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Short communication

Distribution of α-MSH-like immunoreactive cells in the nervous system, Hatschek's pit and other tissues of amphioxus, *Branchiostoma belcheri*

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Abstract

Immunohistochemical localization of α-melanocyte-stimulating hormone (α-MSH) in the nervous system, Hatschek's pit and other tissues of amphioxus (*Branchiostoma belcheri*) was performed using the antibody against synthetic α-MSH. The results revealed that α-MSH-like immunoreactive cells were distributed at the dorsal side and ventral side of brain vesicle, the dorsal side and the surrounding of nerve tube, and in the epithelial cells of Hatschek's pit, the zone 1, 3, and 6 of endostyle and gut. The immunoreactive substance was also found in the primary oocytes of the small and large growth stage of ovary and early stage spermatogenic cells in testis. These findings indicate that α-MSH is an ancient and highly conserved hormone and it is extensively distributed in amphioxus. Although Hatschek's pit in amphioxus does not have a structure of the intermediate lobe of vertebrate adenohypophysis, it has already hosted α-MSH-like endocrine cells, implying that the functional differentiation of α-MSH-like cells occurred earlier than the differentiation of the tissue structure. The results of the present study provided a new evidence for the endocrinology of Hatschek's pit and for the origin and evolution of vertebrate adenohypophysis.

Keywords: Amphioxus (*Branchiostoma belcheri*); Alpha-melanocyte-stimulating hormone (α-MSH); Hatschek's pit; Nervous system; Immunohistochemistry

1. Introduction

Hatschek's pit of amphioxus was postulated to be a homolog of the adenohypophysis of vertebrate [1]. Hatschek's pit hosts five types of endocrine cells which produce and release five kinds of hormones. Among them gonadotropes produce gonadotropins; thyrotropes produce thyrotropin; somatotropes produce growth hormone; lactotropes produce prolactin; and corticotropes produce corticotrophin [2–9]. However, we do not know whether the Hatschek's pit of amphioxus hosts melanotropes produces a pituitary hormone in the intermediate lobe of adenohypophysis of vertebrates derived from post-translational processing of proopiuomelanocortin (POMC) [10], and called as α-melanocyte-stimulating hormone (α-MSH). To answer this question it is very important for us to understand the origin and evolution of adenohypophysis in vertebrates. Thus, we investigated the distribution of α-MSH in the nervous system, Hatschek's pit and other tissues in amphioxus by immunohistochemical method.

2. Materials and methods

2.1. Materials

Amphioxus (*Branchiostoma belcheri*) were collected during the period April 2006 to May 2007 along the Qiongtou coast, Tong'an County, Xiamen, South China. The animals were reared in the laboratory for a week at a temper-

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ature of 25.2 °C, and fed with cultured microscopic algae once a day. After the acclimation, a total of 16 amphioxus in both sexes, with a length of 40.3–50.8 mm, were used in this study.

2.2. Sample preparation

After being anaesthetized at low temperature, the head, middle and tail regions of amphioxus were cut off and fixed in a freshly prepared Bouin's solution without acetic acid for 8–12 h. The tissues were dehydrated through a graded ethanol series, and then embedded in a paraplast. Serial cross sections of 6 μm thickness were cut and mounted on poly-L-lysine-coated slides. Sections with a structure of whole brain vesicle and Hatschek's pit of three different layers (whole, deep and bottom layers) in the head region as well as gonads at different stages and the gastrointestinal tract in the middle region were chosen under a light microscope for immunohistochemical localization.

