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Calcitonin-immunoreactive cells of the digestive tract of the amphioxus are distributed concentrically in a restricted region of the mid-gut

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Abstract

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In amphioxus (*Branchiostoma belcheri*), calcitonin-immunoreactive cells of the digestive tract were distributed concentrically in a 1-mm region of the latter half of the mid-gut. In the mid-gut caecum these cells were also present, but were dispersed throughout the length. The total number of cells in both parts ranged from 280 to 1157, varying from individual to individual, although the number in the mid-gut caecum was only 100–200. These cells were morphologically typical gut endocrine cells. Considering their morphology and the characteristics of their distribution pattern, these cells may be related to some phenomena of digestive processes.

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Introduction

In vertebrates, calcitonin is a hormone which prevents excessive increases of blood Ca levels, by suppressing the activity of osteoclasts and causing the mineralization of bones (Azria, 1989). This hormone is secreted from the parenchymal cells of ultimobranchial glands. Calcitonin-immunoreactive cells, however, are present in the brain of some species of vertebrates (Flynn *et al.*, 1981; Galan Galan *et al.*, 1981a,b; Fischer *et al.*, 1983; Yui, 1983). We previously found calcitonin-immunoreactive cells in the digestive tract of the salamander (*Hynobius nigrescens*) and described them as ectopic calcitonin-immunoreactive cells (Matsuda *et al.*, 1989). Recently, we also demonstrated the existence of cells producing the same calcitonin molecule as produced by the ultimobranchial gland in the small intestine of goldfish, using a reverse transcription polymerase chain reaction method (Okuda *et al.*, 1999). These facts support the idea that calcitonin, in addition to other hormones, is a brain-gut hormone, and the cells have been regarded as APUD (Amino Content and Precursor Uptake and Decarboxylation) cells (Pearse, 1968), or paraneurones (Fujita, 1983).

Protochordates such as sea squirts and amphioxus are phylogenetically interesting animals, since they may share the same ancestor as vertebrates. In these animals it is also known that calcitonin-immunoreactive cells, in addition to various brain-gut hormones, are present in both the central nervous system and gut (Fritsch *et al.*, 1979, 1980, 1982; Reinecke, 1981; Van Noorden, 1984). Girgis *et al.* (1980) have detected a human calcitonin-like molecule in the nervous system of sea squirts and amphioxus using radioimmunoassay. On the basis of these facts, Thorndyke and Falkmer (1985) suggested that brain-gut hormones present in vertebrates can ascend at least to protochordates. In fact, it was recently reported that amphioxus has the ancestral insulin and insulin-like growth factor genes of vertebrates (Chan *et al.*, 1990).

In this context, we studied calcitonin-immunoreactive cells in both the brain and gut of amphioxus. We failed to distinguish those cells in the brain from Hesse's cells which contain brown-black pigments, since the colour of the immunoreaction in the method we adopted was very similar to that of the pigments. On the other hand, we found that calcitonin-immunoreactive cells were distributed in a very

restricted area of the gut in the digestive tract of amphioxus. Therefore, our study describes the morphology, number, and distribution patterns of cells in the digestive tract.

Materials and Methods

Ten individual species of amphioxus (*Branchiostoma belcheri* Gray, 1847), were collected from depths of 15–20 m in Amakusa, Kumamoto Prefecture. They were immature and approximately 4 cm in body length. We anaesthetized them with ethyl 4-amino-benzoate, fixed overnight with Bouin's solution without acetic acid, and stored in 70% alcohol solution. Samples were subjected to the routine paraffin method, cut at 8 μm serially from the snout to the caudal end, and immunostained with a kit for the labelled streptavidin biotin method (Dako, Tokyo, Japan). Polyclonal antisalmon antiserum, whose specificity had been already checked, was also used (Sasayama *et al.*, 1991). The number of calcitonin-immunoreactive cells was examined by counting the number of nuclei whose cytoplasm had reacted to the antiserum.

