การโคลนยีนตัวรับฮอร์โมนโพรแลคตินในปลาช่อนและศึกษาการแสดงออกในภาวะไฮเปอร์ออสโมติก

Cloning of Snakehead Prolactin Receptor Mrna, Expression during Hyperosmotic Condition

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บทคัดย่อ

เพื่อศึกษาบทบาทสำคัญของฮอร์โมนโพรแลคตินในปลาช่อน (*Channa striata*) ผู้วิจัยได้โคลนยีนตัวรับฮอร์โมนโพรแลคตินจากเหงือกและทำการศึกษาลำดับยีนดังกล่าว ตัวรับฮอร์โมนโพรแลคตินในปลาช่อนมีขนาด 2,412 คู่เบส และมีลำดับกรดอะมิโน 618 ตัว ซึ่งประกอบด้วยบริเวณภายนอกเซลล์โดยประกอบด้วยตำแหน่งซิสทีอีน 4 ตำแหน่ง และลำดับ WSXWS ตำแหน่งที่ฝั่งบนเยื่อหุ้มเซลล์ และตำแหน่งของยีนตัวรับที่อยู่ด้านไซโตพลาสซึมซึ่งประกอบด้วยลำดับกรดอะมิโน box 1 และ box 2 ยีนตัวรับฮอร์โมนโพรแลคตินมีการแสดงออกในปริมาณสูงที่เหงือกและไต แต่ระดับการแสดงออกของในเหงือกระหว่างกลุ่มที่เลี้ยงในน้ำจืดและน้ำเค็ม พบว่าระดับการแสดงออกไม่แตกต่างกัน ผลดังกล่าวชี้ให้เห็นว่าฮอร์โมนโพรแลคตินอาจไม่เกี่ยวข้องกับการไปมีผลควบคุมอวัยวะที่ทำหน้าที่ควบคุมสมดุลน้ำและแร่ธาตุในปลาช่อน

ABSTRACT

To investigate the physiological significance of prolactin (PRL) in a snakehead (*Channa striata*), we cloned and characterized cDNAs encoding its PRL receptor (PRLR) from the gills. The snakehead PRLR cDNA consisted of 2,412 bps and encoded a protein of 618 aa, contained an extracellular domain with 4 cysteins and a WSXWS motif, a single transmembrane domain and intracellular domain with box 1 and box 2 regions. Snakehead PRLR mRNA showed highest expression in gills and kidneys, furthermore, the expression levels in gills were not significant difference between fresh water and salt water. The results suggest that PRL might not be involved in the osmoregulatory organs in snakehead.

คำสำคัญ: ฮอร์โมนโพรแลคติน, ปลาช่อน, ยีน *shPRLR*

Keywords: prolactin, snakehead, *shPRLR*

INTRODUCTION

Prolactin (PRL) exhibits the greatest diversity of action of all pituitary hormones (Nicoll and Bern, 1972). In teleost species, it plays an important role in adaptation to fresh water in freshwater and euryhaline teleosts (Clake and Bern, 1980). Many studies were found the role of PRL in teleost species such as killifish (*Fundulus sp.*), stickleback (*Gillichthyes mirabilis*), molly (*Poecilia latipinna*), and tilapia (*Oreochromis mossambicus and O. nioticus*) (Horseman, 1987). PRL acts through binding to a specific cell surface PRLR, which has been cloned in several vertebrate species (Boutin *et al*., 1989; Tanaka *et al*., 1992; Huang and Brown, 2000). These receptors belong to the class I cytokine receptor superfamily. In teleosts, PRLR cDNA have been cloned in tilapia (*O. mossambicus*) (Sandra, *et al*., 1995), gold fish (*Carassius auratus*) (Tse *et al*., 2000), and rainbow trout (*Oncorhynchus mykiss*) (Le Rouzic *et al*., 2001). The PRLR mRNAs were found heavily expression in osmoregulatory organs such as gill, kidney and intestine.

Since the natural water sources in Northeast Thailand have the problem in salinity, in the present study, we cloned cDNA encoding snakehead PRLR from gills, examined the expression in tissues and determined the expression levels in gills between the fish maintained in FW and those transferred to 10 ‰ salinity.

