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Neural Network Influencing the Regulation of Locomotion in the Garden Snail *(Eobania vermiculata)*

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Abstract: The garden snail *Eobania vermiculata* crawls utilizing muscular waves which pass along the sole of the foot from posterior to anterior. In addition to the Pedal nerves supplying the foot, there is a subepidermal neural plexus embedded in foot muscu lature. Light and electron microscopical preparations revealed the sole plexus which consists of ganglionic knots contain neural cell bodies situated on the periphery of neuropile. In the neuropile synaptic junctions of axo-axonic typeare demonstrated. Clear and/or dense core granulated synaptic vesicles are frequently occur in the axon profiles. Single axons containing synaptic vesicles diverge from the neural plexus and contact the muscle cells forming neuromuscular junctions. So presence of peripheral neural network may be necessary for precise, rapid neural control over the small regions of the foot and must facilitate co-ordinated pedal movements. Furthermore, presence of different types of synaptic vesicles is a good evidence for the possible role of these inclusions as sites of excitatory and inhibitory transmitters.

Key words: *Eobania vermiculata*, neural plexus, neuromuscular junctions, synaptic vesicles

Introduction

Land pulmonates, in common with most other gastropods, are primarily adapted for locomotion over and adhesion to relatively hard substrate, and the foot is consequently a muscular organ with a large planter surface, the sole, with which the animal crawls and remains attached to the substratum. During locomotion waves of muscular contractions are produced and travel along the foot as the animal crawls forward (Jones,1975). The foot of these animals is supplied by branches of the pedal nerves descending from the central pedal ganglia. Bundles of nerve fibers branch and form a complicated plexus among the muscle fibers(Regers, 1969; Schulte, 1988 and Essawy et al., 1991). The analysis of locomotion has been further complicated by controversy as to the relative roles of the peripheral and central nervous system.

Reports of some investigators showed that, destruction of the pedal ganglia leads to complete locomotor paralysis in *Helix* but not in Limax (Bullock and Horridge, 1965). In Helisoma, Kater *et al.* (1971) observed contractions in the foot in response to mechanical disturbance even after complete extirpation of the entire central nervous system. More recently, Essawy (1988) reported that, in addition to the peripheral neural elements the activity from the central nervous system is necessary, at least, to initiate and maintain the locomotory waves in the foot musculature of *Arion*.

An electronmicroscopic investigation would help in the understanding of these structures, and research has been undertaken in an attempt to elucidate the morphology of the neuromuscular contacts in the foot of the snail *Eobania vermiculata* inhabiting the Egyptian gardens.

Materials and Methods

Adult specimens of the land snails *Eobania vermiculata* were collected from different gardens of Mamoura, Alexandria, Egypt. The collected snails were maintained in terrarium and fed on lettuce leaves. Head- foot region excised with sharp scissors from alive unanesthetized animals which were fully extended from their shells.

Small pieces of the foot were cut and prepared for light- and electronmicroscopical studies. For the demonstration of the peripheral neural plexus, fresh frozen sections were prepared, cut at 150 μm thickness and stained with gold chloride (Romeis and Mikroskopische, 1968). To throw more light on the structure of the ganglionic knots, histological sections were prepared from materials fixed in Bouin's fluid and stained with Hansen's triple stain (Romeis and Mikroskopische, 1968). For electronmicroscopy, the preparations were fixed in a mixture of 4% formaldehyde and 2.5% glutaraldehyde buffered with 0.1M phosphate buffer (pH 7.6) at 4°C. The specimens were then postfixed for 2h in 1% phosphate - buffered OsO_4. Dehydration was carried in graded series of ethanol and followed by treatment with propylene oxide and embedding in Epon-Araldite mixture. Sections were cut on LKB 2088 ultramicrotome with glass knives. The ultrathin sections were double stained with uranylacetate - lead citrate and were examined under Jeol CX electron microscope.

