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Preliminary study of the key hormones regulating reproduction in the bluefin tuna (BFT): The brain gonadotropin-releasing hormones (GnRHs) and the pituitary gonadotropins, FSH and LH

H. Rosenfeld*, U. Klenke, H. Gordin* and Y. Zohar****
*National Center for Mariculture, PO Box 1212, Eilat 88112, Israel
**Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Columbus Center, 701 East Pratt, St. Baltimore, MD 21202, USA

SUMMARY – As a first step towards understanding the spatio-temporal profiles of the key reproductive hormones in bluefin tuna (BFT; *Thunnus thynnus*), the present study was aimed to assess the available immunological tools to detect the gonadotropin (GtH) LH and the gonadotropin-releasing hormone (GnRH) isoforms in tuna species. Our preliminary studies demonstrated that the heterologous ELISA, developed to measure striped bass LH (stbLH), successfully determined LH levels in pituitaries and plasma of bonito (*Sarda sarda*) and yellowfin tuna (*T. albacares*). All displacement curves were parallel to the standard curve, allowing the use of the system for determining the cognate hormone in tuna species. In addition, our specific ELISAs for GnRH isoforms successfully detected the salmon GnRH (sGnRH) and the sea bream GnRH (sbGnRH) in pituitary extracts of bonito and yellowfin tuna. Unfortunately, we could not detect the chicken GnRH-II (cGnRH-II) isoform. However, as all fish species studied to date exhibit cGnRH-II, we assume that our failure to detect this particular isoform in tuna is technical, and that tuna species, like other perciform fish, possess the three GnRH isoforms: sbGnRH, sGnRH and cGnRH-II.

Key words: *Sarda sarda*, *Thunnus albacares, Thunnus thynnus,* ELISA, GnRH isoforms, LH.

RESUME – *"Etude préliminaire des hormones-clés qui régulent la reproduction chez le thon rouge : Neurohormones GnRH (brain gonadotropin-releasing hormones) et gonadotrophines pituitaires, FSH et LH". Comme premier pas vers la compréhension des profils spatio-temporels des hormones reproductives déterminantes chez le thon rouge (*Thunnus thynnus*), la présente étude vise à évaluer les outils immunologiques disponibles pour détecter la LH gonadotrope (GtH) et les isoformes de GnRH (gonadotropin-releasing hormone) chez les thonidés. Nos études préliminaires ont démontré que l'ELISA hétérologue, mis au point pour mesurer la LH du bar rayé (stbLH), a déterminé avec succès les niveaux de LH dans les glandes pituitaires et le plasma de bonite (*Sarda sarda*) et d'albacore (*T. albacares*). Toutes les courbes de déplacement ont été parallèles à la courbe standard, permettant d'utiliser le système pour déterminer l'hormone apparentée chez les thonidés. En outre, nos ELISAs spécifiques pour les isoformes de GnRH ont détecté avec succès le GnRH de saumon (sGnRH) et le GnRH de daurade (sbGnRH) dans des extraits pituitaires de bonite et d'albacore. Malheureusement, nous n'avons pas pu détecter l'isoforme GnRH-II de poulet (cGnRH-II). Cependant, vu que toutes les espèces de poissons étudiées jusqu'à cette date présentent cette cGnRH-II, nous supposons que c'est pour des raisons d'ordre technique que nous n'avons pas pu détecter cette isoforme particulière chez le thon, et que les thonidés, comme les autres perciformes, possèdent les trois isoformes de GnRH : sbGnRH, sGnRH et cGnRH-II.*

Mots-clés : Sarda sarda, Thunnus albacares, Thunnus thynnus*, ELISA, isoformes de GnRH, LH.*

Introduction

The Bluefin tuna (BFT) is a highly evolved perciform species with exceedingly commercial value; nevertheless, during the last 30 years it has become a threatened species due to over fishing. In order to resolve the contradiction between environmental and fisheries needs, BFT was chosen as a prime candidate for domestication. Yet, it is clear by now that the development of current methods to induce successful reproduction in captive fish has been feasible only through a basic understanding of the species' reproductive endocrinology. Characterizing the pivotal regulators of reproduction, i.e. gonadotropin-releasing hormones (GnRHs) and gonadotropins (GtHs), and monitoring their circulating levels, were found to be indispensable for developing GnRH-based spawning induction

therapies. These therapies were already found to be most effective for spawning-induction in many marine finfish of commercial importance (reviewed by Zohar, 1996; Zohar and Mylonas, 2001).

