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The expression and distribution of a leptin receptor in the central nervous system, digestive organs, and gonads of the giant freshwater prawn, *Macrobrachium rosenbergii*

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ABSTRACT

In the present study, the presence and distribution of leptin receptor (LEP-R) in central nervous system, digestive organs, gonads of giant freshwater prawn, *Macrobrachium rosenbergii*, were investigated with Western blot and immunohistochemistry. By Western blot a LEP-R with a molecular weight (MW) of 100 kDa was detected in the brain, thoracic ganglia, abdominal ganglia, hepatopancreas, all parts of the gastrointestinal tract, ovaries, and testes. In hepatopancreas and foregut, another intense positive band was detected at molecular weight of 30 kDa, which could be an isotype of LEP-R. By immunohistochemistry, LEP-R-ir was detected in the neurons, and neuropils in the brain, thoracic ganglia, and abdominal ganglia. In the gastrointestinal tract, there was intense LEP-R-ir in the apical part of the epithelial cells of the foregut, midgut, and hindgut. In addition, LEP-R-ir was found in the Restzellen(R)cells and Fibrillenzellen(F) cells in the hepatopancreas. In the ovary, LEP-R-ir was detected in early stage of oocytes and mature oocytes. Intense LEP-R-ir was observed in spermatogonia and spermatocytes of the small and orange claw male prawns. In addition, LEP-R was seen in the high epithelium of spermatic ducts from all male morphotypes. In summary, the detection of the LEP-R-ir suggests the existence of a LEP-R in several organs of *M. rosenbergii*. Through binding with leptin peptide, LEP-R may be an important signaling molecule that has critical functions in modulating and controlling food intake, energy expenditure, and reproduction in this prawn.

1. Introduction

Leptin receptor (LEP-R), also known as cluster of differentiation 295 (CD295), is a single transmembrane domain receptor of the cytokine receptor family with a molecular weight of approximately 130 kDa. In human, it is encoded by the LEP-R gene (Tartaglia et al., 1995). LEP-R functions as a receptor for the adipocyte derived-hormone, leptin, which regulates fat storage, food intake and energy expenditure by acting through the LEP-R in the hypothalamus (Frühbeck et al., 1998; Ahima and Flier, 2000; Caprio et al., 2001). Binding of leptin to this receptor can induce its conformational changes in the intracellular domain, resulting in activation of JAK-STAT pathway, intracellular factor-regulated kinases, as well as MAPK (Houseknecht and Portocarrero, 1998). Up to now, six different isoforms of LEP-R have been identified: LEP-Ra, b, c, d, e, and f, based on the alternative splicing of the LEP-R mRNA (Lee et al., 1996; Halaas and Friedman, 1997; Ahima and Flier, 2000; Caprio et al., 2001; Dardeno et al., 2010). LEP-R isoforms have either a common extracellular domain or lack the transmembrane domain (Caprio et al., 2001). The longest isoform (LEP-Rb) with a molecular weight of 125 kDa, contains box1/box2 JAK/STAT binding sites which is capable of signal transduction (Burguera et al., 2000). In contrast, the short isoforms (LEP-Ra, c, d, e, and f, 100 kDa) contain only box1 JAK binding sites for JAK and Mitogen-Activated Protein Kinases (MAPK) activation (Schaab and Kratzsch, 2015). This isoform acts as a leptin binding protein, which plays a role in leptin transport across the blood-brain barrier (Smith and Waddel,

