006; Hausman et al., 2012), serum, gastric mucosa (Stengel et al., 2009) and pancreatic beta cells (Gonzalez et al., 2010).

Reproduction typically puts havoc energy demands in the pregnant or laying animal. In mammals, nutrition and the food availability are the two most important factors that influence reproduction (Bronson, 1989). Inadequate nutrition can delay sexual maturation, inhibit the ovulatory cycle and reproductive performance. Specifically, low energy level hinders down the secretions of hypothalamic-pituitary-gonadal axis (Foster and Nagatani, 1999). As reproduction imposes additional nutrient needs during pregnancy or egg production, females store fat prior to the onset of reproduction in order to satisfy the additional energy demand that is not met by daily food intake. However, a chronic imbalance of high energy intake exceeding energy expenditure causes the deposition of excess adipose tissue and subsequently hinders fertility (Ahima, 2006; Friedman and Halaas, 1998). Thus both chronic under-nutrition and over-nutrition are detrimental to the health and reproductive capability. The central nervous system regulates body energy homeostasis through a variety of hormones that have complex orexigenic and anorexigenic effects (Berthoud and Morrison, 2008; Crowley, 2008).

The search for hormones that can link energy intake and body stores with reproductive function is ongoing, and several hormones that are involved in both nutrition and reproduction are coming up simultaneously to complete the linking of energy metabolism and reproduction. In this context, nesfatin-1, secreted by

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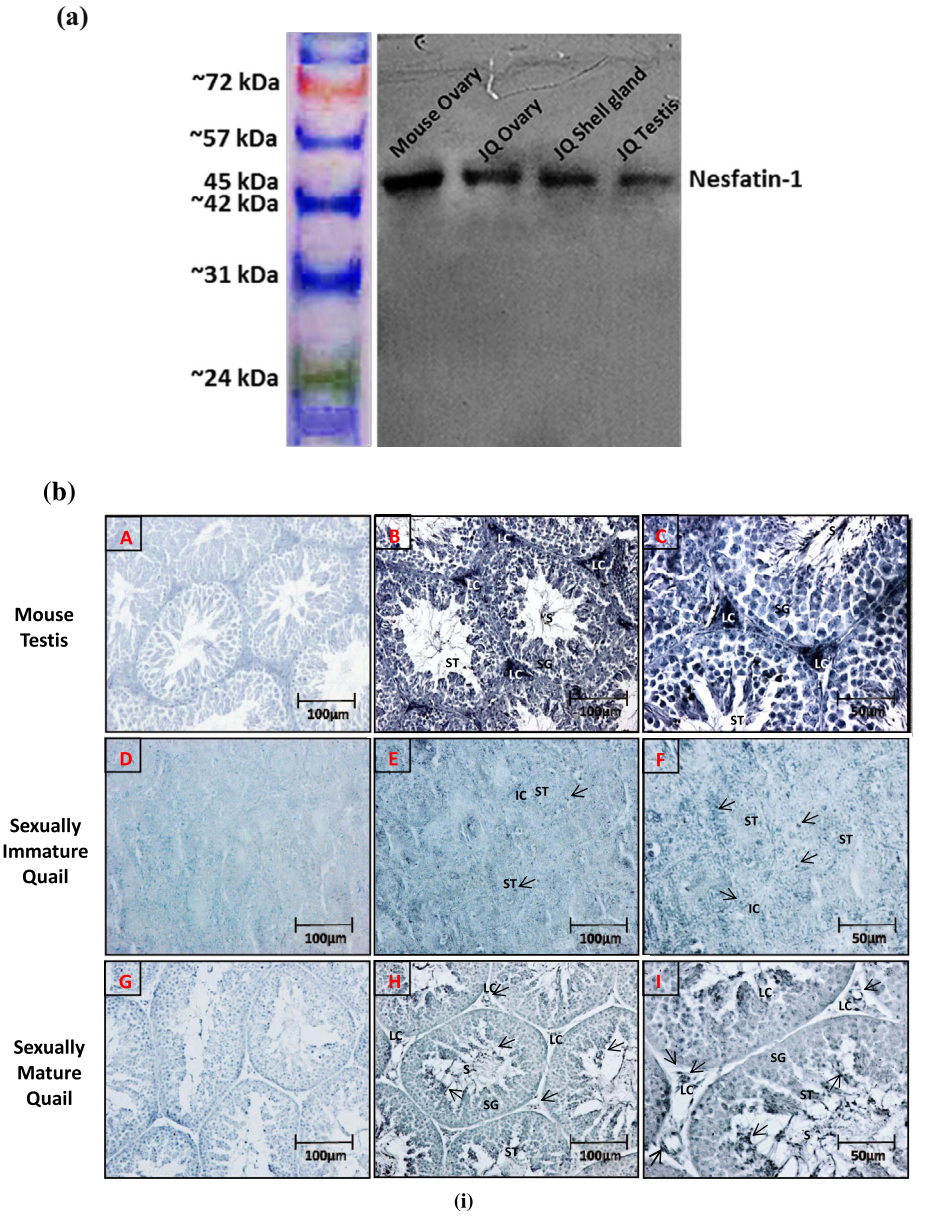

