A Morphological Study of the Ultimobranchial Body in the Grass Parakeet

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Summary. As parafollicular cells can be stained by Grimelius' silver impregnation and by lead-hematoxylin, the ultimobranchial body cells, homologous to them, seem to also react positively to these methods. In the present study of the ultimobranchial body of the grass parakeet, mirror-image sections were stained by both methods and compared. While about 50% of the cells reacted positively to both Grimelius' silver impregnation and lead-hematoxylin staining, only about 20% were positive to the former and about 30% were positive to the latter only. This result indicating heterogeneity among the cells of the ultimobranchial body of the grass parakeet suggests a possible occurrence of cells secreting substances other than calcitonin.

It is well-known that the ultimobranchial body of mammals exists only during the fetal stage and then merges into the thyroid gland shortly before birth. Soon after this mergence, parafollicular cells come into existence in the area where the ultimobranchial body disappeared. Thus, it has frequently been suggested that the parafollicular cells have an endodermal origin; i.e., ultimobranchial origin (GODWIN, 1937; SATO, 1959; PEARSE and CARVALHEIRA, 1967; SUGIYAMA et al., 1969). PEARSE and POLAK (1971), on the other hand, have suggested that some cells of neural crest origin move into the ultimobranchial body to develop into parafollicular cells, and thus claim a neuroectodermal origin for the cells.

Unlike the ultimobranchial body of mammals, that of lower vertebrates—including birds—remains independent of the thyroid gland after birth, being involved in the secretion of calcitonin, similar to the parafollicular cells (DUDLEY, 1942; SEHE, 1960; COPP et al., 1967; CHAN et al., 1969). PEARSE (1966, 1968, 1969) has long been advocating the amine precursor uptake and decarboxylation (APUD) theory by noting the existence of serotonin (5-HT), an amine, in addition to calcitonin in the parafollicular cells and the ultimobranchial body. The existence of somatostatin, a peptide hormone, in parafollicular cells has also been recently confirmed in a number of animals (HÖKFELT et al., 1975; NOORDEN et al., 1977; YAMADA et al., 1977; BUFFA et al., 1979; ALUMETS et al., 1980; KUSUMOTO, 1980). Furthermore, the existence of tyrosin hydroxylase (TH), a catecholamine-synthesizing enzyme, in the parafollicular cells of the dog and in the ultimobranchial body of the grass parakeet has been confirmed by the present authors (YAMADA et al., 1983) by using an immunofluorescent method, thus supporting the

existence of these cells as being of the neuroectodermal origin suggested by PEARSE. It has been now made clear that parafollicular cells and the ultimobranchial body contain various peptide hormones and amines.

In the field of light microscopy, various attempts have been made to differentiate parafollicular cells by staining. Today, Grimelius' silver impregnation (GRIMERIUS, 1968) and lead-hematoxylin (SOLCIA et al., 1969) are widely used for staining parafollicular cells, although it has been pointed out that these methods may not be effective for the cells of all animals (BUFFA et al., 1979).

In this connection, it was hypothesized that if these two methods were effective for staining parafollicular cells, they should also be suitable for the ultimobranchial body, which possesses secretory functions similar to the parafollicular cells. Accordingly, the ultimobranchial bodies of the grass parakeet as well as quail were subjected to these two methods. This study was considered to form the basis for immunohistochemical and electron microscopic studies presently being carried out by the authors.

MATERIALS AND METHODS

The grass parakeets used in this experiment were about 15 cm in length and 30–40 g in weight. The anesthetized grass parakeets were incised from the cervical region to the throat, and the thyroid glands and the surrounding tissues including the trachea, esophagus, and blood vessels were removed. The tissues were fixed with a GPA solution for 3 hrs., embedded in paraffin, made into horizontal mirror-image sections of 6 μ m, and subjected to hematoxylin-eosin staining, PAS reaction, Grimelius' silver impregnation, and lead-hematoxylin staining. As control samples, parafollicular cells of the thyroid gland of the dog were stained by Grimelius' silver impregnation and lead-hematoxylin. The measurement of the ultimobranchial body and other organs was carried out on the hematoxylin-eosin stained samples. The ventro-dorsal depth and left-right width were measured with a manometer for microscopes and on micrographs. The cranio-caudal length was computed out by multiplying the thickness of a segment by the number of the segments. Based on the data obtained, a schema was drawn up