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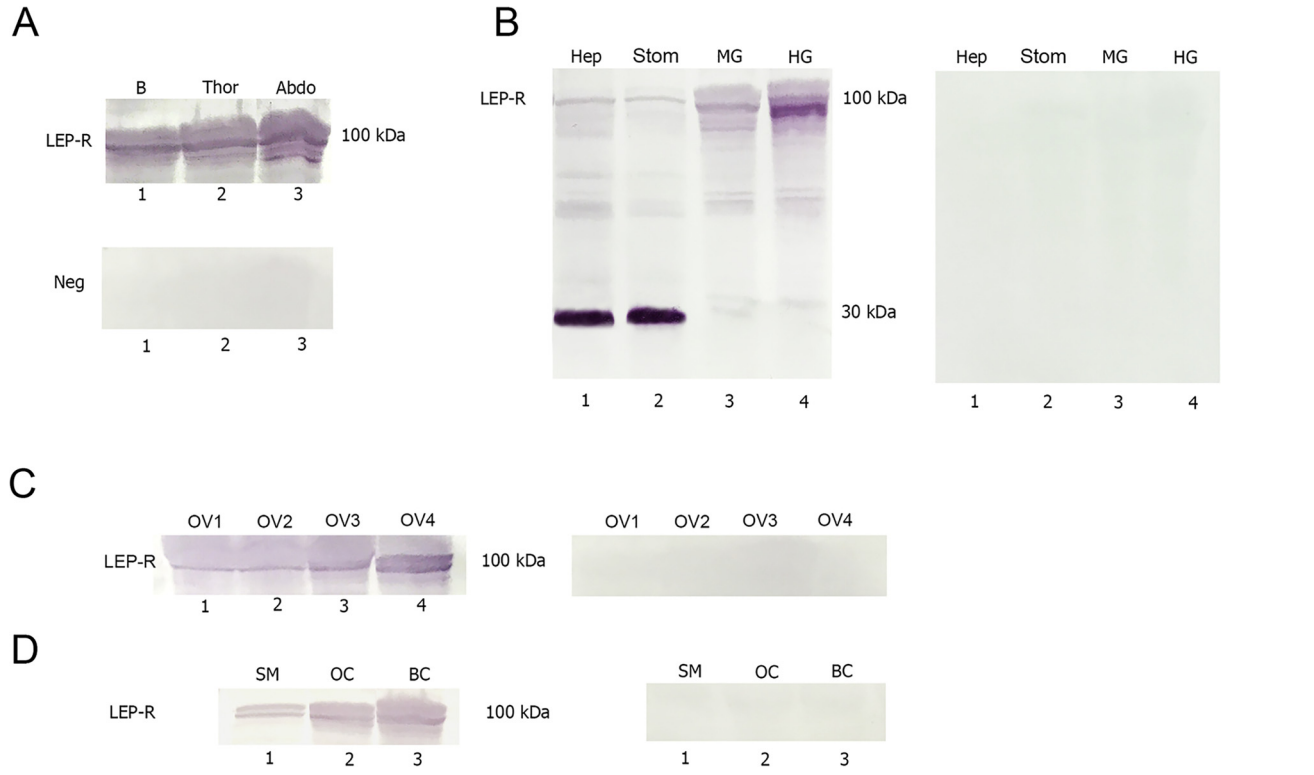

Fig. 1. Western blot analysis of leptin receptor in the CNS, gastrointestinal tract, and gonad of *M. rosenbergii.* The proteins were run on 10% gel under reducing condition. The positive LEP-R band mainly corresponds to a 100 kDa (A) Upper panel lanes 1–3 show positive LEP-R band at 100 kDa in the brain, thoracic ganglia, and abdominal ganglia respectively. Lower panel lanes 1-4 show no positive signal in the negative controls. (B) LEP-R positive bands in the gastrointestinal tract including hepatopancreas, stomach (foregut), midgut, and hindgut, respectively. Left panel lanes 1 and 2 show an intense band at 30 kDa in hepatopancreas and stomach (foregut) and 100 kDa band in midgut and hindgut, whereas in right panel lanes 1–4 show no positive signal in the negative controls. (C) Left panel lanes 1–4 show positive LEP-R signal in the homogenates of oocyte stage 1–4. Lane 4 shows intense immunoreactive LEP-R band in oocyte stage 4. Right panel lanes 1–4 show no positive signal in the negative controls. (D) Left panel lanes 1–3 show positive LEP-R immunoreactive band in testes from all three male morphotypes. Right panel lanes 1–3 show no positive signal in the negative controls. *LEP-R* leptin receptor, *B* Brain, *Th* Thoracic ganglia, *Ab* Abdominal ganglia, *Hep* Hepatopancreas, *Stom* Stomach, *MG* Midgut, *HG* Hindgut, *Oc1* early previtellogenic oocytes, *Oc2*: late previtellogenic oocytes, *Oc3* early vitellogenic oocyte, *Oc4* mature oocytes. *SM* small male, *OC* orange claw male, *BC* blue claw male.