Results

Figure 1 shows a schematic drawing of the digestive tract of amphioxus. The digestive tract branches out to the right side at the portion called the mid-gut, ranging 2–4 mm. This branch, the mid-gut caecum, is 5–9 mm in length. Calcitonin-immunoreactive cells were found in the mid-gut and the mid-gut caecum, intermingled with the columnar epithelial cells. The basal portion, which includes the nucleus, was located at the serosal side. The apical portion of the cytoplasm was elongated in the direction of the gut lumen (Fig. 2A). Consequently, the shape of the cell was very elongated; the width was around 1.5–4 μm, and the height roughly 15–84 μm. The apical portion of the cytoplasm was occasionally protruded into the lumen (Fig. 2B). Calcitonin-immunoreactive cells tended to be located in the ventral side of the gut (Fig. 2C). Furthermore, the cells were sometimes present in large numbers in the pit-like portions of the mid-gut in 9 out of 10 individuals (Fig. 2D). However, pit-like portions did not always contain calcitonin-immunoreactive cells.

Calcitonin-immunoreactive cells in the mid-gut were found only in the 1 mm region of the latter half (Fig. 3). In contrast, in the mid-gut caecum, the cells were dispersed throughout. The total number of cells in both parts ranged from 288 to 1157, varying in each individual. In comparison, the number of cells in the mid-gut caecum was only 100–200. In total, the difference in the number of cells of each individual was often four times as many. However, the distribution pattern was common to all individuals.

Discussion

Fritsch *et al.* (1980) reported calcitonin-immunoreactive cells in the digestive tract of the sea-squirt *Ciona intestinalis*. However, this species showed no definite distribution pattern. Alternatively, Thorndyke and Probert (1979) found that calcitonin-immunoreactive cells distributed concentrically in the pharynx of the sea squirt, *Styela clava*. These cells discharged their secretory granules when they were exposed to high-Ca environment, in the same way that calcitonin cells react in the ultimobranchial gland of vertebrates. Therefore, the authors suggested that a group of the calcitonin-immunoreactive cells found in the pharynx of the sea squirt might be the ancestor of the ultimobranchial gland of vertebrates. This viewpoint seems to be very interesting, since ultimobranchial glands are one of the derivatives of the pharynx in vertebrates (Pearse and Carvalheira, 1967). In amphioxus examined here, calcitonin-immunoreactive cells were distributed concentrically in a very narrow region in the latter half of the mid-gut, although they were scattered in the gut in other species of amphioxus (Reinecke, 1981).

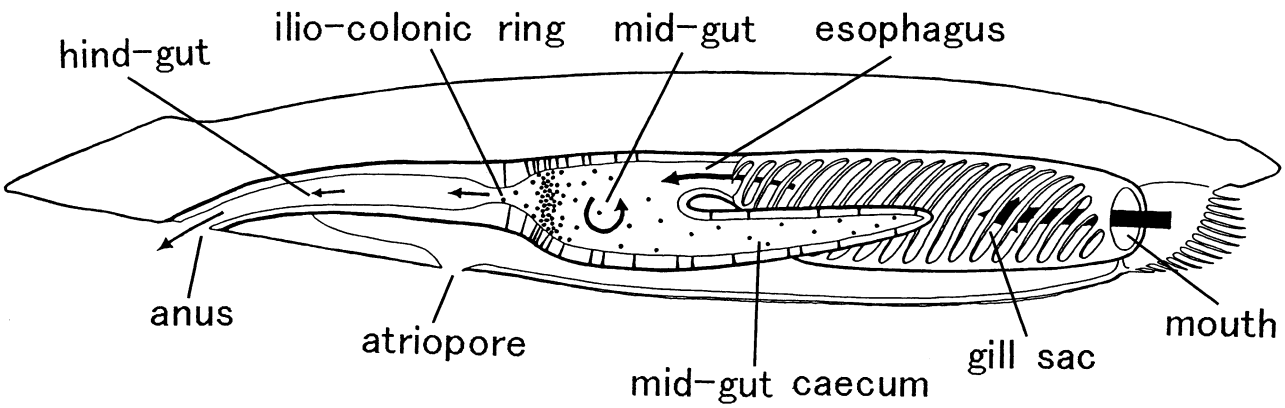

Fig. 1—Schematic drawing of the digestive tract of amphioxus, indicating processes of digestion. Black spots in the mid-gut and mid-gut caecum show the region in which calcitonin-immunoreactive cells are present.

