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Distribution of immunoreactive adenohypophysial cell types in the pituitaries of the Atlantic and the Pacific hagfish, *Myxine glutinosa* and *Eptatretus burgeri*

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Abstract

The hagfish is considered the most primitive vertebrate known, living or extinct. It remains an enigma whether adenohypophysial hormones similar to those of more advanced vertebrates are present in the hagfish pituitary gland or not. The present study aimed to detect immunoreactive adenohypophysial hormones in the hagfish pituitary gland, using antisera to tetrapod and fish adenohypophysial hormones as immunohistochemical probes. For this purpose, two species of hagfish, the Atlantic hagfish, *Myxine glutinosa*, and the Pacific hagfish, *Eptatretus burgeri*, were used. In both species, three different types of immunoreactive cells were detected in the adenohypophysis. (1) The first type of cells was gonadotropin (GTH)-like cells which were stained by antisera to LH-related GTHs, such as ovine LHβ, human LHβ, bullfrog LH, salmon LHβ and sturgeon LHβ in both species of hagfish. (2) The second type of cells that were detected was growth hormone (GH)/prolactin (PRL)-like cells. In *M. glutinosa* the cells were stained by antisera to salmon GH, salmon PRL, sturgeon GH, sturgeon PRL, blue shark GH, and lamprey GH. In *E. burgeri* the cells were only stained by anti-human GH and anti-sturgeon PRL. (3) The last type of cells was adrenocorticotropin (ACTH)-like cells. These cells were stained by antisera to lamprey ACTH and human β-endorphin. In both species of hagfish, GTH-like cells were relatively abundant, and were distributed throughout the adenohypophysis, whereas GH/PRL-like and ACTH-like cells were few in number in the adenohypophysis. Based on these findings, we suggest that hagfish may have retained ancestral characteristics of key anterior pituitary hormones.

Keywords: Hagfish; Agnatha; Pituitary; Adenohypophysis; Growth hormone; Adrenocorticotropin; Gonadotropin; Immunohistochemistry

1. Introduction

Lampreys and hagfish are the only two extant representatives of the oldest class of vertebrates, Agnatha (jawless fishes). The agnathans probably arose as the first vertebrates about 550 million years ago (Forey and Janvier, 1993), immediately after the evolutionary explosion of multicellular organisms in the Cambrian period. The pituitary system in agnathans had been an enigma until the identification of the corticotropin (ACTH) and melanotropins (MSHs) in the sea lamprey (Takahashi et al., 1995a). Unlike gnathostome vertebrates, ACTH and MSHs were found to be encoded in two distinct genes in the lamprey (Heinig et al., 1995; Takahashi et al., 1995b). Nevertheless, ACTH-like and MSH-like cells were found in the pars distalis and the pars intermedia, respectively, as well as those of gnathostome verte-

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brates (Nozaki et al., 1995). More recently, growth hormone (GH) has been isolated from the sea lamprey pituitary gland (Kawauchi et al., 2002; Sower and Kawauchi, 2001). The lamprey GH shows approximately 22% sequence identity to GHs of gnathostomes, 16–20% identity with somatolactins (SLs) and 15–17% with prolactins (PRLs) (Sower and Kawauchi, 2001). Kawauchi et al. (2002) have suggested that GH is the only member of the GH family in the lamprey, and thus GH seems to be the ancestral hormone that came first in the molecular evolution of the GH family. The presence of gonadotropin (GTH) is also strongly suggested in the lamprey pituitary (Nozaki et al., 1999, 2001; Sower, 1990) and has been tentatively identified (unpublished).