2.3. Immunohistochemical staining

Sections were de-waxed, hydrated and incubated in 3% H_2O_2 for 10 min to remove endogenous peroxidase. After being rinsed in distilled water, the sections were immersed in 0.01 M PBS (pH 7.2–7.4) for 5 min and incubated with normal goat serum (1:10 dilution) for 10–20 min at room temperature to reduce nonspecific staining. Then the sections were incubated at 4 °C for 24–36 h with a polyclonal antibody against synthetic α-MSH (1:3000 dilution, purchased from Sigma Chem. Co., USA). After being rinsed with PBS, the sections were reacted sequentially with the secondary antibody (a product of Wuhan Boster Biological Technology Company), then with streptavidin-biotin-peroxidase complex (SABC, 1:100) for 20 min. Visualization of antigens was achieved with 3′,3-diaminobenzidine (DAB)/H_2O_2 solution.

Negative controls included replacing the primary antibody with PBS or normal rabbit serum in the SABC reaction.

3. Results

The results of immunohistochemical staining demonstrated that Hatschek's pit, the nervous system and other tissues of amphioxus were strongly immunopositive for α-melanocyte-stimulating hormone, the sections of negative control showed a negative reaction.

3.1. Localization of α-MSH in the nervous system

The nervous system of amphioxus consists of the brain and the spinal cord (nerve tube). The brain vesicle is the expanding part in front of the spinal cord, and extends along the right side of the notochord to the dorsal edge of Hatschek's pit. The extended brain tissue is called infundibulum. Our results showed that α-MSH-like immunoreactive nerve cells were localized on the brain vesicle and

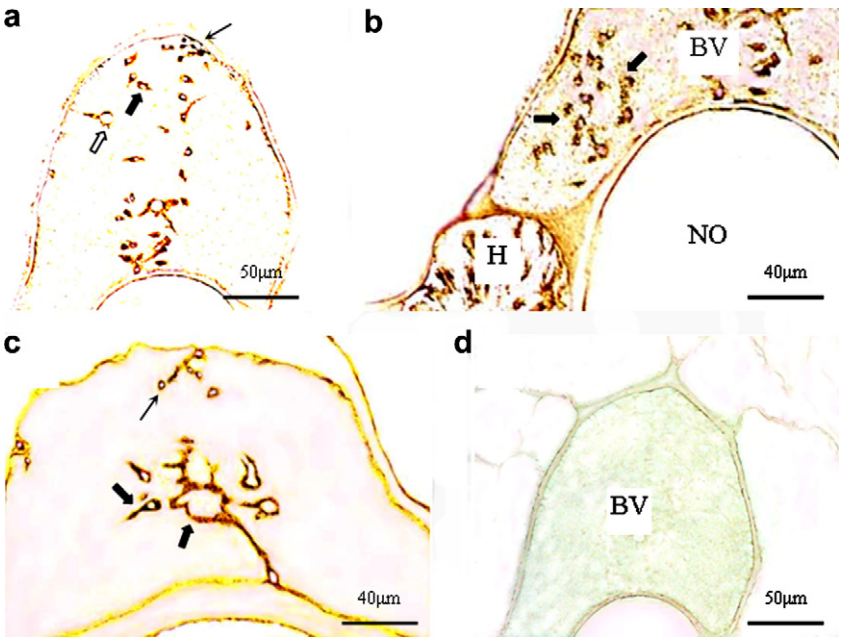

Fig. 1. Distribution of α-MSH-like immunoreactive nerve cells in the nervous system of amphioxus. (a) Cross section of head region of a male amphioxus showing small size α-MSH-like immunoreactive nerve cells (thin arrow) on the dorsal-right side; medium size (thick arrow) and large size positive cells (hollow arrow) on the left side; also the different size immunopositive nerve cells and nerve fibers on the ventral side. (b) Cross section of head region in male amphioxus showing α-MSH-like immunoreactive nerve cells located on the infundibulum part of brain vesicle (arrow). (c) Cross section of the middle part of a female amphioxus showing α-MSH-like immunoreactive nerve cells were located on the dorsal side (thin arrow) of the nerve tube and the surrounding area (thick arrow) of central tube. (d) The control section over the head region of a male amphioxus, showing an immunonegative reaction to α-MSH antibody. BV, brain vesicle; H, Hatschek's pit; NO, notochord.