The Fish Biology Journal MEDAKA, 7: 43–46 (1995)

The location and morphology of the ultimobranchial gland in medaka, *Oryzias latipes*

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(Received July 28, 1995)

Abstract The location and morphology of the ultimobranchial gland in medaka *Oryzias latipes* were examined. The ultimobranchial gland was found in the tissue of the thin transverse septum which separates the pericardial cavity from the abdominal cavity, thus in close association with the cardio-vascular system. In the anterior part of the body, the ultimobranchial tissue begins from where the hepatic vein joins the sinus venosus. After that, the ultimobranchial gland is elongated upwards and attached to the left and right Cuvierian ducts, in a sheet-like way. The ultimobranchial gland looks Y-shaped. In the posterior part of the body, the ultimobranchial gland ends at the same level as the head kidney.

Introduction

Calcitonin is a hormone which lowers blood Ca^{2+} levels. This hormone is secreted from the thyroid gland in mammals, while is secreted from the ultimobranchial gland in non-mammalian vertebrates. In 13 species of vertebrates, the primary structure of calcitonin has been determined (seven species of mammals, one species of bird, four species of bony fishes, and one species of cartilaginous fish). In bony fishes, the amino acid sequence of calcitonin is known for the salmon, eel, goldfish and sardine. Calcitonins of the two latter species were clarified by us (Sasayama *et al.*, 1993; Suzuki *et al.*, 1994). Those four fishes are situated at primitive positions in the phylogenetic tree of bony fish, and are closely related to one another (Nelson, 1994). On the other hand, medaka (*Oryzias latipes*) belongs to the family Adrianichtyidae of the order Beloniformes, and is taxonomically distant from those four fishes (Nelson, 1994). Furthermore, medaka has often been used as a model of fishes in the field of experimental biology, as this species has various useful characteristics in its physiology (Naruse *et al.*, 1994). Therefore, based on the above considerations, we decided to purify medaka calcitonin from the ultimobranchial gland. In medaka, however, the location of the ultimobranchial gland is not yet known. In the past, we found by rat-bioassay that there was the calcitonin activity in extracts from pharyngeal tissue of medaka (Sasayama *et al.*, 1991a). This finding suggested that in medaka the ultimobranchial gland is located in that position. Nevertheless, the ultimobranchial gland could not be identified when tissue preparations were made by ordinary staining methods (Sasayama, unpublished).

The purpose of this study is to determine the exact position of the ultimobranchial gland and to examine its morphology, using immunohistochemistry with an anti-salmon calcitonin antiserum.

Materials and Methods

Common medaka of both sexes of both albino and wild types were purchased from commercial source. Some were sexually mature, some were not. Their body lengths ranged from 2.6 to 3.5 cm, and body weights ranged from 0.2 to 0.5 g. After anesthetizing with 1/3,000 tricaine methanesulfonate (Sigma), the portion centered around the pharynx, from the heart to the anterior part of the liver, was dissected out and fixed in Bouin's solution without acetic acid for about 12 h. These samples were dehydrated and embedded in paraffin, according to a routine method, and sectioned at 6μ serially. These were stained with the labeled streptavidin biotin method (LSAB) using an anti-salmon antiserum. When the antiserum was absorbed with synthetic salmon calcitonin, the ultimobranchial gland of medaka was not stained. Delafield's hematoxylin was applied as counterstain. Details of this immunohistochemical method have been descried (Sasayama *et al.*, 1991b).

Results and Discussion

In several species of bony fish, the location and morphology of the ultimobranchial gland have been reported. In the goldfish, the ultimobranchial gland is found in the ventral surface of the central part of the pharynx (Sasayama and Oguro, 1987).