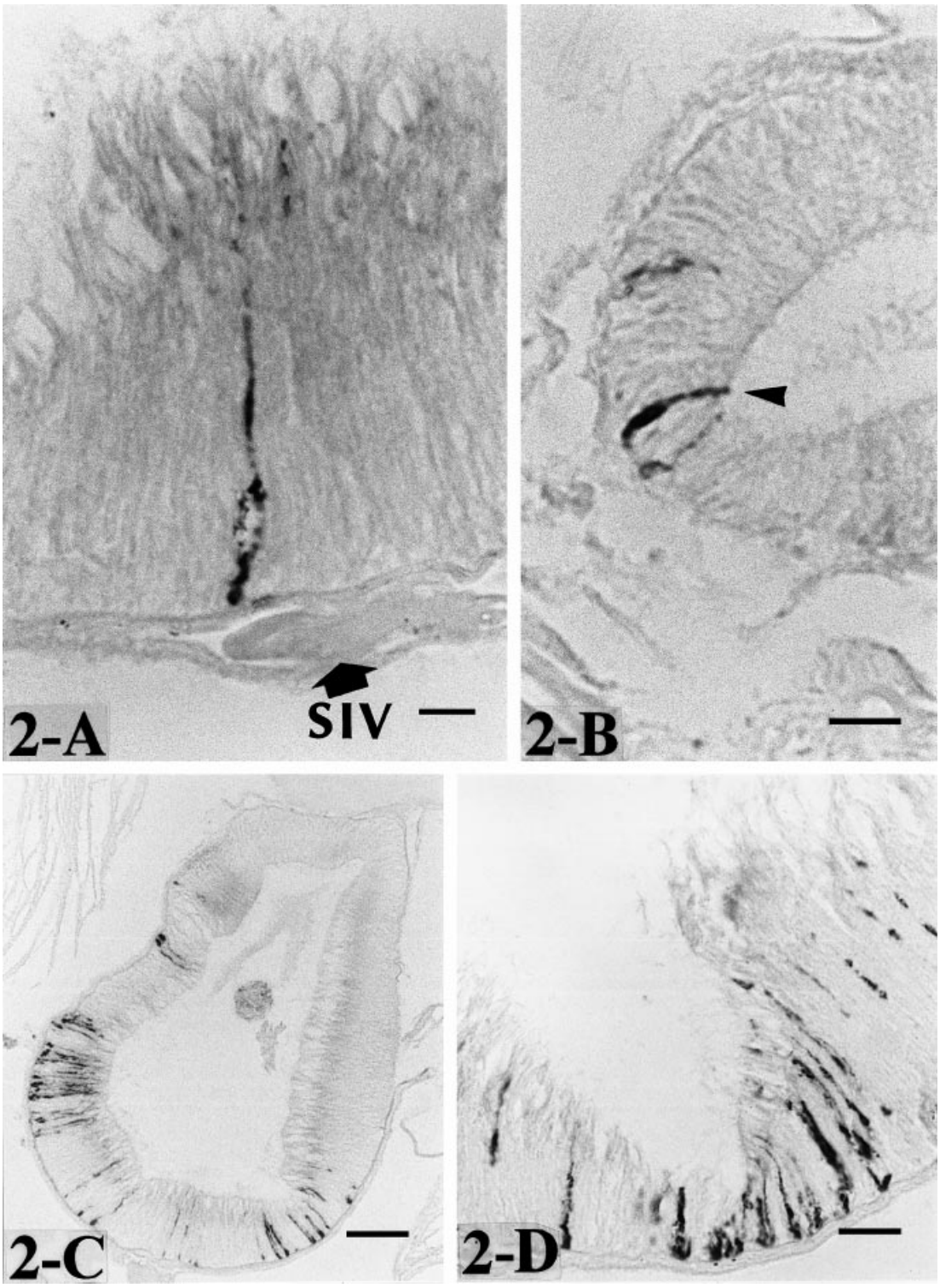

Fig. 2—**A**. Photograph showing a typical calcitonin-immunoreactive cell in the mid-gut. Note that the basal portion of the cell is bordered with the subintestinal vein (SIV) (arrow head). Bar = 10 μm —**B**. Photograph showing a protrusion (arrow head) of the apical portion of the cytoplasm of a calcitonin-immunoreactive cell to the lumen in the mid-gut caecum. Bar = 10 μm —**C**. Photograph showing a tendency for calcitonin-immunoreactive cells to be distributed in the ventral side of the mid-gut. Bar = 50 μm —**D**. Photograph showing a group of calcitonin-immunoreactive cells found in a pit-like portion of the mid-gut. Bar = 20 μm.