In nature, the Atlantic BFT are managed as separated eastern and western stocks, which parallel their spawning grounds, Mediterranean and Gulf of Mexico, respectively (Block *et al*., 2001). Recently, it was demonstrated that the eastern stock of BFT reaches its sexual maturity at the age of 3 years. Females of the concurrent stock are characterized by asynchronous gonadal development and daily spawning cycles (Abascal *et al*., this issue), similar to the reproductive strategy of the sea bream (Zohar and Gordin, 1979; Gothilf *et al*., 1997).

Two distinct GtHs, FSH and LH, were isolated from the pituitaries of tuna species (Koide *et al*., 1993; Okada *et al*., 1994; Garcia-Hernandez *et al*., 1997), which share the highest sequence identity with the respective hormones of other perciform fish, i.e. striped bass, tilapia and sea bream. In addition, the pituitaries of immature BFT were found to express both gonadotropins, each in a distinct gonadotropic cell type (Kagawa *et al*., 1998; Rodriguez-Gomez *et al*., 2001), resembling the situation found in tilapia (Melamed *et al*., 1998) and sea bream (Elizur *et al*., 2000). However, despite the isolation and characterization of tuna LH and FSH, until now no assays have been developed to monitor these hormones in tunas, and therefore, no information is available concerning their profiles during the reproductive cycle or in response to hormonal manipulation.

Three distinct forms of GnRH, namely: chicken GnRH-II (cGnRH-II), salmon GnRH (sGnRH) and sea bream GnRH (sbGnRH), have been characterized in the brains of all perciform fish studied to date (Powell *et al*., 1994; Gothilf *et al*., 1995; White *et al*., 1995; Weber *et al*., 1997). Based on the resemblance of tuna reproductive features to that of several well-studied perciform fish, it is expected that the same identity of GnRH forms is conserved in BFT, however no information on this subject is available so far.

In order to expedite the study on the reproduction in BFT, the current study examined whether heterologous assay systems, available in our laboratories, are suitable for monitoring hormone profiles in the tuna.

Materials and methods

Validation of the stbLH ELISA for tuna pituitary and plasma samples

Pituitary and plasma LH content was measured using an ELISA developed to measure stbLH (Mañanós *et al.*, 1997). Pituitaries from two male bonito and one male yellow fin tuna were sonicated in 200 μl of double distilled (dd) H_2O. The samples were assayed at five serial dilutions (1/2,500, 1/5,000, 1/10,000, 1/20,000, 1/40,000).

Validation of the GnRH ELISAs for tuna pituitary samples

Pituitaries of two male bonito and one male yellow fin tuna were analysed for GnRH content using an ELISA, previously developed for the quantification of cGnRH-II, sGnRH, and sbGnRH isoforms (Holland *et al*., 1998). Briefly: pituitaries were sonicated in 200 μl dd H_2O and extracted with an equal volume of 4N acetic acid. After spin down, supernatants were collected, lyophilized and reconstituted in EIA-buffer. The samples were assayed at five serial dilutions (1/4, 1/8, 1/16, 1/32, and 1/64).

Results and discussion

In order to test the possibility of using the stbLH ELISA for LH measurement in tuna species, displacement curves obtained with serial dilution of pituitary and plasma extracts from bonito and yellowfin tuna were compared with the stbLH standard curve (Fig. 1). A clear linearity was obtained in the dilution of the plasma or pituitary of the bonito (A, C and D) and the yellowfin tuna (B). Moreover, these dilution curves exhibited parallelism with the standard stbLH enabling the determination of LH in tuna species.