It is unpaired, and its shape is amorphous. It is pinkish white in color. In the fully matured female, it is possible to see the ultimobranchial gland with the naked eyes, because it is highly vascularized (Sasayama, unpublished). In the chum salmon, the ultimobranchial gland is located abdominally, where the transverse septum joins the esophagus (Sasayama *et al.*, 1989). It is paired. In the adults, it is possible to see it with naked eyes, as the gland looks pale white (Sasayama, unpublished). In other species, the ultimobranchial gland is also present in the transverse septum forming a sheet-like tissue (Pang, 1971).

In medaka, even under the dissecting microscope, the presence of the ultimobranchial gland could not be confirmed. By an immunohistochemical method, however, we confirmed that the ultimobranchial gland of medaka is also present in the transverse septum. The gland is very thin and composed of only a few layers of cells (Fig. 1). The septum is lined by black peritoneum on the abdominal side. When cross sections of medaka were made from the anterior part of the body, they showed that the ultimobranchial gland begins where the hepatic vein joins the sinus venosus (Fig. 2). The ultimobranchial gland is an elongated and sheet-like tissue stretching from the sinus venous to the left and right Cuvierian ducts. It looks a Y-shaped tissue (Fig. 2). The ultimobranchial tissue ended at the beginning of the head kidney. It is rare for the ultimobranchial gland to spread to the ventral part of the esophagus. Thus, in medaka, the ultimobranchial gland is in close association with cardiac vessels (Fig. 3a,b). In the frog, calcitonin is secreted into blood capillaries

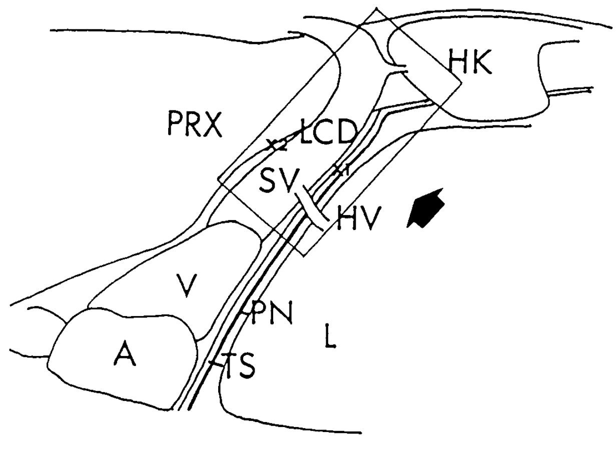

Fig. 1. Schematic drawing of a sagittal section of the throat area of medaka. The ultimobranchial gland is shown in X1. X2 is explained in Fig. 6. The square area with arrow is explained in Fig. 2. A, atrium; HK, head kidney; HV, hepatic vein; L, liver; LCD: left Cuvierian duct; PN, peritoneum; PRX, pharynx; SV, sinus venosus; TS, transverse septum; V, ventricle.

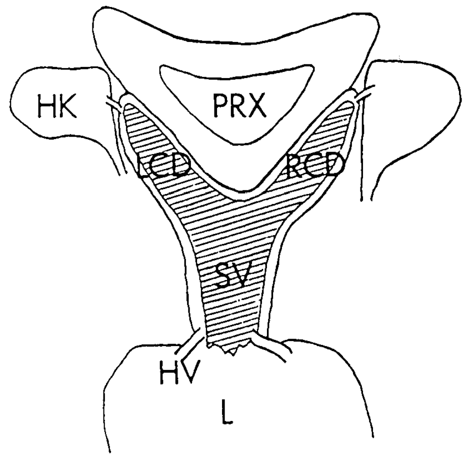

Fig. 2. Schematic drawing to show the spread of the ultimobranchial gland as a Y-shaped sheet, as viewed the squared portion of Fig. 1 in the arrow direction. The shaded area is the ultimobranchial gland. RCD: right Cuvierian duct. Other abbreviations are the same as in Fig. 1.

which envelop the ultimobranchial parenchyma (Robertson, 1988). Taking into consideration the case of female goldfish mentioned earlier, the morphology of the ultimobranchial gland in medaka may be advantageous for monitoring the Ca^{2+} levels in the blood stream and calcitonin secretion. These observations suggest that, in medaka, the ultimobranchial gland plays an important physiological role. It is interesting that the female ultimobranchial gland of the goldfish during the reproductive season is larger than that of male goldfish (Oguri, 1973).

