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Fine Structural Analysis of the Neuromuscular Junction in the Venomous Organ of the Spider, *Agelena limbata* (Araneae: Agelenidae)

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Fine structure of the neuromuscular junction in the venomous organ of the spider, *Agelena limbata*, was studied using high magnification electron microscope. The motor nerve endings at neuromuscular contact area composed of neurons and neuroglial cells were located between musculature and extracellular sheath of the venom gland. At the synaptic contact between a motor axon and a muscle fiber in the musculature, spherical synaptic vesicles were prominent in the nerve terminal. The sarcoplasm beneath the neuromuscular synapse has a granular appearance and lacks myofilaments. And the main axon gives off a branch between the muscle fibers.

The synaptic regions of this organ are located close to the myofilaments unlike to other chelicerate classes. Moreover the postsynaptic complex of vesicles and membrane invaginations present in other synaptic regions are absent from these regions in this venomous organ.

KEY WORDS: Fine Structure, Neuromuscular Junction, Spider Venom Gland, *Agelena limbata*

All spiders with any kind of venom apparatus considered as venomous, but this does not mean that all of them are dangerous (Sutherland, 1972). Russell *et al.* (1973) considered only about 100 spider species as actually dangerous to man.

It has been known that the *Latrodectus* venom acts upom neuromuscular transmissions which release the transmitter acetylcholine from synaptic vesicles and extravesicular agglomerations (Clark *et al.*, 1970; Frontali *et al.*, 1976; Fritz *et al.*, 1980; Leung *et al.*, 1989; Hurlbut *et al.*, 1990). On the other hand, Sutherland (1972) established that the toxin of *Atrax robustus* acts upon neuromuscular endings, but differing from the toxin of *Latrodectus*, the venom of *Atrax robustus* changes the electrical field in nervous membranes (Spence *et al.*, 1977; Morgan and Carroli, 1977; Scheer *et al.*, 1986; Guth *et al.*, 1990).

It is clear that the spider venom is produced by one pair of venom gland of the cephalothorax (Maretić, 1987), and its secretion is regulated by the nervous system (Watanabe and Meldolesi, 1983; Love *et al.*, 1986). A large number of bioactive components from spider venoms have been isolated and characterized by several investigators (McCrone and Hatala, 1967, Bücherl, 1969; Bettini and Maroli, 1978), however the neuromuscular regulation of the venom glands is not yet fully understood.

A few years ago numerous neuromuscular connections were discovered within the venom glands of this arachnids (Moon, 1992a, b). But functional significance of these neuromuscular junctions is still obscure. Therefore, this paper reports detailed observations of the fine structure of neuromuscular junction and morphological aspects of the venom secretion within the venom

glands of the spider, *Agelena limbata*.

Materials and Methods

Adult spiders (*Agelena limbata* Thorell) of both sexes were collected from the suburbs of Seoul and rared in special cages of the Biology Department of Dankook University, Cheonan. Species identification was kindly performed by the Korean Institute of Arachnology, Seoul, Korea.

Spiders were sacrificed after paralysing with CO_2, and a Ringer's solution consisting of 160 mM NaCl, 7.5 mM KCl, 4 mM $CaCl_2$, 1 mM $MgCl_2$, 4 mM NaCl, 7.5 mM glucose, pH 7.4 (Groome *et al.*, 1991) was used during dissections. The venom glands were obtained from anterior part of the cephalothorax, and pre-fixed in a mixture of 2.5% paraformaldehyde and 2% glutaraldehyde buffered with 0.1 M Sorensen's phosphate buffer at pH 7.4 (Karnovsky, 1965). Specimens were post-fixed in 1% Osmium tetroxide for 2 hours at 4°C, dehydrated in ethanol and propylene oxide, and embedded in Polybed 812 (Polysciences, Inc., U.S.A.).

Semithin sections stained with toluidine blue were used to study the gross morphology. Ultrathin sections were cut with diatome or glass knives and picked up on grids without supporting film. Sections were double stained with uranyl acetate and lead citrate. Thin sections were examined using a JEM 100CX-II electron microscope (JEOL Ltd., Japan) at 80 kV.

Results

The venomous apparatus of the spider, *Agelena limbata*, is composed of chelicera and paired venom glands in the cephalothorax. Each glands is surrounded by bundles of striated muscular fibers resting on a extracellular sheath (or basal membrane). The gland musculature is covered by a thin adventitia which contains cross-banded collagen-like fibrils oriented in a circular pattern.

A sarcoplasmic reticulum and a transverse tubular system are very abundant. Between the musculature and extracellular sheath of the venom gland, the motor nerve endings at neuromuscular contact area which composed of neurons and neuroglial cells are located (Fig. 1). Motor axon among the musculature of the venom gland has numerous cytoplasmic components. A conspicuous Golgi apparatus is located very close to the granules. The Golgi cisternae usually appear to be filled with a dense material which is pinched off to form electron dense granules (Fig. 2).

Numerous synaptic contacts are formed by the axon. Glial processes occur between adjacent synapses. Each motor axon gives off numerous branches which extend over the muscle fiber surface, and each branch forms multiple synaptic contacts. Characteristically a lot of extracellular spaces were formed by an indentation of the muscle fibers or by a slight expansion of the extracellular sheath outward from the fiber surface (Fig. 3).