2002; Schaab and Kratzsch, 2015).

The leptin receptor overlapping transcript (LEP-ROT) or endospanin, a 131 amino acid protein, is co-transcribed with LEP-R, without similarity to the LEP-R (Bailleul et al., 1997). The LEP-ROT expression have been investigated in some fish species, i.e., *Takifugu rubripes, Oryzias latipes,* and *Pelteobagrus fulvidraco* (Kurokawa et al., 2008; Wong et al., 2007; Kurokawa and Murashita, 2009; Gong et al., 2013). For invertebrates, the Ensembl Metazoa annotation has identified 48 genes which contain LEP-ROT homologous proteins (Kersey et al., 2016). LEP-ROT sequences have highly conserved regions among invertebrate species (ie., *Drosophilla melanogaster, Saccharomyces cerevisiae*.) (Londraville et al., 2017). However, there was no report on function of this protein in invertebrates, including decapod crustaceans. As well, the information on LEP-R in decapod crustaceans, including *M. rosenbergii* was very scarce. In Chinese mitten crab, *Eriocheir sinensis*, LEP-R gene was first identified from the hepatopancreas EST library, and it shares high sequence identity to LEP-R gene from vertebrate species, including *Xenopus levis*, *Nasonia vitripennis* and *Macaca mulatta*, suggesting that LEP-R may be present and play an important role in the regulation of reproductive maturity in this species (Jiang et al., 2009, 2010). Moreover, LEP-R gene expression has been detected in various tissues, including intestine, thoracic ganglia, hepatopancreas and gonads of *E. sinensis* (Jiang et al., 2010). As well, the LEP-R genes expression has also been detected in the same tissues of *Litopeneas vannamei*, indicating that LEP-R may be involved in the nutritional regulation of metabolism and reproduction of decapod crustaceans (Deng et al., 2017). From these studies, it was hypothesized that the LEP-R gene found in these species is LEP-ROT which may have function similar to the LEP-R in vertebrates (Jiang et al., 2010; Deng et al., 2017). In *M. rosenbergii*, the analyses of neuropeptides and their receptor genes in the eyestalk, central nervous system, and ovary transcriptomes, indicated the existence of LEP-R in this prawn (Suwansaard et al., 2015). However, up to now, there was no direct evidence on the presence and distribution of LEP-R in tissues of *M. rosenbergii*. Therefore, the aim of this study was to prove the existence of LEP-R in the tissues of this prawn using Western blot and immunohistochemical detection. The data gained could contribute insight and further stimulus for studying the function of LEP-R in controlling the feeding and reproduction in this prawn.

2. Materials and methods

2.1. Experimental animals and tissue collections

The mature male (N = 50) and female (N = 50) *M. rosenbergii* were purchased from a central market in Ayutthaya province, Thailand. The animals were held in plastic tanks and acclimatized for one week. The prawns were anesthetized on ice for 3 min before tissue collection. For Western blotting, several tissues including brain, thoracic ganglia, abdominal ganglia, gastrointestinal tract [including stomach (foregut), midgut and hind gut], testes and ovaries were collected and frozen at -80°C until used. For immunohistochemical detection, similar tissues were collected, fixed in 4% paraformaldehyde in 0.1 M phosphate buffer saline (PBS) (containing $NaH_2PO_4.2H_2O$ 5.14 g, $Na_2HPO_4.H_2O$