The hagfish is considered the most primitive vertebrate known, living or extinct (Forey and Janvier, 1993). In addition to their primitive external body features, the pituitary gland is also considered to be primitive and not well developed. The adenohypophysis of the hagfish consists of clusters of cells embedded in connective tissue below the neurohypophysis. The adenohypophysis and the neurohypophysis are completely separated by the thick layer of connective tissue (Ball and Baker, 1981; Holmes and Ball, 1974). Differentiation of the pars distalis and the pars intermedia seen in gnathostomes and lampreys is not observed in hagfish. The hagfish adenohypophysis consists of relatively undifferentiated follicles. In addition, there is no recognizable pars intermedia nor a median eminence (Gorbman, 1983). Moreover, it has not been established whether the hagfish pituitary gland contains tropic hormones of any kind (Gorbman, 1983).

Surgical hypophysectomy had no observable effect on gonadal structure in *Eptatretus stouti* (Gorbman and Tsuneki, 1975; Matty et al., 1976). Matty et al. (1976) identified only limited abnormalities in the testes and ovaries of 150 hypophysectomized hagfish during a 7-month study. In this study, gametogenesis appeared to be unaffected by hypophysectomy suggesting that the hagfish gonad was independent of hypophysial gonadotropic control. However, Patzner and Ichikawa (1977) observed a decrease in the number of follicles containing spermatocytes and only a few follicles containing spermatides in hypophysectomized hagfish when compared to sham operated hagfish. Their results suggested that the development of the hagfish gonad was under hypophysial gonadotropic control.

Among hagfishes, only *Eptatretus burgeri* live in shallower water less than 50 m in depth, and show a seasonal migration and a seasonal development of gonads, and thus it is thought that the development of gonads is under the influence of the hypothalamo-hypophysial system (Ichikawa et al., 2000; Kobayashi et al., 1972; Nozaki et al., 2000; Patzner and Ichikawa, 1977). Therefore, the identification of pituitary hormones in hagfish will be necessary to determine the functionality of hagfish.

Therefore, as a first step in the identification of possible anterior pituitary hormones, the aim of this study was to determine if there were immunoreactive adenohypophysial cell types in the pituitary gland of the hagfish. For this purpose, antisera to tetrapod and fish adenohypophysial hormones were used as immunohistochemical probes.

2. Materials and methods

2.1. Animals

The Atlantic hagfish, *Myxine glutinosa* ($n = 14$, 44–70 cm in total length), were collected in traps in the Gulf of Maine 10 miles east of the Isle of Shoals, New Hampshire in June 1999. They were transported to the marine station of the University of New Hampshire and were kept in seawater for 1–5 days before sacrifice. The Pacific hagfish, *E. burgeri* ($n = 17$, 30–60 cm in total length), were collected at the Koajiro Bay near the Misaki Marine Biological Station of the University of Tokyo in May 1999. They were transported to the Sado Marine Biological Station of Niigata University and were kept in seawater for 1–2 days before sacrifice.

2.2. Tissue preparations

Animals were killed by decapitation. After rapid removal of the dorsal fibrocranium and exposure of the dorsal surface of the brain, the dissected brain and the attached pituitary were fixed in Bouin-Hollande sublimate solution (Romeis, 1948) for about 24 h. The fixed tissues were dehydrated through a series of increasing concentrations of ethanol. Deposits of mercuric chloride were removed by treatment of tissues with iodine-potassium iodide in 90% ethanol for 24 h. Tissues were embedded in Paraplast, and serial sagittal sections of 6 μm were mounted on gelatin coated glass slides.

2.3. Antisera and immunohistochemistry

Rabbit antisera raised against various adenohypophysial hormones or their subunits were used. The sources and working dilutions of each antiserum used is shown in Tables 1–3. The immunoreactive validity and optimal working dilution of each antiserum were previously determined by immunostaining of paraffin sections of the pituitary gland of responsible animals (Kawauchi et al., 2002; Nozaki et al., 1990, 1995, 1999; Ominato and Nozaki, 2002). Immunohistochemical staining was performed by use of a Vectastain avidin-biotin peroxidase complex (Elite ABC) kit. In brief, sections were deparaffinized in xylene, hydrated in a graded ethanol series, and washed in phosphate-buffered saline (PBS). All procedures were performed at room temperature, and