Table 1. Measurements of the neck organs in μ m in the grass parakeet (body weight about 34 g)

		Cra-Cau	Ven-Dor	L-R
L	T	1164	795	719
	P	831	588	534
	C	351	367	340
	U	594	439	341
R	T	1254	890	663
	P	1042	548	668
	C	470	281	286
	U	318	143	209

L: left side organs, R: right side organs, T: thyroid gland, P: para-thyroid gland, C: carotid body, U: ultimobranchial body, Cra-Cau: cranio-caudal length, Ven-Dor: ventro-dorsal thickness, L-R: left and right breadth.

Fig. 1. Light micrographs of an ultimobranchial body (*U*), carotid body (*C*) and parathyroid gland (*P*) in the grass parakeet. Hematoxylin-eosin staining. $\times 150$

Fig. 2. Higher magnification of the ultimobranchial body. Solcia's lead-hematoxylin staining. $\times 300$

showing various organs in the cervical region and their positions in relation to one another.

RESULTS

The shapes of the thyroid gland, parathyroid gland, and carotid body of the grass para-

Fig. 3. Light microscopic mirror-image pictures of an ultimobranchial body (*U*), carotid body (*C*), parathyroid gland (*P*) and thyroid gland (*T*) in the grass parakeet. $\times 120$ **a.** Solcia's lead-hematoxylin staining, **b.** Grimelius' silver impregnation in a subsequent section of Figure 3a.

keet were either ovoid or spherical. In contrast, the ultimobranchial body was irregular in shape. This irregularity in the shape might be related to the lack of a capsule around the ultimobranchial body (Fig. 1, 2). The ultimobranchial bodies were located near the bifurcation of the branchiocephalic artery and the common carotid artery, and lay parallel to the carotid artery and carotid vein. Adjacent to the vagus nerve, and located in a descending order were the thyroid gland, parathyroid gland, carotid body, and ultimobranchial body. The organs on the left side were located closer as a whole to the pileum than those on the right side. The thyroid gland and the parathyroid gland were often found adjacent to each other. Slightly apart from those organs were the carotid body and the ultimobranchial body. The ultimobranchial body on the left was generally better developed than that on the right, but the other organs showed virtually no differences in size. The thyroid gland was the largest of all the organs, and the parathyroid gland second largest. The third and fourth largest organs differed on the left and right sides; i.e., on the left side the ultimobranchial body was the third largest and the carotid body the fourth, but on the right side this order was reversed (Table 1).

A histological examination revealed that the ultimobranchial body had no capsules. Some cells existed independently, while others formed clusters connected by connective tissues rich in capillary vessels. Follicles of various sizes formed by single-layered

Fig. 4. **a** and **b.** Light micrographs in serial sections of an ultimobranchial body in the grass parakeet. **a.** Solcia's lead-hematoxylin staining. **b.** Grimelius' silver impregnation. Arrow heads: Lead-hematoxylin positive and Grimelius negative cell clusters. Arrows: Grimelius positive and Lead-hematoxylin negative cell clusters.

follicular cells were also observed (Fig. 1, 2). There were colloidal substances and cellular debris in the follicular lumen (Fig. 2).

The ultimobranchial body was distinguished from the other organs by hematoxylin-eosin staining. The shapes of the cells were either spherical or ovoid. The nucleus appeared light and the cytoplasm was stained in a reddish color (Fig. 1). The colloidal substances in the follicular lumen and some of the connective tissues reacted positively to PAS staining. Grimelius' silver impregnation and lead-hematoxylin staining produced different results depending on the time of fixation. Samples fixed for 3 hrs were stained much better than those fixed for 6 hrs or for 24 hrs. In either method, only the cytoplasm reacted positively; the nucleus showed no reaction (Fig. 3, 4). Follicular cells, cilia of the follicular cells, and the colloidal substance and cellular debris in the follicle showed no reactions to either method. Examination of the mirror-image sec-

tions revealed that, among the cells which showed some kind of stain ability, about 50% reacted positively to both Grimelius' silver and lead-hematoxylin, about 20% only to Grimelius, and about 30% only to lead-hematoxylin. There were a considerable number of cells showing no reactions to either method (Fig. 4).