In the eel (Lopez *et al.*, 1968), goldfish (Sasayama *et al.*, 1984) and chum salmon (Sasayama *et al.*, 1989), the ultimobranchial gland is composed of small follicles. In a small-sized fish, killifish, the ultimobranchial gland is found in the transverse septum as a sheet-like tissue (Pang, 1971). The ultimobranchial gland is also sheet-like in medaka, although cell clumps or stratification were observed in some areas. Where the ultimobranchial gland begins, peritoneum is diminished, since the hepatic vein penetrates the transverse septum and joins the sinus venosus. At this position, in almost all cases, the ultimobranchial gland takes a form of a small clump of cells (Fig. 4). In the Cuvierian ducts, the ultimobranchial gland is a sheet-like tissue (Fig. 5). No difference was recognized between the sexes and states of maturity. However, this may be because the ultimobranchial tissue observed in one tissue preparation is very limited, due to its sheet-like structure. In medaka, it is also sometimes hard to distinguish the parenchymal cells of the ultimo-

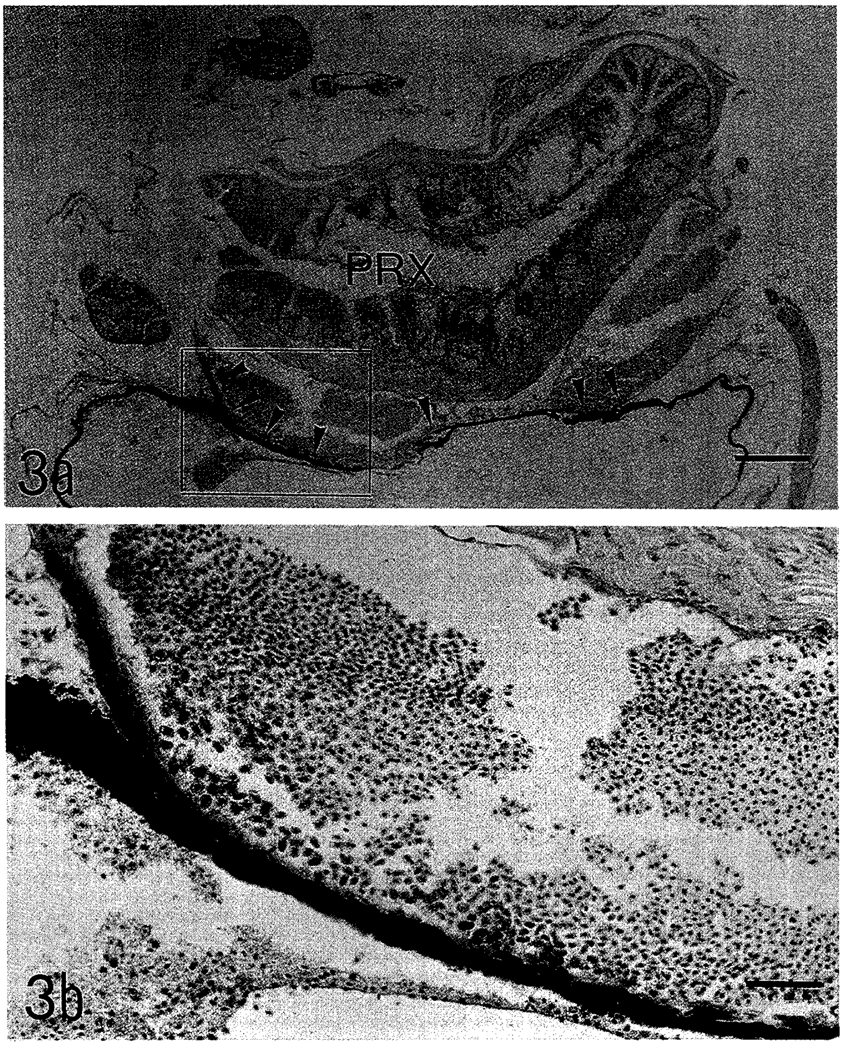

Fig. 3. (a) A pharyngeal portion including Cuvierian ducts attached to the ultimobranchial gland which is stained brown (arrowheads) by LSAB method. The black membrane is the peritoneum. The bar is 200 μm. (b) The ultimobranchial gland in a high magnification. Bar, 40 μm.

branchial gland from cells of the transverse septum and epithelium of blood vessels, since ultimobranchial cells responded so intensely to the antiserum, and the cells of the septum were co-stained (Fig. 5). In medaka, when the function of the ultimobranchial gland is discussed morphologically, observation by the electronmicroscope may be necessary. Thus, the reason why the ultimobranchial gland could not be found in medaka in the past may reside in the fact that the ultimobranchial gland is hard to distinguish from other tissues on account of its morphology.

Furthermore, in one individual, a small number of ultimobranchial cells were found beneath the pharynx (Fig. 6), as seen in the goldfish. It is known that in amphibians, the ultimobranchial gland is formed beneath the pharynx by invagination of the pharyngeal epithelium (Maurer, 1888; Sasayama *et al.*, 1976). Although early development of the ultimobranchial gland has not been studied in medaka, the ultimobranchial gland may form at first beneath the pharynx as in amphibians. After that, with development of the sinus venosus, most of ultimobranchial cells may be transferred onto the Cuvierian ducts. More studies are necessary to examine whether this is true.

Acknowledgments

We thank Miss R. Okuda, a graduate student of Kanazawa University, for her technical helps on making the immunohistochemical preparations.