Results

Gold chloride preparations (Fig. 1) revealed that, in the foot musculature of *Eobania vermiculata* bundles of nerve fibers branch and form a coarse-meshed plexus with interstices of the order of a millimeter or more. The plexus includes a large number of nerve cells, lying both in the nodal accumulations (ganglionic knots) and along the strands. Sections of the ganglionic knots (90-110 μm in diameter) show that, they contain from 2-18 neural cell bodies situated on the periphery of the neuropile (Fig 2). Under the electronomicroscope, the cell bodies have large, round or ovale centrally placed nuclei with predominating euchromatin (Fig. 3). The cytoplasm of these cells showed numerous oval shaped dense mitochondria and well developed golgi bodies (Fig. 4). The center of the ganglionic knot is occupied by large neuropile areas. In neuropile, the axon endings are characterized by well-known cytoplasmic inclusions, notably an accumulation of synaptic vesicles (Fig 5). According to the size and morphology of these vesicles, different types of axon terminals were distinguished.

Type 1: Axons containing clear, agranular (V_1) vesicles with an average diameter of 30-90 nm (Figs. 5 & 7). These vesicles donot apparently present any content.

Type 2 : Axons containing dense core-granulated vesicles (V_2, Fig 5). The granules do not fill the vesicles completely but between the membrane and the inner granule there is a clear rim of variable width. This type of vesicles can be subdivided into (a) large dense–core granulated vesicles with an average diameter of 130 nm and (b) small dense- core granulated vesicles with an average diameter of 90 nm (Fig 6).

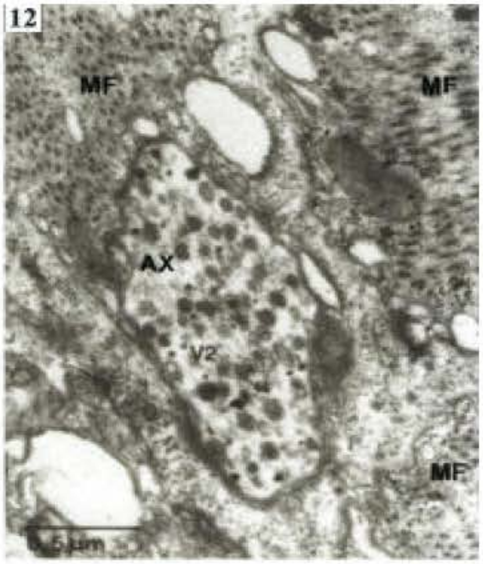

Fig. 12: Section through the foot musculature, showing an axon profile (Ax) containing dense-core granulated vesicles and forming neuromuscular junction with a muscle fibre (MF)

Type 3 : Axons containing mainly agranular clear vesicles, but some dense- core granulated vesicles are also found (Figs. 6 & 8).

Type 4: Axons containing mainly dense- core granulated vesicles mixed with some clear vesicles (Figs. 6 & 9).

In addition to the above mentioned types of axon-terminals, ther e are several axon profiles without synaptic vesicles but with mitochondria and prominent neurotubules (Figs. 5 & 9).

As in other gastropods, the synaptic junctions in the peripheral neural network of *Eobania* are often of axo-axonic (Figs. 7 & 8). Few axo-somatic contacts on the peripheral neurons are also demonstrated (Fig. 9).

In the axo-axonic junctions, synaptic contacts take place between complex axonal presynaptic ending and thin branchings of post synaptic axons. The synaptic zone proper is characterized in these synapses chiefly by : a well defined synaptic cleft, increased density of the opposed pre- and post synaptic membranes and the orientation of the synaptic vesicles toward the presynaptic membrane (Figs. 7 & 8).

Several thin nerve fibers containing synaptic vesicles diverge from the plexus and run in close contact with the smooth muscle fibers of foot (Figs. 10-12). Some of these nerve endings are completely surrounded by the plasma membrane of a muscle cell (Fig. 11). The neuromuscular junctions have a presynaptic structure containing clear or dense-core granulated synaptic vesicles, a narrow synaptic cleft and unspecialized synaptic membranes (Figs. 11 & 12).