MATERIALS AND METHODS

1. Animals and tissues

Snakeheads (*C. striata*) were obtained from local hatchery and were acclimated in dechlorinated-freshwater for 2 weeks by feeding with *Artemia salina* twice a day.

2. cDNA cloning of snakehead PRLR

Total RNA was extracted from the gill by using TRIzol reagent (Invitrogen) and cDNA were synthesized according to manufacturer's instructions. PCR was carried out using degenerate primers (forward: 5'- CGNTCNCCNGARAARGARACNTT-3', reverse: 5'-CCNCCNGTNCCNGGN CCNAARAT-3'). The PCR products were cloned into pGEM-T easy vectors (Promega) and sequenced. The 5' and 3' regions of snakehead PRLR cDNA were cloned by using the 5'- and 3'- rapid amplification of cDNA ends (RACE) method. For 5'-RACE, total RNA from the gills was reverse-transcribed using SMART RACE cDNA Amplification kit (Clontech).

3. PRLR mRNA levels in hyperosmotic condition

Two groups of snakeheads were exposed to fresh water and 10 ‰ NaCl dissolved-water for 7 days (n =10 each). For quantitative analysis of PRLR expression, the gill was dissected out for RNA extraction. The mRNA level of PRLR was determined using real time-PCR (iCycler iQ; Bio-Rad) was performed with SsoFast EvaGreen Supermix (Bio-Rad) according to the manufacturers' instructions. Relative expression was calculated from the threshold cycle (CT) values and twofold change was considered significant.

4. Statistics

Significant mean effects of treatment ($P < 0.05$) were followed up by Student's t test using a computer program, GraphPad Prism 5.0 (San Diego, CA, USA).

RESULTS AND DISCUSSION

The snakehead PRLR cDNA consisted of 2,412 bps, containing an ORF encoding 618 aa (DDBJ/EMBL/GenBank DNA databases with the Accession No. K051404).

