Ultrastructure of the Corpuscular Nerve Ending in the Lymph Heart of the Turtle (*Pseudemys scripta elegans*)

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Summary. Corpuscular nerve endings were found in the tunica externa of the lymph heart of a turtle (*Pseudemys scripta elegans*). They consisted of axon terminals and surrounding cellular lamellae. Serial thin sections of one such presumable sensory corpuscle were made and reconstructed for the three-dimensional analysis of the corpuscle, with special reference to the axon terminals. It was revealed that axon terminals ramified at several points within the corpuscle and formed varicosities of various sizes ranging from 1.0 to 3.0 μm in diameter in their courses. Such varicosities contained an abundance of mitochondria as well as clear and granular vesicles 500 to 800 $\text{\AA}$ in diameter. Finger-like protrusions often projected from the axon terminals into the interlamellar spaces. The lamellae consisted of several thin cytoplasmic processes of modified Schwann cells. The corpuscle was enclosed in an incomplete capsule consisting of a thin layer of cellular processes. The corpuscular nerve endings may represent pressoreceptors in the wall of the lymph heart.

The lymph heart is a pulsating chamber located at the sites where lymph vessels enter the veins. This occurs in many classes of vertebrates, except mammalia. There have been many light (see review by KAMPMEIER, 1969) and electron microscopic studies (KAWAGUTI, 1967; RUMYANTSEV and SHMANTZAR, 1967; SCHIPP and FLINDT, 1968; LINDNER and SCHAUMBURG, 1968; SATOH and NITATORI, 1980) on the structure and on the development of the lymph heart. As most of these studies are on the muscle fibers and their motor innervations, available reports on the sensory innervation of the lymph heart are quite limited in number. ITO (1944) observed Meissner-like corpuscles on the wall of the lymph heart of turtles and snakes by means of a silver impregnation method. SATOH and NITATORI (1980) observed corpuscular nerve endings consisting of axon terminals intimately surrounded by multiple-stacked lamellae in the tunica externa of the turtle lymph heart. These lamellated corpuscles were thought to represent pressoreceptors in the lymph heart. No report has been available on the detailed descriptions of the ultrastructure of such pressoreceptors.

In this study, a three-dimensional analysis was performed in one such lamellated corpuscle by reconstruction of serial thin sections for electron microscopy. Special reference was given to the ramification pattern and the varicosity formation of the axon terminals within the corpuscle.

MATERIALS AND METHODS

Red-eared turtles (*Pseudemys scripta elegans*), 4-5 g in body weight, were used in this study. After they were chilled in ice-cold water to an anesthetic state, the caudal part of the carapace was removed, and the pulsating posterior lymph heart was immediately excised and fixed by immersion for a few minutes in an ice-cold fixative containing 1.25% glutaraldehyde and 4% paraformaldehyde in 1/15 M phosphate buffer (pH 7.4). The specimens were then postfixed in a 1% osmium tetroxide solution in the same buffer for 90 min. After a brief washing in the buffer, the specimens were dehydrated through a graded ethanol series and embedded in Epon 812 through n-butyl glycidyl ether as an infiltration medium.

Fig. 1. Diagram of the *Pseudemys*, showing the site of the lymph heart. **a.** The small rings indicate the position of two posterior lymph hearts (*L*). **b.** Part of the scales covering the lymph heart was removed. The lymph heart (*L*) lies on the posterior limb muscles (*M*), and pumps the lymph into the vein which is a branch of the vena circumflexa ileum (*VCI*), receiving vena ischiadica (*VI*).

schematic drawing of the lamellated corpuscle was made with three-dimensional visualization (Fig. 9).

RESULTS

The two posterior lymph hearts of the turtle are located in the connective tissue just below the rear-most large median plate of the carapace resting on the muscles of each posterior limb (Fig. 1a, b). These lymph hearts pour lymph into the branches of the vena circumflexa ileum, which flows into the vena portae renalis.