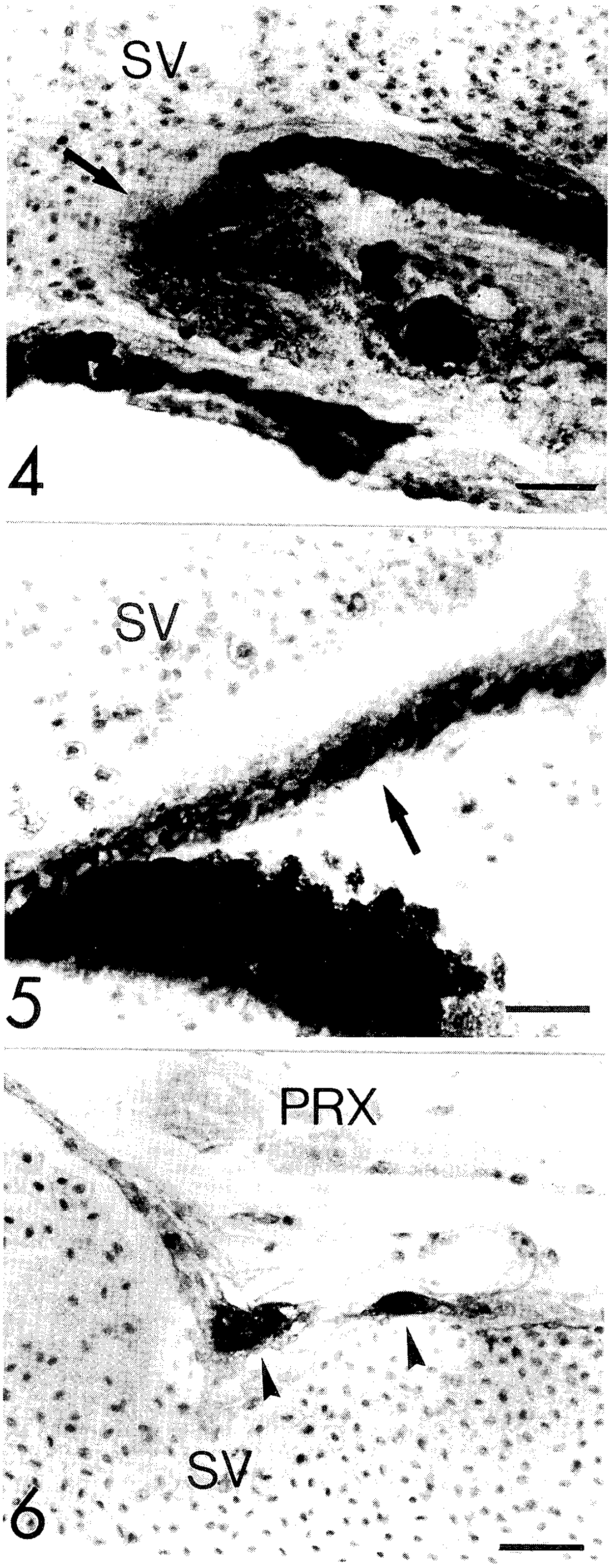

Fig. 4. A clump of ultimobranchial tissue (arrow) at the beginning of the gland. Bar, 20 μm.

Fig. 5. The sheet-like tissue of the ultimobranchial gland attached to the sinus venosus (arrow). Bar, 20 μm.

Fig. 6. Ultimobranchial tissue found beneath the pharynx (arrowheads). The location of this area is shown in Fig. 1 as X2. Bar, 20 μm.

References

Lopez, E., J. Deville and E. Bagot (1968) *C.R. Acad. Sci. Paris,* **267D**: 1531–1534.

Maurer, F. (1888) *Morph. Jahrbuch.,* **13**: 296–382.

Naruse, K., M. Sakaizumi and A. Shima (1994) *Fish Biol. J. MEDAKA,* **6**: 47–52.

Nelson, J.S. (1994) In: *Fishes of the World,* John Wiley & Sons, Inc., New York, pp. 264–279.

Oguri, M. (1973) *Bull. Jap. Soc. Sci. Fish.,* **39**: 851–858.

Pang, P.K.T. (1971) *J. Exp. Zool.,* **178**: 89–99.

Robertson, D.R. (1988) *Gen. Comp. Endocr.,* **71**: 349–358.