DISCUSSION

There have been a number of reports on the ultimobranchial body of the chicken, with the themes of discussion varying from its origin to the ultrastructure (STOECKEL and PORTE, 1967, 1969; MALMOVIST *et al.*, 1968; CHAN *et al.*, 1969; CHAN, 1971, 1978; YOUSHAH and CAPEN, 1971; ISLER, 1973). Since there has been no report on the grass parakeet, a comparison between the chicken and the grass parakeet was made in this paper.

A brief diagram describing the location of organs was presented by DUDLEY (1942), with more detailed diagrams by NICKEL *et al.* (1977) including the sizes of the ultimobranchial bodies of the chicken, goose, and pigeon.

The location of the ultimobranchial body and the relative sites of the thyroid gland, parathyroid gland, and carotid body in the grass parakeet matched those of the chicken (STOECKEL and PORTE, 1967). The size of the ultimobranchial body of the grass parakeet was considerably smaller than that of the birds reported by NICKEL *et al.* (1977), a fact easily accounted for when considering the body sizes of the birds.

The fact that the organs on the left side were located closer to the plicum than those on the right side and that the ultimobranchial body on the left was better developed than that on the right coincided with the data on reptiles.

Concerning the secretory functions of the ultimobranchial body, WATZKA (1933) suggested the possibility of an endocrine function for this organ, and COPP *et al.* (1967) reported the secretion of calcitonin based on their experiment. By immunofluorescence methods, the present authors confirmed the occurrence of calcitonin and tyrosine hydroxylase in the cells of the body (YAMADA *et al.*, 1983).

The ultimobranchial body of the grass parakeet was histologically characterized by its forming cell groups lacking a capsule and rich in connective tissues and capillary vessels. These characteristics were similar to those of fish and reptiles (TAKAGI and YAMADA, 1977, 1982).

The existence of follicles noted by HODGES *et al.* (1971) was confirmed in this study, which revealed a number of follicles of various sizes formed by cylindric or cubic follicular cells possessing cilia. PAS-positive colloidal substances and cellular debris were recognized in the follicular lumen. NAGY and SWARTZ (1966) also found PAS-positive substances in the follicular lumen of the chicken. HODGES *et al.* (1971) observed follicles with substances containing complex acid mucopolysaccharides and mucoproteins, but found no traces of secretion. Reports by ALMQVIST *et al.* (1971) and ISLER (1973) concluded that these follicles had no relation to the secretion of calcitonin. Though YOUSHAH and CAPEN (1971) and ISLER (1973) made references to substances resembling cellular debris, they claimed that the follicles were not related to the secretion of calcitonin. As has already been noted by the present authors, the secretion of calcitonin is more likely related to the cell clusters rich in capillary vessels.

BUFFA *et al.* (1979) applied Grimelius' silver impregnation and Davenport's improved method to thyroid parafollicular cells in the dog, guinea pig, rabbit and human and observed that the parafollicular cells of all mammals except the rabbit reacted positively to both methods. The parafollicular cells of the rabbit, which showed histo-

logical similarities to the pancreatic D-cells, reacted positively to Davenport's improved method but negatively to Grimelius' method. They contained both calcitonin and somatostatin; i.e., approximately 80% of the calcitonin immunoreactive cells reacted to the anti-somatostatin sera. According to BUFFA et al. (1979), however, the parafollicular cells of the other animals, and including man, were positive only for calcitonin.

KAMEDA (1968) subjected the parafollicular cells of the dog and rat to Davenport's improved method and found that the number of parafollicular cells per 100 follicular cells was far greater than the number counted after the conventional staining method (ROHR and HASLER, 1968). MATUZAWA (1966) studied the ultrastructure of the parafollicular cells of the rat and reported that the secretory granules in these cells appeared dark when they were double-fixed with glutaraldehyde and osmium tetroxide, while they appeared as vacuoles when fixed with osmium tetroxide alone. The same was true in D-cells of the pancreatic islets.

Considering the data in the reports mentioned above and the present finding that while about 50% of the cells reacted positively to both Grimelius and lead-hematoxylin, and, about 20% reacted to Grimelius only and about 30% reacted positively to lead-hematoxylin only, there seems to be a possibility that the ultimobranchial body of the grass parakeet contains cells which might secrete substances other than calcitonin. This problem must be elucidated by immunofluorescent methods.

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