Though the wall of the *Pseudemys* lymph heart is very thin and transparent, it consists of three tunicae, as observed by electron microscopy: 1) tunica intima, composed of a continuous layer of endothelial cells and supporting connective tissue, 2) tunica media, consisting of slender striated muscle fibers, with a few peripherally located nuclei, and 3) tunica externa, containing fibroelastic tissue penetrated by blood vessels. A group of lamellated corpuscles was observed in the tunica externa (Fig. 2), measuring about $50\mu\text{m}$ in length and about $20\mu\text{m}$ in width. These lamellated corpuscles were situated with the long axis oriented almost obliquely in the direction of the lymph heart. The lamellated corpuscles consisted of ramified axon terminals and the surrounding multiple-stacked lamellae. Thin cellular processes formed an incomplete sheath or a capsule around these lamellated corpuscles.

Serial thin sections, approximately $0.1\mu\text{m}$ thick, were cut on a Porter-Blum Mt-2 Ultramicrotome and mounted on single hole grids. After staining with uranyl acetate and lead citrate they were observed in a JEM 100B electron microscope. A three-dimensional reconstruction of the axon terminals of the lamellated corpuscle was accomplished by observing approximately one hundred thin serial sections. The outline of the corpuscle and the profiles of the axon terminals were copied down on transparent acrylic sheets. These sheets were consecutively piled on each other to obtain the tri-dimensional images of the axon terminals, as well as of the whole corpuscle (Fig. 4). Then the

Fig. 2. Low electron micrograph showing the wall of a *Pseudemys* lymph heart. Lamellated corpuscles exist in the fibroelastic tunica externa of the lymph heart. A1-5 nerve fibers surrounded by multiple-stacked lamellae (L), C capsule of lamellated corpuscle, Lu lumen of the lymph heart, E endothelium of the lymph heart, M striated muscle fibers in the tunica media, BV blood vessel. The serial sectioning started at this level. $\times 1,900$

Fig. 3. a-d. Electron micrographs of consecutive serial sections showing the two axon terminals, A1 and A2 in Figure 2. About $0.9\mu\text{m}$ interval exists between Figures 2 and 3a, about $2.7\mu\text{m}$ between Figures 3a and 3b, about $3.8\mu\text{m}$ between Figures 3b and 3c, and $2.8\mu\text{m}$ between Figures 3c and 3d. It is evident that both axon terminals have two enlargements within each $10\mu\text{m}$ length of their axon courses. *my* Myelin-figure body, *P* finger-like protrusion of the A1 axon terminal. $\times 6,500$

Fig. 4. The reconstruction of the axon terminal of A1 and A2 shown in Figure 3. The electron micrographic images were drawn on acrylic sheets by selecting one out of every 5 micrographs, thus obtaining 20 drawings from 100 micrographs of serial thin ($0.1\mu\text{m}$ thick) sections. Accordingly the gap between adjacent acrylic sheets represents about $0.5\mu\text{m}$ of the actual depth. *L* profile of the outermost lamella, *P* finger-like protrusion of the A1.

Fig. 5. a-e. Serial samplings of A3 shown in Figure 2. About $1.0\mu\text{m}$ intervals exist between adjacent micrographs from 5a to 5e. About $1.0\mu\text{m}$ interval also exists between Figure 5a and Figure 2. Arrows indicate the cross sections of branches of the axon terminal (A3). A part of the axon terminal was not covered by lamella (arrowhead in Fig. 5e). my Myelin-figure bodies in axon terminals and lamellae. $\times 5,200$

The axon terminals branched out and were repeatedly swollen within the corpuscle (Fig. 3a-d, 4, 5a-e, 9). Based on the reconstruction of the serial sections, several varicosities of 1.0 to 3.0 μm in diameter and containing many mitochondria and glycogen granules (Fig. 6, 7), were observed in the axon leading to the axon terminal. Myelin-figure bodies and multivesicular bodies were also found in the central region of the varicosities (Fig. 3c, 5c). Neurofilaments and neurotubules were less numerous. In a few cases, parts of the swollen axon terminals were not covered by lamellae (Fig. 5e). Such parts were covered only by a basal lamina. A cluster of clear vesicles (about 500 $\text{\AA}$ in diameter) was found in the periphery of the varicosities (Fig. 6, 7). Granular vesicles (about 800 $\text{\AA}$ in diameter) were rare.

