



## Immunohistochemical detection of gonadotropin-like material in the hagfish pituitary, correlated with their gonadal conditions

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**Key words:** hagfish, agnatha, pituitary gland, gonadotropin, pituitary-gonadal axis, immunohistochemistry

### Abstract

In the present study, immunoreactive gonadotropin (GTH)-like material was examined in the hagfish pituitary, in correlation with their gonadal conditions. For this purpose, three species of hagfish, *Myxine glutinosa*, *Eptatretus burgeri* and *Paramyxine atami*, were used. In all species, GTH-like cells were observed in the adenohypophysis. They were stained with several antibodies to LH-related GTHs, such as ovine LH $\beta$ , human LH $\beta$ , bullfrog LH, and salmon GTHII $\beta$ . In the subsequent study, the relationship between the amount of GTH-like material and gonadal conditions was examined in *Paramyxine atami*. The adenohypophysis of adult animals exhibited well-developing features, and about half of the adenohypophysial cells were stained intensely with anti-ovine LH $\beta$ . On the other hand, the adenohypophysis of juveniles exhibited less developed features with few or no cells stained with anti-ovine LH $\beta$ . These results suggest that a GTH-gonadal system is truly present in the hagfish.

### Introduction

Since hagfish are members of the most primitive group of living vertebrates, studies on their reproduction are indispensable for understanding phylogenetic aspects of vertebrate reproduction. Nevertheless, our knowledge of the reproductive physiology of the hagfish, especially of the pituitary-gonadal axis, is almost completely lacking. The lack of information is mostly due to the fact that hagfish pituitary function is difficult to assess from cytological and physiological studies (Gorbman 1983). Moreover, none of the adenohypophysial hormones have been isolated from the hagfish pituitary, and thus it remains an enigma whether adenohypophysial hormones similar to those of more advanced vertebrates are present in the pituitary or not.

Interestingly, previous hypophysectomy studies between *Eptatretus stouti* and *Eptatretus burgeri* showed different results. *Eptatretus stouti* live in deep water more than 100 m in depth of the eastern Pacific coast, and do not show a seasonal gonadal development (Gorbman and Dickhoff 1978). Hypophysec-

tomy in this species had no effect on the gonadal development (Matty et al. 1976). On the other hand, *Eptatretus burgeri* live in shallower water less than 50 m in depth of the western Pacific coast, and show a seasonal migration and a seasonally synchronized gonadal development (Ichikawa et al. 2000; Nozaki et al. 2000). Hypophysectomy in *Eptatretus burgeri* resulted in retardation of spermatogenesis (Patzner and Ichikawa 1977). Thus, some species of the hagfish may have a functional pituitary gland associated with seasonal reproductive development. In the present study, immunoreactive gonadotropin (GTH)-like material was examined in the hagfish pituitary gland, in correlation with their gonadal conditions.

### Materials and methods

Three species of hagfish were used. The Atlantic hagfish, *Myxine glutinosa* were collected off the coast of Maine, USA, in June 1999. The Pacific hagfish, *Eptatretus burgeri* were collected at the Koajiro Bay