Sasayama, Y., H. Noda and C. Oguro (1976) *Dev. Growth Differ.,* **18**: 467–471.

Sasayama, Y., C. Oguro, R. Yui and A. Kambegawa (1984) *Zool. Sci.,* **1**: 755–758.

Sasayama, Y. and C. Oguro (1987) In: *Atlas of Endocrine Organs* (A. Matsumoto and S. Ishii, eds.), Springer-Verlag, Tokyo, pp. 83–92.

Sasayama, Y., K. Matsuda, C. Oguro and A. Kambegawa (1989) *Zool. Sci.,* **6**: 607–610.

Sasayama, Y., T. Koizumi, C. Oguro, A. Kambegawa, H. Yoshizawa and W. Magtoon (1991a) *Nippon Suisan Gakkaishi,* **57**: 851–855.

Sasayama, Y., A. Katoh, C. Oguro, A. Kambegawa and H. Yoshizawa (1991b) *Gen. Comp. Endocr.,* **83**: 406–414.

Sasayama, Y., K. Ukawa, H. Kai-ya, C. Oguro, Y. Takei, T.X. Watanabe, K. Nakajima and S. Sakakibara (1993) *Gen. Comp. Endocr.,* **89**: 189–194.

Suzuki, N., Y. Nose, Y. Kase, Y. Sasayama, Y. Takei, H. Nagawasa, T.X. Watanabe, K. Nakajima and S. Sakakibara (1994) *Zool Sci.,* **9**: 785–789.