Small finger-like protrusions (about 0.4 μm in diameter and about 1.0 μm in length) extended from the axon terminals and penetrated into spaces between the lamellae (Fig. 3a, 7). The protrusions contained longitudinally-oriented microfilaments and occasional clear vesicles of about 500 $\text{\AA}$ in diameter. Some of the protrusions extended through the stack of lamellae to the connective tissue. The basal lamina on such protrusions was obscure.

The axon terminals were intimately surrounded by about three to ten layers of cytoplasmic processes (lamellae) of lamellar cells. The gap between the axon terminal and the inner-most lamella measured 170 $\text{\AA}$, and desmosome-like junctions were recognized between these two elements (Fig. 6, 7). The outermost lamella was thicker than the inner lamellae and contained a nucleus with a conspicuous nucleolus. Mitochondria,

Fig. 6. Higher magnification of the terminal swelling of the axon terminal (*A4*) shown in Figure 2. The terminal swelling contains peripherally located spherical mitochondria (*m*) and glycogen particles. The arrow indicates the basal lamina covering the outermost lamellae. *V* cluster of clear and granular vesicles, *D* desmosome-like structures, *P* finger-like protrusion of the axon terminal (*A*). $\times 21,600$

Fig. 7. A finger-like protrusion projecting from a terminal varicosity of the axon (*A5*) shown in Figure 2. The protrusion contains axillary running microfilaments (*f*) and clear vesicles (*V*). *CV* coated vesicles, *Cav* caveolae of lamellae, *D* desmosome-like structure, *mb* multivesicular body, *C* capsule, arrow: basal lamina. $\times 32,000$

Fig. 8. Micrograph showing a thin cytoplasmic process (*C*) of a capsular cell lying between the striated muscle fiber (*M*) and the corpuscle (see Figure 3). Basal lamina (arrow) is observed on the surface of the lamellar cell (*L*) and muscle fiber, but not on the capsular cell. *A1* axon terminal shown in Figure 2. $\times 23,000$

Golgi apparatus, well-developed rough endoplasmic reticulum, glycogen particles, myeline-figure bodies and microfilaments were noted in the lamellar cell body. The inner lamellae contained only filamentous materials and free ribosomes. The basal lamina was distinct on the outer surface of the lamellar cell bodies. Between the individual lamellae the basal lamina was generally absent, and collagen fibrils were not observed. The individual lamellae were separated by gaps of approximately $250 \text{ \AA}$ in width. Desmosome-like structures were sometimes found between the lamellae (Fig. 6). Caveolae and coated vesicles were not prominent in the lamellae.

The lamellated corpuscle was surrounded by an incomplete sheath or a capsule consisting of thin cellular processes. Such cellular processes had a higher electron density than the lamellae. Cell organelles were scanty (Fig. 2). Even where the corpuscle was located very close to the striated muscle fibers of the tunica media, the thin cellular process was interposed between the corpuscle and the muscle fibers (Fig. 8). Capsular thin cellular processes lacked a distinct basal lamina.

DISCUSSION

A previous study by means of silver impregnation showed that Meissner-like corpuscles

Fig. 9. Schematic drawing of three-dimensionally reconstructed axon terminals (A1-5 shown in previous figures). These arborize and form varicosities within the corpuscle. L lamellae surrounding axon terminals, P finger-like protrusions.

(Nervenknäuel) occurred in the tunica media and tunica externa of the lymph hearts of turtles (*Clemmys* and *Geoclemys*) (Ito, 1944). Two types of Meissner-like corpuscles have been described so far: "simple small" ($60\text{ }\mu\text{m}$ in length and $20\text{ }\mu\text{m}$ in width) and "complex large" ($100\text{ }\mu\text{m}$ in length and $60\text{ }\mu\text{m}$ in width) corpuscles. The former type receives two to three myelinated nerve fibers. Ito (1944) observed branchings and nodular dilatations of axon terminals in the corpuscles.