Discussion

Light microscopical preparations showed that, the Pedal nerves of *Eobania vermiculata* descend toward the sole of the foot, occasionally branching. They then more or less abruptly go over into a coarse-meshed plexus. This kind of subepidermal neural plexus has been described in the foot of some gastropod molluscs (Schulte, 1988 and Essawy *et al.*, 1994). The plexus includes a large number of ganglionic knots in which the neural cell bodies are situated on the periphery of a central neuropile. This corresponds to the structure of central nerve ganglia of molluscs (Bullock and Horridge, 1965 and Steffens, 1980). According to Bidermann (1906), the more heavily ganglionated structure of the neural plexus allows more rapid and fine control of the small waves passing along the sole of the foot. Beside the ganglionic knots, solitary nerve cells are also found in the foot of *Eobania*. Peripheral neurons of different types and functions have been described in some muscles of other gastropods species (Bullock and Horridege, 1965 and Bailey *et al.*, 1975)

Electronomicroscopical examination of the neuropile of ganglionic knots demonstrates the presence of synaptic junctions which are often of axo- axonic type. Many axon profiles contain clear and/or dense core-granulated synaptic vesicles. Large variety of vesicle types are recommended in molluscan neuropiles (Bullock and Horridge, 1965; Musio and Bedini, 1990).

According to the hypothesis that each vesicle type could contain a different transmitter, it was reviewed that monamines (Serotonin [SHT], dopamine) are associated with granulated vesicles whereas acetylcholine (ACh) seems present mainly in clear vesicles (Gerschenfeld, 1973).

The single nerve fibers which constitute the terminal plexus in the foot musculature enter into close apposition with the muscle cells forming neuromuscular junctions. These junctions have unspecialized synaptic membranes, a narrow synaptic cleft and a presynaptic structure containing clear or dense-core granulated vesicles.Absence of the morphological specialization of the pre- and postsynaptic membranes is also a common feature of vertebrate autonomic nerve-smooth muscle junctions. Presynaptic endings containing clear and granulated vesicles have been described in the neuromuscular junctions of gastropods smooth muscles (Gerschenfeld,1973; Plesch, 1977; Benedeczky *et al.*, 1987 and Halasy *et al.*, 1988).

As in a variety of molluscan muscles, the foot musculature is controlled by excitatory and inhibitory nerve fibers (Bullock and Horridge, 1975 and Postma, 1946). Molluscan hearts appear to be influenced by both inhibitory fibers releasing acetylcholine and excitatory fibers releaxing 5-hydroxy tryptamine (5-HT) (Welsh, 1953). In the lamellibranch mollusc, *Mytilus edulis*, 5-HT can cause relaxation of tension in smooth muscle displaying a catch (Twar og, 1954). while acetylcholine was found to produce contraction of the same muscle (Twarog, 1967). In addition to monamines and acetylcholine other myoactive neuropeptides have been demonstrated in the nervous elements innervating different peripheral tissue in molluscs(Elkes, 2000; Buckett *et al.*, 1990 and Muneoka *et al.*, 2000).

In the absence of appropriate physiological and pharmacological studies on the gastropod foot, it is not certain which group of nerve fibers are excitatory or inhibitory. Results revealed frequent occurrence of nerve terminals containing different synaptic vesicles and contacting muscle fibers. This finding calls attention to the possibility that presence of peripheral neural network is necessary for precise, rapid neural control over small regions of the foot and must facilitate co-ordinated pedal movements. Furthermore, presence of different types of synaptic vesicles in the axon-terminals is a good evidence for the possible role of these inclusions as sites of excitatory and inhibitory transmitters.

References

Bailey, C.H., V.F. Castellucci, J. Koister and E.R. Kandel, 1979. Cellular studies of peripheral neurons in siphon skin of *Aplysia californica*. J. Neurophysiol., 42: 530-557.

Benedeczky, I., K. Halasy and M. Csoknya, 1987. Fine structure of the neuro muscular junctions in the alimentary tract of phylogenetically different animal species. Acta Biologica Hungarica, 38: 363-382.

Biedermann, W., 1906. Studien zur vergleichenden Physiologie der peristaltischen Bewegung. II. Die innervation der schnechensohle. E. Pflüger, Archiv für Physiologie, 111: 251-297.

Buckett K, J., M. Peters, G. J. Dockray, J. Van Minnen and P.R. Benjamin, 1990. Regulation of heart beat in *Lymnaea* by motorneurons containing FMRFamide-like peptides. J Neurophysiol., 63: 1426-1435.