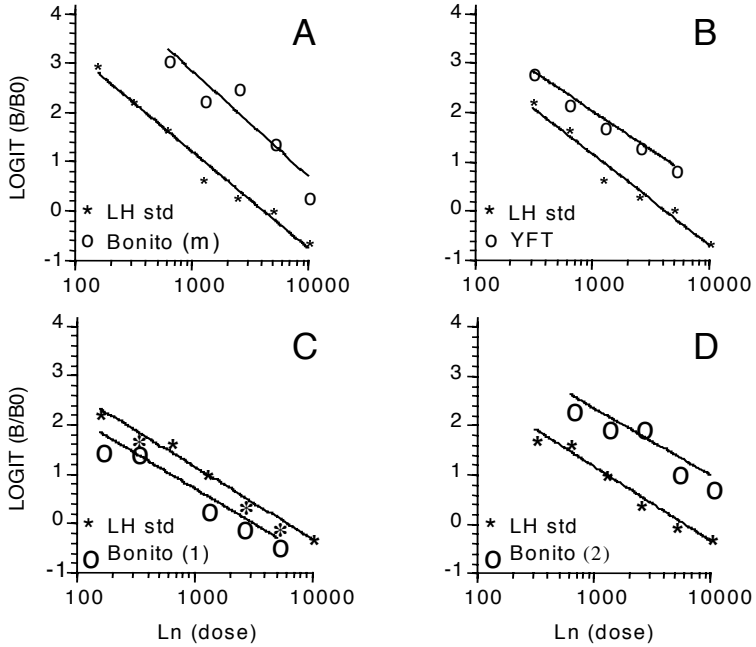

Fig. 1. Displacement curves for standard native stbLH and serial dilutions of plasma (A, B) and pituitary extract (C, D) samples from male bonito (A, C, D) and yellowfin tuna (B). The LOGIT function was utilized to transform standard curve to a linear plot. Each point is the mean of two determinations.

A similar parallelism was obtained in linearized displacement curves of the standards sGnRH and sbGnRH as compared to serial dilutions of pituitary extracts of bonito (Fig. 2) and yellowfin tuna (data not shown). However, using the same procedure we could not detect cGnRH-II.

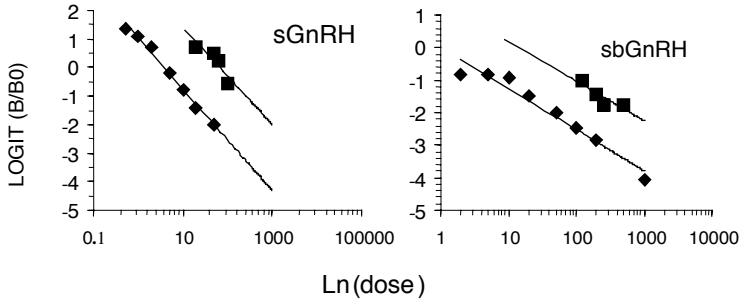

Fig. 2. Displacement curves for standard sGnRH and sbGnRH and serial dilutions of pituitary samples from male bonito. The LOGIT function was utilized to transform standard curve to a linear plot. Each point is the mean of two determinations.

Thus far, we have demonstrated the ability of sGnRH and sbGnRH ELISAs to detect the respective hormones in tuna species. Yet, we ascribe our disability to monitor the cGnRH-II isoform to technical problems (which can be solved once additional samples will be obtained) and assume, therefore, that tuna species, like all other perciformes, are characterized by three GnRH forms, namely: sGnRH, sbGnRH and cGnRH-II. Our assumption is based on accumulating data, which indicate that cGnRH-II is synthesized in all studied fish, including the more phylogenetically ancient ones (Carolsfeld *et al*., 2000).

Although our findings are of preliminary nature, we believe that acquisition of immunological tools necessary to analyse these key hormones will be useful in the development of spawning-induction therapies for BFT, and will alleviate many experiments based on trial and error. Therefore, the findings should be considered an important step leading to BFT domestication.

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