Recently, SATOH and NITATORI (1980) found in the tunica externa of the *Pseudemys* lymph heart the occurrence of Meissner-like corpuscles which corresponded in structure to the "simple small corpuscle" of Ito (1944). The three-dimensional reconstruction of their fine structure performed in this study revealed 1) the arborizations and varicosity-like dilatations of axon terminals within the corpuscle, 2) densely packed lamellae which surrounded the axon terminals without intervening basal lamina or collagen fibrils, 3) relatively few caveolae on the lamellae and 4) an incomplete capsule of thin cellular processes. It remained unclear in this study how many nerve fibers enter the corpuscle of the *Pseudemys* lymph heart.

Until now swellings of the receptor axons have been observed only at the terminal tip regions in the lamellated receptors of frog skin (DÜRING and SEILER, 1974), Herbst corpuscles (ANDERSEN and NAFSTAD, 1968; HALATA, 1971; SAXOD, 1978; CHOCHKOV, 1978), Meissner corpuscles (IDE, 1976), Krause simple Endkolben, Pacinian and Golgi-Mazzoni corpuscles (CHOCHKOV, 1978). On the other hand varicosity-like expansions of axon terminals within the corpuscle have been observed only in the lamellated mechanoreceptors of reptilian skin (DÜRING, 1973). It may be considered that the varicosity-like dilatations of the axon terminals represent a characteristic feature of lamellated corpuscles in lower vertebrates. Arborization of the axon terminal within the corpuscles seems to occur more generally in sensory corpuscles such as Meissner corpuscle (IDE, 1976), genital and Golgi-Mazzoni corpuscles (HALATA, 1975; CHOCHKOV, 1978).

Densely packed lamellae surrounding the axon terminals and the lack of basal lamina and collagen fibrils interposed between the lamellae have also been observed in the lamellated cutaneous mechanoreceptor of reptiles (DÜRING, 1973, 1974). Intervening basal lamina or collagen fibrils between the individual lamellae have been observed in Meissner corpuscles (IDE, 1976), Krause simple Endkolben, Pacinian corpuscles (CHOCHKOV, 1978), genital end bulbs (MUNGER, 1971), and lamellated receptors of frog skin (DÜRING and SEILER, 1974). It has been reported that the interlamellar space of the Herbst corpuscle contains collagen fibrils, but not basal lamina (ANDERSEN and NAFSTAD, 1968; SAXOD, 1978). The lamellar cells in the mechanoreceptors described above were thought to have been derived from the Schwann cell (HALATA, 1975; IDE, 1977; SAXOD, 1978).

The lack of basal lamina and the incompleteness of the capsule of the lamellated corpuscle in turtle lymph heart coincide with the findings in the Meissner corpuscle (HALATA, 1975; IDE, 1976) and the lamellated nerve endings in reptilian skin (DÜRING, 1973, 1974). In the present study it is uncertain whether the capsule is continuous with the perineurium.

The lamellated corpuscles as described above have been considered to be mechanoreceptors (probably pressoreceptors in this case) which have rapidly-adapting characteristics (MUNGER, 1971; DÜRING and SEILER, 1974; CHOCHKOV, 1978; SAXOD, 1978). Although physiological details of the pressoreceptor of a reptilian lymph heart have not been studied, the receptor seems to contribute to the control of the lymph heart activity.

It has been reported that amphibian lymph hearts, which are better developed than reptilian ones, have no corpuscular nerve endings in the heart wall. This fact might obscure the importance of the corpuscular afferent nerves on the lymph heart function. However, the presence of a relatively large number of unmyelinated nerve fibers in the amphibian lymph heart wall (SATO and NITATORI, 1980) might indicate that at least some afferent nerve regulations are present in amphibian lymph hearts.

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