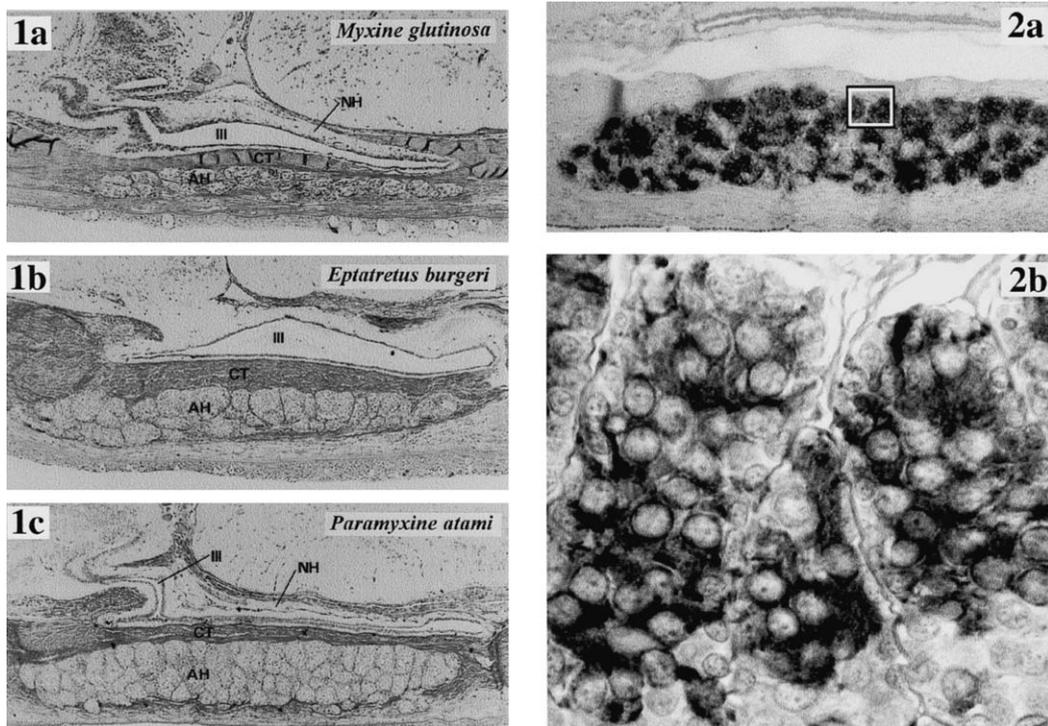


Figure 1a-c. Sagittal sections of the pituitary gland of three species of hagfish. (a) *Myxine glutinosa*; (b) *Eptatretus burgeri*; (c) *Paramyxine atami*. Notice that the thickness of the adenohypophysis (AH) are largely different among species. Hematoxylin-eosin stain.

Figure 2(a) A sagittal section of the pituitary gland of *Paramyxine atami*, stained with anti-ovine LH $\beta$ . The area outlined by a rectangle is enlarged and shown in b.

near the Misaki Marine Biological Station of the University of Tokyo in May 1999. The Pacific hagfish, *Paramyxine atami* were obtained from fishermen at Iwafune and Oyashirazu Fishing Ports facing on the Japan Sea, Niigata, during the period from July 2000 to December 2002. The animals were killed by decapitation after being anesthetized by immersion in 2-phenoxyethanol. The brain and the attached pituitaries were immersed in Bouin-Hollande sublimate solution for about 24 hr. The fixed tissues were dehydrated, embedded in Paraplast, and serial sagittal sections of 7  $\mu\text{m}$  were mounted on gelatin-coated glass slides. Immunohistochemical staining was performed by use of a Vectastain avidin-biotin peroxidase complex (ABC Elite) kit.

To obtain further insights into the functional significance of GTH-like material in the hagfish pituitary, the relationship between the amount of GTH-like material and gonadal conditions was examined in *Paramyxine atami*. At first, seasonal changes in gonadal activities were studied using specimens collected monthly throughout one year. Results showed no clear seasonality in gonadal development in either males or

females (see Results and Discussion). Thus, in the subsequent studies, three groups of male *Paramyxine atami*, each comprised of at least eight animals, were used: 1) Adults more than 40 cm in total length with developing testis of which gonadosomatic index ( $\text{GSI} = \text{testicular weight} / \text{body weight} \times 10^5$ ) was greater than 200; 2) Adults more than 40 cm in total length without developing testis of which GSI was less than 150; and 3) Juveniles smaller than 35 cm in total length.

## Results and discussion

There was a remarkable difference in the size and thickness of the adenohypophysis among hagfish species: *Paramyxine atami* had a well-developed adenohypophysis, which consisted of many clusters of cells, embedded densely in the connective tissue (Figure 1c), whereas *Myxine glutinosa* had a poorly-developed adenohypophysis, which consisted of small numbers of clusters of cells, embedded sparsely in the connective tissue (Figure 1a). The adenohypophysis of *Eptatre-*

*tus burgeri* was intermediate in size between above-mentioned two species (Figure 1b). Thus, although it is generally considered that the pituitary gland of the hagfish exhibits largely degenerative features, it is likely that *Paramyxine atami* has a functional adenohypophysis.

Following immunohistochemistry, a considerable number of GTH-like cells have been observed in the adenohypophysis of all species. They have been stained with several antibodies to LH-related GTHs, such as ovine LH $\beta$ , human LH $\beta$ , bullfrog LH, and salmon LH $\beta$ . Especially, in *Paramyxine atami* about half of the adenohypophysial cells were stained intensely with antibodies to ovine LH $\beta$  and human LH $\beta$  (Figure 2).

A total of 948 specimens of *Paramyxine atami* collected monthly during the period from August 2001 to June 2002 were analyzed. Gonadal development occurred in both males and females larger than 38 cm in total length. However, considerable individual variation in gonadal development was observed in any season of the year, and thus there was no clear seasonality in gonadal development. In adults with developing testis with their GSI more than 200, about half of the adenohypophysial cells were stained intensely with anti-ovine LH $\beta$  (Figure 2). On the other hand, in adults without developing testis, a less intense positive

reaction to anti-ovine LH $\beta$  was observed in the adenohypophysial cells. The adenohypophysis of juveniles exhibited less developed features with few or no adenohypophysial cells stained with anti-ovine LH $\beta$ . These results suggest that a GTH-gonadal system is truly present in the hagfish.

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