Bullock, T.H. and G. A. Horridge, 1965. Structure and function in the nervous system of invertebrates. Vol. II. Freedman and Company, San Francisco und London,

Chase, R., 2000. Structure and function in the cerebral ganglion. Microscopy Research and Technique, 49: 511-520.

Elkes, K., 2000. Ultrastructural aspects of peptidergic modulation in the peripheral nervous system of *Helix pomatia*. Microscopy Research and Technique, 49: 534-546.

Essawy, A.E, N. Abdelmeguid and Y. Hassan, 1994. The ultrastructure of the neural plexus in the foot musculature of the desert snail *Eremina ehrenbergi*. Functional and Developmental Morphology, 4: 17-19.

Essawy, A., 1988. Neuroanatomische und physiologische untersuchungen zur Lokomotion von *Arion empiricorum* (L.). Disseration Technische Universität Braunschwig.

Fujisawa, Y., Y. Furukawa, S. Ohta, T.A. Ellis, N.C. Dembrow, L. Li, PD. Floyd, J.V. Sweedler, H. Minakata, K. Nakamura, F. Morishita, O. Matsushima, KR. Weiss and F.S. Vilim, 1999. The *Aplysia mutilus* inhibitory peptide-related peptides: Identification, cloning, processing, distribution and action. J. Neurosci, 19: 9618-9634.

Gerschenfeld, H.M., 1973. Chemical transmission in invertebrate central nervous system and Neuromuscular junctions. Physiological Reviews, 53: 1-119.

Halasy. K., I. Benedeczky, E. Fekete, L. Toth and R. Gabriel, 1988. Enteric neuromuscular junctions : Comparison of ultrastructural features in different phylogenetic groups. Neuroscience, 25: 147-162.

Ierusalimsky, V.N and I.S. Zakharov,1994. Mapping of neurons participating in the innervation of the body wall of the snail. Neurosci Behav Physiol., 1994; 24: 33-46.

Jones, H.D., 1975. Locomotion, In : Pulmonates. (Fretterv, Peak J. eds). New York, Academic Press, 1-32.

Kater, S.B., C. Heyer and J. Hegmann, 1971. Neuromuscular transmission in the gastropod mollusc *Helisoma trivolvis* : Identification of motoneurons. Z. vergl. Physiol., 74: 127-139.

Musio, K. and C. Bedini, 1990. Fine structure and axonal organization in the buccal ganglia nerves of *Aplysia* (Mollusca, Gastropoda). Zoomorphology, 110: 17-26.

Muneoka, Y., F. Morishita, Y. Furukawa, O. Matsushima, M. Kobayashi, M. Ohtani, T. Takahashi, E. Iwakoshi, Y. Fujisawa and H. Minakata, 2000. Comparative aspects of invertebrate neuropeptides. Acta Biol Hung, 51: 111-132.

Plesch, B., 1977. An ultrastructural study of the innervation of the musculature of the pond snail *Lymnaea staganalis* L. with reference to peripheral neurosecretion. Cell and Tissue Res., 183: 353-369.

Postma, N., 1946. De L' influence du ganglion susoesophagien sur les functions due pied a l' escagot (*Helix pomatia* Linne). Arch Neerl Zool., 7: 471-495.

Romeis, B., 1968. Mikroskopische Technik. R. Olden burg Verlag, München und Wien.

Rogers, D.G., 1969. Fine structure of smooth muscle and neuromuscular junctions in the foot of *Helix aspersa*. Z.Zellforsch. 99:315-335.

Schulte, W.I., 1988. Strukturelle untersuchungen des Nervennetz in Fuss von *Helix pomatia*. Diplomarbeit Technische Universität Braunschweig.

Steffens, H., 1980 The buccal ganglia of *Helix pomatia* L. Anatomy and histology. Zoomorphologie, 95: 195-212.

Twarog, B.M., 1954. The response of a molluscan smooth muscle to acetylcholine and 5-hydroxytryptamine. J Cell Comp Physiol., 44: 141-1673.

Twarog, B.M., 1967. The regulation of catch in molluscan muscle. J Gen Physiol., 50: 157-169.

Welsh, J.H., 1953. Excitation of the heart of *venous mercenaria*. Naunyn-schmiederbergs Arch Exp Path Pharmak, 219: 23-29.