EVOLUTIONARY ORIGIN OF A FUNCTIONAL GONADOTROPIN IN THE PITUITARY OF THE MOST PRIMITIVE VERTEBRATE, HAGFISH

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Introduction:

Reproduction in jawed vertebrates (gnathostomes) is controlled by a hierarchically organized endocrine system called the hypothalamic-pituitary-gonadal (HPG) axis. The HPG axis, which is specific to vertebrates, is considered to be an evolutionary innovation and seminal event that emerged prior to or during the differentiation of the ancestral jawless vertebrates (agnathans). In gnathostomes, two functional gonadotropins (GTHs), luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are secreted from the pituitary and stimulate the gonads inducing the synthesis and release of sex steroid hormones, which in turn elicit growth and maturation of the gonads. The extant representative of the agnathans, hagfish, represent the most basal and primitive vertebrate that diverged over 550 million years ago. They are of particular importance in understanding the evolution of the HPG axis related to vertebrate reproduction. Nevertheless, our knowledge of the endocrine regulation of reproduction in the hagfish has been poorly understood. The objective of this study was to identify a pituitary glycoprotein hormone (GPH) and to examine its possible functions for gonadal activities in the most primitive vertebrate, the hagfish.

Methods:

The brown hagfish, *Paramyxine atami*, is sold in local markets in Niigata district, Japan. Pituitary cDNA library was prepared and the cDNA clones were

Fig.1. Correlation between pituitary GPH activities and gonadal development in hagfish. (A, B) Cellular activities of GPHβ cells in the hagfish pituitary. Note that intense immunoreactions are observed in mature female (B), while faint reactions presented in juvenile (A). (C, D) Relative GPHα and GPHβ gene expressions in the pituitary of female (C) and male (D) hagfish. Open bars represent GPHα gene expressions and filled bars represent GPHβ gene expressions. Note that two GPH transcripts in both sexes increase in well accordance with the developmental stage of the gonad.

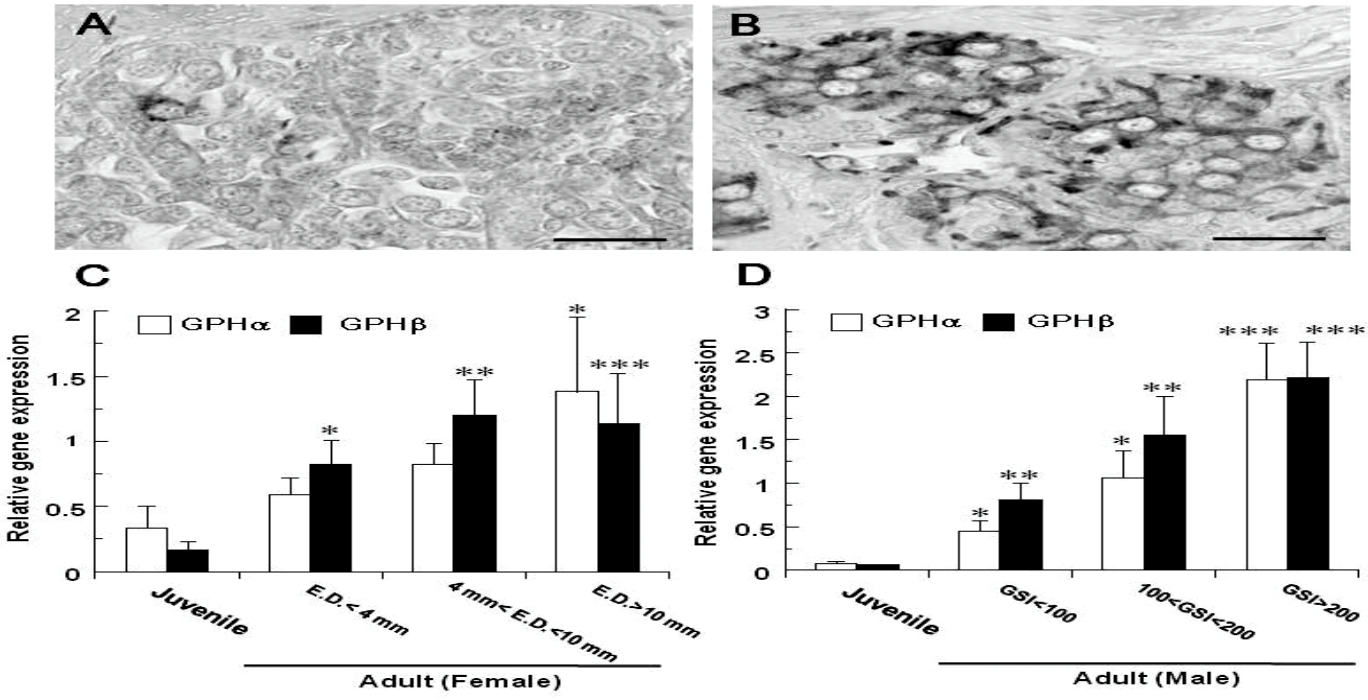
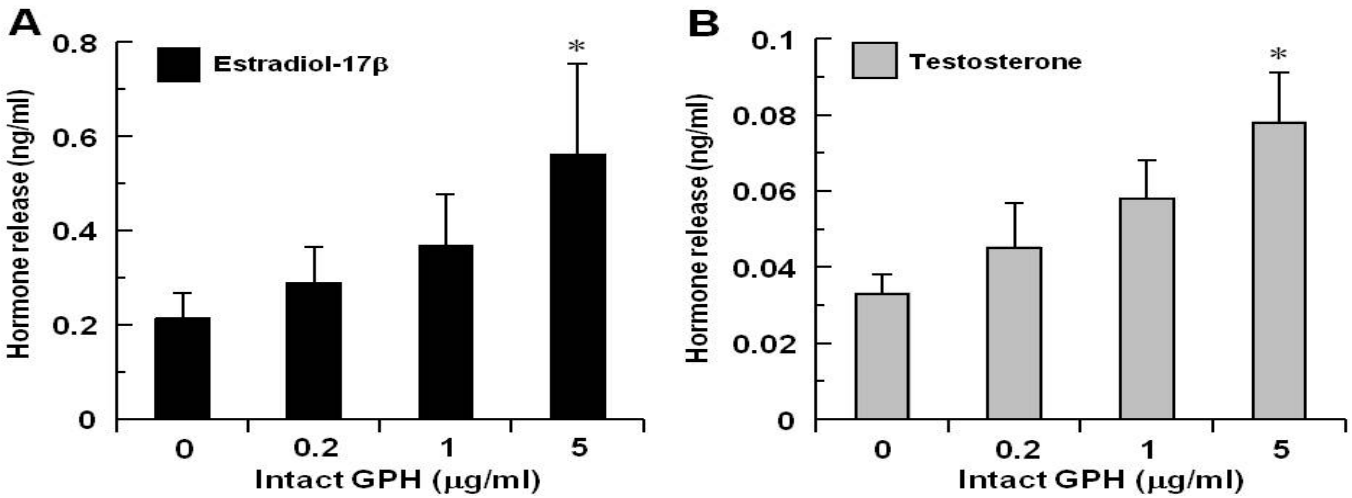


