軟骨魚類におけるカルシトニンの生理作用に関する研究

軟骨魚類におけるカルシトニンの生理作用に関する研究

東野将也，関口俊男

〒927-0553 鳳珠郡能登町小木 金沢大学 環日本海域環境研究センター 臨海実験施設
Masaya HIGASHINO, Toshio SEKIGUCHI: Study on the physiological function of calcitonin in cartilaginous fishes

Introduction

Cartilaginous fish possesses a skeletal system composed of cartilage. Most of the cartilaginous fish inhabits seawater. Cartilaginous fish maintain a steady serum Ca^{2+} level even though exposed to high Ca^{2+} conditions. However, hormonal regulation of Ca^{2+} homeostasis is yet to be fully clarified in cartilaginous fish. Calcitonin (CT) is a hypocalcemic hormone synthesized in the thyroid C-cells and ultimobranchial glands in mammals and non-mammals, respectively. In mammals, hypercalcemic condition induces the release of CT from the thyroid C-cells. CT suppresses the osteoclastic activity in the bone and reduces blood Ca^{2+} levels. The hypocalcemic function of CT is conserved from teleost to mammals. Although cartilaginous fishes also synthesize CT in the ultimobranchial gland, they have no endoskeleton regulated by the coordination of the osteoblast and osteoclast. Therefore, the target cell and physiological function of CT remain unclear in cartilaginous fishes. The present study aims to evaluate the physiological role of CT through the establishment of assay systems for the plasma Ca^{2+} and CT concentrations in the red stingray, *Hemitrygon akajei*.

Results and discussion

1. *Establishment of an assay system for evaluation of Ca^{2+} homeostasis in red stingray*

To evaluate the mechanism to regulate blood Ca^{2+} concentration, I attempted to establish the *in vivo* assay system in the red stingray. Oral administration of high Ca^{2+} consome solution increased plasma calcium levels. The peak of plasma Ca^{2+} concentration was detected at 6 hours after treatment. Thereafter, plasma Ca^{2+} concentration slowly declined. Treatment with the control consome solution did not increase the plasma Ca^{2+} level. These results confirmed that the oral administration of high Ca^{2+} consome solution increases plasma Ca^{2+} levels. Therefore, I established an *in vivo* assay system to evaluate gene function related to Ca2+ homeostasis in red stingrays.

2. *Tissue distribution analysis of genes associated with* Ca^{2+} homeostasis *by using RT-PCR*

To obtain a clue of the molecular mechanism of plasma Ca^{2+} regulation in cartilaginous fish, tissue distribution analysis of genes related to Ca^{2+} homeostasis was performed by RT-PCR. Transient receptor potential vanilloid 5 (TRPV5), transient receptor potential vanilloid 6 (TRPV6), and calcium-sensing receptor (CaSR)

were selected. TRPV5 functions as a Ca^{2+} channel on the apical region of kidney epithelial cells and is involved in Ca^{2+} reabsorption. RT-PCR analysis indicated that TRPV5 mRNA was expressed in the gill, stomach, and kidney, suggesting that TRPV5 is associated with Ca^{2+} homeostasis in stingrays. TRPV5 mRNA was also detected in the brain, implying that TRPV5 might possess a neural function. Mammalian TRPV6 is a calcium channel involved in Ca^{2+} absorption in the intestine. The expression patterns of red stingray TRPV6 mRNA were similar to that of mammalian TRPV6, suggesting that red stingray TRPV6 might possess similar functions with mammalian TRPV6. In mammals, CaSR belongs to the metabotropic G-protein coupled receptor family and senses the extracellular Ca^{2+}. It is reported that mammalian CaSR is involved in the Ca^{2+} sensing and release of parathyroid hormone in the parathyroid gland. The red stingray CaSR mRNA was detected in the various organs. These tissue distributions of CaSR mRNA are similar to that of mammals, suggesting that CaSR also acts as a Ca^{2+} sensor in red stingrays.

3. Expression analysis of red stingray CT.

To evaluate the hormone function related to Ca^{2+} homeostasis, I focused on CT. A nucleotide sequence of red stingray CT was determined by mRNA-sequence analysis of the ultimobranchial gland. Red stingray CT comprised 32 amino acid sequences. The comparison of the amino acid sequence of red stingray CT and other vertebrate CTs revealed that vertebrate CT share the common motif, including the N-terminal circular region and the C-terminal Pro amide. In addition, tissue distribution analysis demonstrated that red stingray CT mRNA is exclusively detected in the ultimobranchial gland. Next, to evaluate the concentration of CT peptide, I attempted to establish the Enzyme-Linked Immunosorbent Assay (ELISA) using anti-stingray CT antiserum. Experimental conditions, including antiserum concentration and standard red stingray CT peptide concentration were determined. Further, CT levels of the ultimobranchial gland were detected using this assay system, suggesting that ELISA can be used to measure CT levels. Plasma CT levels were also detectable.

Conclusion

In the present study, I established the *in vivo* assay system to evaluate the physiological function of maintaining a steady blood Ca^{2+} level. Moreover, tissue distribution of channel and transporter genes involved in the blood Ca^{2+} homeostasis was clarified. Finally, I focused on CT as a calcemic hormone. The full-length nucleic acid sequence of red stingray CT was determined from transcripts of the ultimobranchial gland. Tissue distribution analysis by RT-PCR revealed that red stingray CT mRNA was predominantly expressed in the ultimobranchial gland. Furthermore, I established ELISA assay system to measure the CT peptide concentration.

In the future, measurement of plasma CT level and expression of Ca^{2+} homeostasis-related gene will be performed using the *in vivo* assay system that administrates high Ca^{2+} consome. These studies will contribute to clarifying the mechanism of Ca^{2+} homeostasis in cartilaginous fish.

本研究は，金沢大学大学院自然科学研究科自然システム学専攻　東野将也氏の学位論文の一環として行われた。