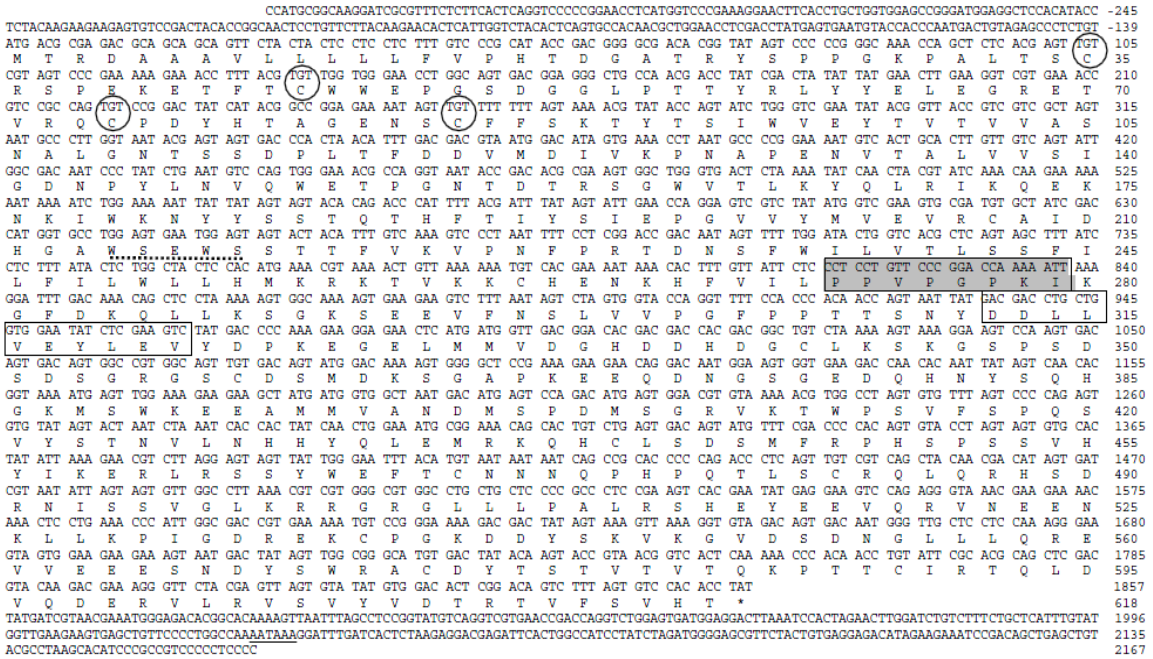

Figure 1 Nucleotide and deduced amino acid sequence of the snakehead PRLR *(shPRLR)*. Nucleotide (upper sequence) and aa (lower sequence) are numbered on the right. Conserved cysteine residues (circled), WS motif (underlined with a dot line), Box 1 (boxed in shaded) and Box 2 (boxed), the stop codon (marked with an asterisk), and underline aataaa is the poly-A adding sequence

The 5'-UTR and 3'-UTR consisted of 245 and 313 nucleotides, respectively. The deduced amino acid sequence was composed of an extracellular domain with two pairs of cysteines and a WSXWS motif, a single transmembrane domain and an intracellular domain with highly conserved box 1 and box 2 regions (Figure 1). In vertebrate species, PRLRs conserve structural features common to the members of the class I cytokine receptor superfamily (Bole-Feysot *et al*., 1998).

Figure 2 shows the expression of PRLR was observed in the liver, hepatopancrease, kidney gill, gastrointestinal tract, urinary bladder and brain but predominantly expressed in the kidney and the gill of snakehead. It should be noted that high expression of those PRLR in the tissues are closely associated with osmoregulation, suggesting the involvement of PRL in regulation of hydromineral balance in these organs.

Since PRL has been shown to have the effects on gill and our RT-PCR analysis also shown highly expressed of PRLR in the snakehead's gill, we had determined the expression levels of PRLR in gills compared between hypoosmotic (FW) and hyperosmotic condition (SW) to blood. The present result reveals there was no significant different between 2 conditions (Figure 3). This result could exhibit that PRL does not involve osmoregulation control in stenohaline snakehead fish.

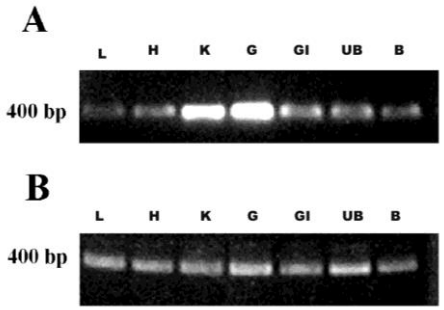

Figure 2 Expression of *shPRLR* in various tissues of snakehead fish using RT-PCR (A). Expression of β-actin, an internal control (B). L, liver; H, hepatopancrease; K, kidney; G, gill; GI, gastrointestinal tract; UB, urinary bladder and B, brain.

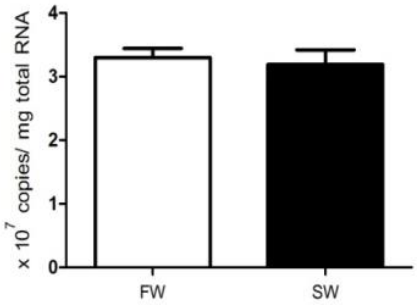

Figure 3 Expression levels of PRLR mRNA in the gills of snakehead maintained in fresh water (FW) and those transferred to salt water (SW). Data are expressed as means±SEM.

CONCLUSION

The cloned cDNAs encoding snakehead PRLR (shPRLR) from the gill was determined its nucleotide and deduced amino acid sequence. The conserve structural features of shPRLR show a common to the member of the class I cytokine receptor superfamily, such as two pairs of cysteine residues, a WSXWS motif, a single transmembrane domain with the box 1 and box 2. The *shPRLR* mRNA showed highest expression in gill and kidney. Furthermore, *shPRLR* levels in gills of snakeheads were not significant difference between fresh water and 10% NaCl-dissolved water.

ACKNOWLEDGEMENTS

This work was supported by funds from TRF-CHE research grant for New Scholar (project No. MRG5580008 to L. Nakkrasae), Faculty of Science, Khon Kaen University to L. Nakkrasae, and Graduated School, Khon Kaen University to K. Wisetdee

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