Fig. 2. Biological activity of native GPH from hagfish pituitary with *in vitro* bioassay. *In vitro* effects of native GPH on the releases of estradiol-17β (A) and testosterone (B) from organ cultured testis. It is notable that intact GPH stimulates the release of sex steroids from cultured testis *in vitro,* indicating "gonadotropic action" of hagfish GPH.



randomly selected. A total of 2304 clones were used for the sequence analysis. The molecular phylogenetic analysis of vertebrate GPHs was constructed using Maximum likelihood method. Cellular characterization of GPH-producing cells in hagfish pituitary was demonstrated by *in situ* hybridization and immunohistochemistry. We also examined correlation between pituitary GPH gene expression and gonadal development by quantitative real-time PCR assay. Purification and characterization of native GPH from hagfish pituitaries was performed, and the maturing testis was then cultured in the medium with or without native hagfish GPH at the same conditions. The concentrations of estradiol-17β (E2) and testosterone (T) in the medium were measured by Time-Resolved Fluoroimmunoassay (TR-FIA).

Results and Discussion:

The hagfish GPH consists of two subunits, α and β, which are synthesized and colocalized in the same cells of the adenohypophysis. The cellular and transcriptional activities of hagfish GPHα and β were significantly correlated with the developmental stages of the gonad (Fig. 1). The purified native GPH induced the release of gonadal sex steroids *in vitro* (Fig. 2). These results provide evidence that the hagfish GPH identified here has stimulatory effects on steroidogenesis and is thus considered a "GTH-like hormone" that can regulate gonadal functions. From our phylogenetic analysis, we propose that ancestral glycoprotein alpha subunit 2 (GPA2) and beta subunit 5 (GPB5) gave rise to GPHα and GPHβ of the vertebrate glycoprotein hormone family respectively. Based on the sequence and phylogenetic analyses, the identified hagfish GPHα- and β-subunits appear to be the typical pituitary GPHα and GPHβ subunits of gnathostomes. We hypothesize that the identity of a single functional GPH of the hagfish, hagfish GTH, provides critical evidence for the existence of a pituitary-gonadal system in the earliest divergent vertebrate that likely evolved from an ancestral, pre-vertebrate exclusively neuroendocrine mechanism by gradual emergence of a new control level, the pituitary, that is not found in the Protochordates.

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Conclusion:

We report the first identification of a glycoprotein hormone (GPH) in the pituitary of hagfish, the earliest divergent extant lineage and most primitive vertebrate. It seems most likely that an ancestral GPH gave rise to only one GTH in hagfish and that multiplicity of GPHs arose later during the early evolution of gnathostomes. We hypothesize that this functional pituitary GPH found in hagfish helps to delineate the evolution of the complex neuro/endocrine axis of reproduction in vertebrates. Furthermore, we propose that this HPG system likely evolved from an ancestral, pre-vertebpituitary gland.