Fig. 1. (a) Validation of the polyclonal nesfatin-1 protein in the ovary, testis and shell gland of Japanese quail through western blot along with the mouse ovary as positive control. Ovary, testis and shell gland tissue samples were pooled from five animals and the experiment was repeated thrice. (b) Immunohistochemistry of nesfatin-1 in (i) testis: upper panel: A–C: sexually mature mouse testis, A-negative control; middle panel: D–F: sexually immature quail testis, D-negative control; lower panel: G–I: sexually mature quail testis, G-negative control; *Note, intense immunostaining for nesfatin-1 in the leydig cells of mouse testis. Sexually immature quail testis shows weak immunostaining while sexually mature quail testis revealed moderate immunostaining in leydig cells, spermatids and sperms.* (ii) Ovary: upper panel: A–C: sexually mature mouse ovary, A-negative control; lower panel: D–F: sexually mature quail ovary, D-negative control. *Quail ovary shows positive immunostaining for nesfatin-1 in the stromal cells only while mouse ovary shows intense immunostaining in both stromal as well as granulosa cells,* and (iii) uterus/shell gland of mouse and bird: upper panel: A–C: sexually mature mouse uterus, A-negative control; lower panel: D–F: sexually mature quail shell gland, D-negative control. *As in the uterus of mouse, shell gland of quail also shows positive immunostaining for nesfatin-1 in the endometrial glandular region.* (ST-seminiferous tubules, SG-spermatogonia, LC-leydig cells, IC-interstitial cells, S-sperm, O-oocyte, GC-granulosa cells, TC-theca cells, SC-stromal cells, M-myometrium, E-endometrium).

adipose tissue, may be the modulator of reproductive status, due to its potential roles in obesity (Ramanjaneya et al., 2010), inflammation (Ozsavci and Ersahin, 2011) and type II diabetes mellitus (Li et al., 2010).

Main physiological function of nesfatin-1 is the regulation of feeding by reducing food intake (Oh-I et al., 2006; Brailoiu et al., 2007; Maejima et al., 2009) possibly via modulating the glucose sensing neurons in the hypothalamus (Chen et al., 2012). It is reported to reduce body weight gain in rodents (Stengel et al., 2011) and their levels are found to be elevated in obese murine and human adipose tissue (Tan et al., 2011). Intracerebroventricular (icv) injection of nesfatin-1 decreased food intake and provided antibodies that bind nesfatin-1 stimulated feeding in rats along with increased body weight (Oh-I et al., 2006). Oh-I et al. (2006) suggested that as an anorexigenic factor, nesfatin-1 may modulate energy balance. On the other hand, it has been also reported that nesfatin-1 might be involved in regulating the reproductive aspects of rodents/mammals (García-Galiano et al., 2012; Kim and Yang, 2012). In this context, short term fasting and sustained under-nutrition has been correlated with reduced NUCB2 mRNA and peptides expression in the hypothalamus of pubertal female rats (García-Galiano et al., 2010a). These workers also reported