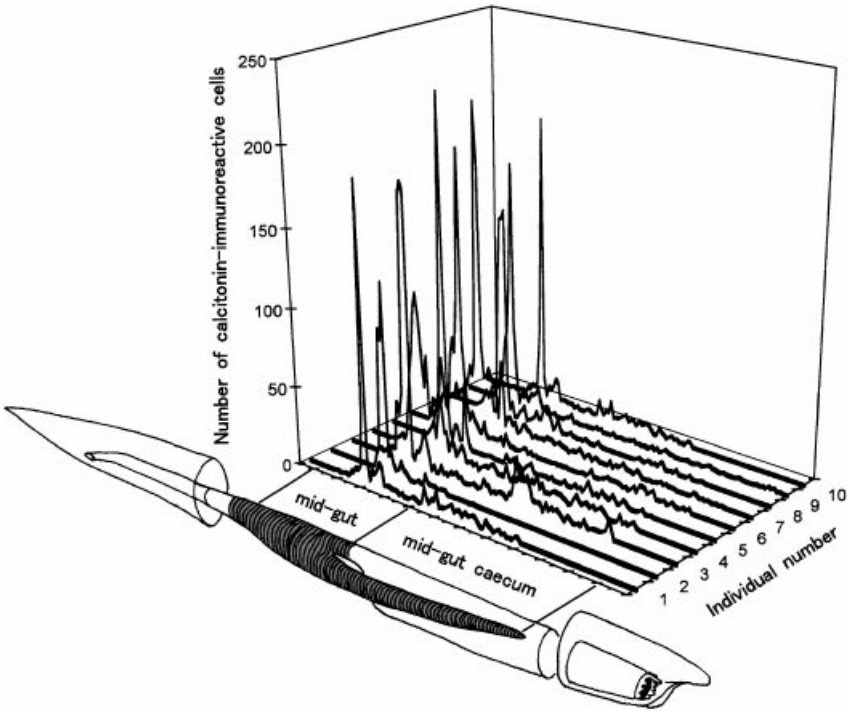

Fig. 3—Distribution pattern of calcitonin-immunoreactive cells in the mid-gut and mid-gut caecum.

In amphioxus, the processes of digestion proceed as follows (Barrington, 1965) (see Fig. 1). Ingested food is sent from the oesophagus to the mid-gut by the movement of the cilia of the epithelial cells. Food in the mid-gut is rotated by the ciliary movement and intermingled with digestive juice secreted from the mid-gut. A large quantity of digestive juice is then poured from the mid-gut caecum, after which food is transferred to the hind-gut, where material is absorbed. Therefore, in the latter half of the mid-gut where calcitonin-immunoreactive cells are concentrated, food passes through immediately after digestion has taken place. Consequently, calcitonin-immunoreactive cells located in this position may be related to the regulation of digestive processes. It is interesting to note that the location of these cells is similar to that of cells in the small intestine of goldfish (Okuda *et al.*, 1999). When examining the goldfish, the number of these cells was compared between individuals administered consomme soup into the small intestine and individuals administered saline solution, the number was found to be significantly larger in the former group than in the latter (Okuda *et al.*, 1999).

In this study, a group of calcitonin-immunoreactive cells were sometimes found in pit-like structures of the mid-gut. In cockroaches (*Periplaneta americana*), a similar structure found in the mid-gut is regarded as a centre of regeneration of the epithelium (Iwanaga *et al.*, 1981; Nishitsutsuji-Uwo and Endo, 1981). In this portion, there is a stem cell which regenerates epithelial cells. Therefore, since cells just after regeneration are low in height, this area appears as a pit-like structure. Gut endocrine cells such as pancreatic polypeptide producing cells are also differentiated from this stem cell in cockroaches (Nishitsutsuji-Uwa and Endo, 1981). Hence, in the digestive tract of amphioxus, the pit-like structures may be a centre for the regeneration of epithelial cells and the calcitonin-immunoreactive cells also. At present, however, we do not have any reliable evidence to support this hypothesis.

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