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Short communication

Distribution of ghrelin-like immunoreactive cells in amphioxus, *Branchiostoma belcheri* – A study of immunohistochemistry

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Abstract

The distribution of ghrelin-like immunoreactive cells in amphioxus (*Branchiostoma belcheri*) was investigated by using immunohistochemical staining with rabbit antiserum against synthetical mammalian ghrelin. The results showed that ghrelin-like immunoreactive cells were distributed widely in the nervous system, Hatschek's pit, wheel organ, digestive tract and gonads (ovary and testis). In nervous system, ghrelin-like immunoreactive neurons and their protrusions were distributed specifically on the dorsal side, ventral side and funnel part of brain vesicle, with a few dispersive immunoreactive nerve cells and their fibers in nerve tube. Ghrelin-like immunoreactivities were also detected in Hatschek's pit epithelial cells and wheel organ cells, with positive substance located along cell membrane. In digestive tract, ghrelin-like immunoreactive cells existed in hepatic diverticulum, anterior and posterior region of midgut, and could be classified into two types, closed- and opened-type endocrine cells. The number of positive cells was most in hepatic diverticulum, secondary in posterior region of midgut and least in anterior region of midgut. In gonads, ghrelin-like immunoreactive substance was detected in oogonia, oocytes and follicle cells in ovary at the small and large growth stages and in early spermatogenic cells and Sertoli cells in testis. The extensive distribution of ghrelin-like cells in amphioxus suggested that these kinds of cells are conservative in evolution and diversified in function. At the same time, we found for the first time that ghrelin-like immunoreactive cells existed in brain vesicle and Hatschek's pit, which provided new morphological evidence for the existence of an activation pathway between brain vesicle and Hatschek's pit for the regulation of growth hormone excretion.

Keywords: Ghrelin; Amphioxus; Nervous system; Hatschek's pit; Digestive tract; Gonad

1. Introduction

Ghrelin, a 28-amino acid peptide, was originally isolated and purified from rat stomach by Kojima et al. and was identified as a natural endogenous ligand for the growth hormone secretagogue receptor (GHS-R) that functions in the stimulation of growth hormone (GH) release [1,2]. Thus, ghrelin is thought to be the stimulating system for hypothalamic regulation of pituitary GH excretion and to be a third regulator for GH release. Ghrelin had been demonstrated to be distributed in various tissues in vertebrates, mammalian (including human) [3–5], avian [6], amphibian [7] and fish [8–10]. It exerts many physiological functions. However, data about ghrelin in reptile and cyclostomata are not available now, and we do not know whether ghrelin exists in cephalochordate amphioxus, which is a transitional animal evolving from invertebrate to vertebrate. In this study, we carried out an immunohistochemical localization of ghrelin in nervous system (brain vesicle and nerve tube), Hatschek's pit, wheel organ, digestive tract and gonads (ovary and testis) of amphioxus using the antiserum against synthetical mammalian ghrelin,

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which helped us to understand the distribution and function of ghrelin in amphioxus.

2. Materials and methods

2.1. Animals

Amphioxus (*Branchiostoma belcheri*) were collected during a period from May 2005 to April 2006 along the Qiongtou coast, Tongan' County, Xiamen, South China. Animals were reared in laboratory for a week and fed on cultured algae twice a day. A total of 20 amphioxus of both sexes, with a length of 43.8–56.6 mm, were used in this study.

2.2. Preparation of samples

After being anaesthetized at low temperature, the animals were fixed for 24 h in freshly prepared Bouin's solution without acetic acid. Specimens were cut into head, middle and caudal part in the process of dehydration through a graded ethanol series, and the head and middle part were subsequently embedded in paraplast. Paraplast blocks were sectioned at 6–7 μm thickness and mounted on poly-L-lysine-coated slides. Sections with the structure of brain vesicle, nerve tube, Hatschek's pit, different parts of digestive tract and gonads (ovary and testis) at different developmental stages were picked out under a microscope and selected for immunohistochemical staining.

2.3. Immunohistochemical staining

Sections were stained by the streptavidin–biotin–peroxidase (SABC) method. Deparaffinized sections were incubated in 3% H_2O_2 for 10 min to remove endogenous peroxidase. After heat-induced antigen retrieval and rinsing with distilled water, the sections were immersed in 0.01 mol/L PBS (pH 7.2–7.4) for 5 min, and then incubated with normal goat serum (1:10 dilution) for 15 min at room temperature. The sections were reacted with the polyclonal antibody against synthetical mammalian ghrelin (1:150 dilution, purchased from Wuhan Biological Technology Company) at 4 °C for 36 h. After being rinsed with PBS, sections were incubated for 20 min at room temperature with goat anti-rabbit IgG, which was followed by incubation for 20 min again with a streptavidin–biotin–peroxidase complex. Visualization of antigens was achieved with diaminobenzidine (DAB)/H_2O_2 solution. Negative controls included replacing the primary antibody with normal rabbit serum or PBS or omitting the primary antibody in the SABC reaction.

3. Results

The results of immunohistochemical staining showed that ghrelin-like immunoreactive cells were distributed in nervous system, Hatschek's pit, wheel organ, digestive tract, testis and ovary of amphioxus.

3.1. Ghrelin-like immunoreactive neurons in nervous system

The nervous system of amphioxus has a structure of solid tube traversing the whole body and it can be divided into brain vesicle and nervous tube (spinal cord). The results of immunohistochemical staining showed that ghrelin-like immunoreactive neurons were located on the dorsal side and ventral side of brain vesicle and on the funnel part near Hatschek's pit extending from the right side of brain vesicle. In these neurons deep brown immunoreactive substance was located along cell membrane but the nucleus showed negative reaction (Fig. 1(a) and (b)). On the longitudinal sections of nerve tube, positively stained neurons and their protrusions and nerve fibers were dispersedly distributed on the dorsal and ventral side and center of the nerve tube (Fig. 1(c)).

3.2. Ghrelin-like immunoreactivity in Hatschek's pit

The Hatschek's pit of amphioxus is the primitive pituitary of vertebrate and consists of three kinds of cells: (1) epithelial cells at the basal part with a prismatic or irregular shape. They appear in an irregular crisscross arrangement of 2–3 layers. Result of immunohistochemical staining showed that strong ghrelin-like immunoreactivity was detected on each layer of epithelial cells and wheel organ cells (Fig. 1(d)). At the same time, on the longitudinal section of brain vesicle and Hatschek's pit different sizes of neurons in the funnel part of brain vesicle were positive for ghrelin staining, which closely contacted the epithelial cells in Hatschek's pit (Fig. 1(e)). The control section with the primary antibody replaced by normal rabbit serum was immunonegative (Fig. 1(f)).

3.3. Ghrelin-like immunoreactive cells in digestive tract

The amphioxus digestive tract is a straight tract which consists of pharynx, short oesophagus, hepatic diverticulum, anterior and posterior region of midgut, hindgut and anus. Ghrelin-like immunopositive cells were found to be distributed in the hepatic diverticulum, anterior and posterior region of midgut, with the number most in hepatic diverticulum, secondary in the posterior region of midgut and least in the anterior region of midgut. The distribution patterns of these positive cells were different in different positions of digestive tract. Most cells in hepatic diverticulum were spindle-shaped with slightly bulgy apex and base parts, and a very fine middle part. Cytoplast of some cells extended to the base membrane (Fig. 2(a)). These cells were typical openedle-type gastrointestinal endocrine cells and could also be seen in the anterior region of midgut (Fig. 2(b)). There were two kinds of immunopositive cells in the posterior region of