The efferent nerves reach the secretory epithelial cells traversing the extracellular gland sheath. Each nerve is formed by three components which are axons, glial cells, and a basal lamina. The surface of the glial cells is covered with a prominent basal lamina, which often contains distinct collagen fibrils. The smaller axon branches are embedded beneath the extracellular sheath of the sarcoplasmic membrane. The axon branches are enveloped by glial cell processes except at the point of synaptic contact between axon and muscle fiber (Figs. 4,5).

Along the musculature of the venom gland, neuromuscular synaptic contacts are formed by a motor axon and the muscle fibers. The main axon gives off a branch between the muscle fibers. At the synaptic contact, spherical synaptic vesicles were prominent in the nerve terminal. The muscle fibers are composed of two types of smaller units, thick and thin myofilaments, and have 8-12 thin myofilaments around each thick myofilament. The sarcoplasm beneath the neuromuscular synapse has a granular appearance and lacks myofilaments (Fig. 6).

The synapses have the usual details, including clusters of spherical and electron lucent vesicles of 45-60 nm diameter, axon and muscle fiber membrane densities and dense material in the

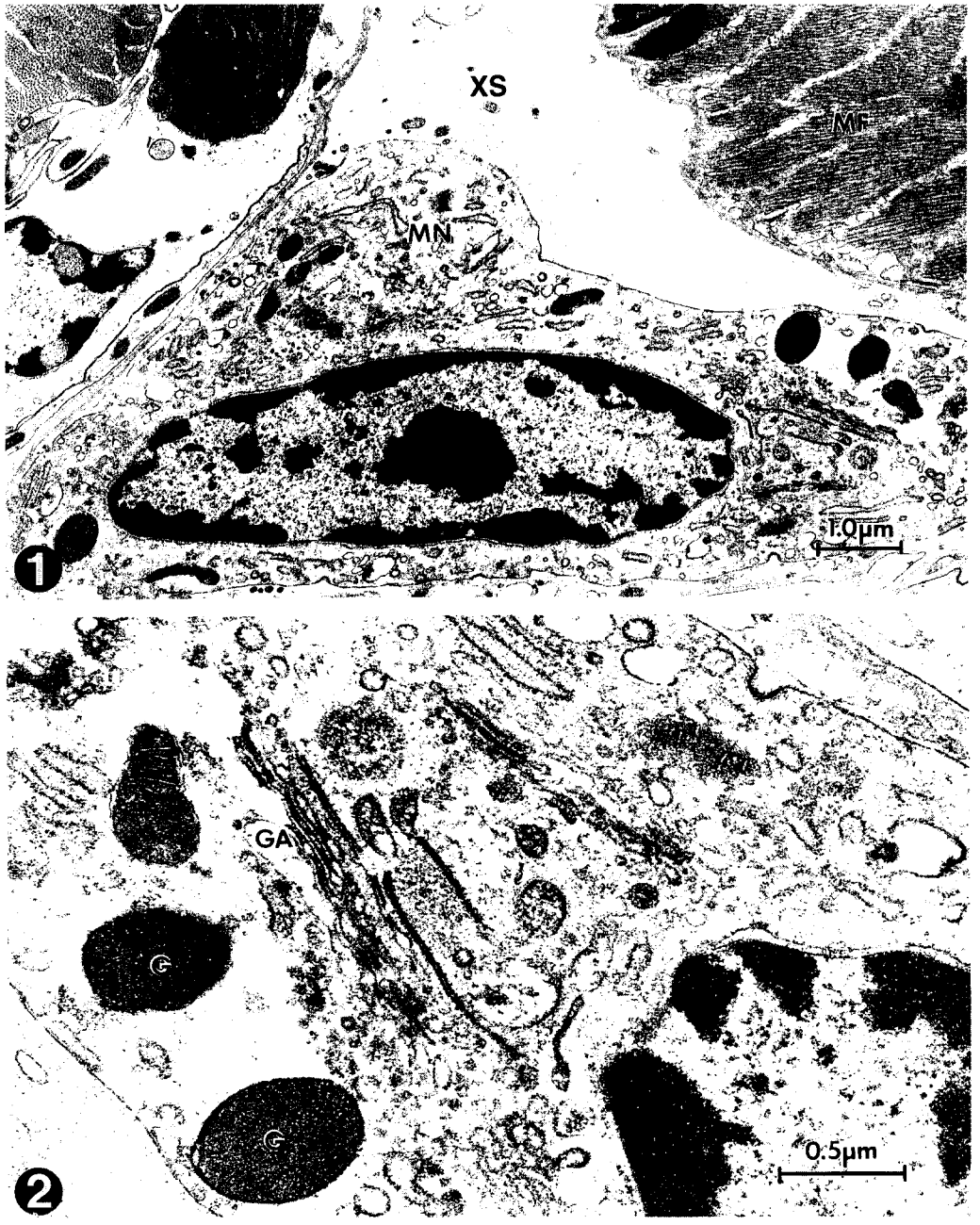


Fig. 1. Electron micrograph of neuromuscular synapses formed by a motor axon (AX) and two muscle fibers (MF) in the musculature of the venom gland in *Agelena limbata*. The main axon gives off a branch between the muscle fibers. Between the musculature and extracellular sheath of the venom gland, the motor nerve endings at neuromuscular contact area which composed of neurons and neuroglial cells are located. (× 12,000)

Fig. 2. High magnification electron micrographs of the cytoplasmic components of a motor axon of the venom gland. The motor axon has numerous cytoplasmic components. A conspicuous Golgi apparatus (GA) is located very close to the granules. The Golgi cisternae usually appear to be filled with a dense material (arrows) which is pinched off to form electron dense granules (G). (× 35,000)

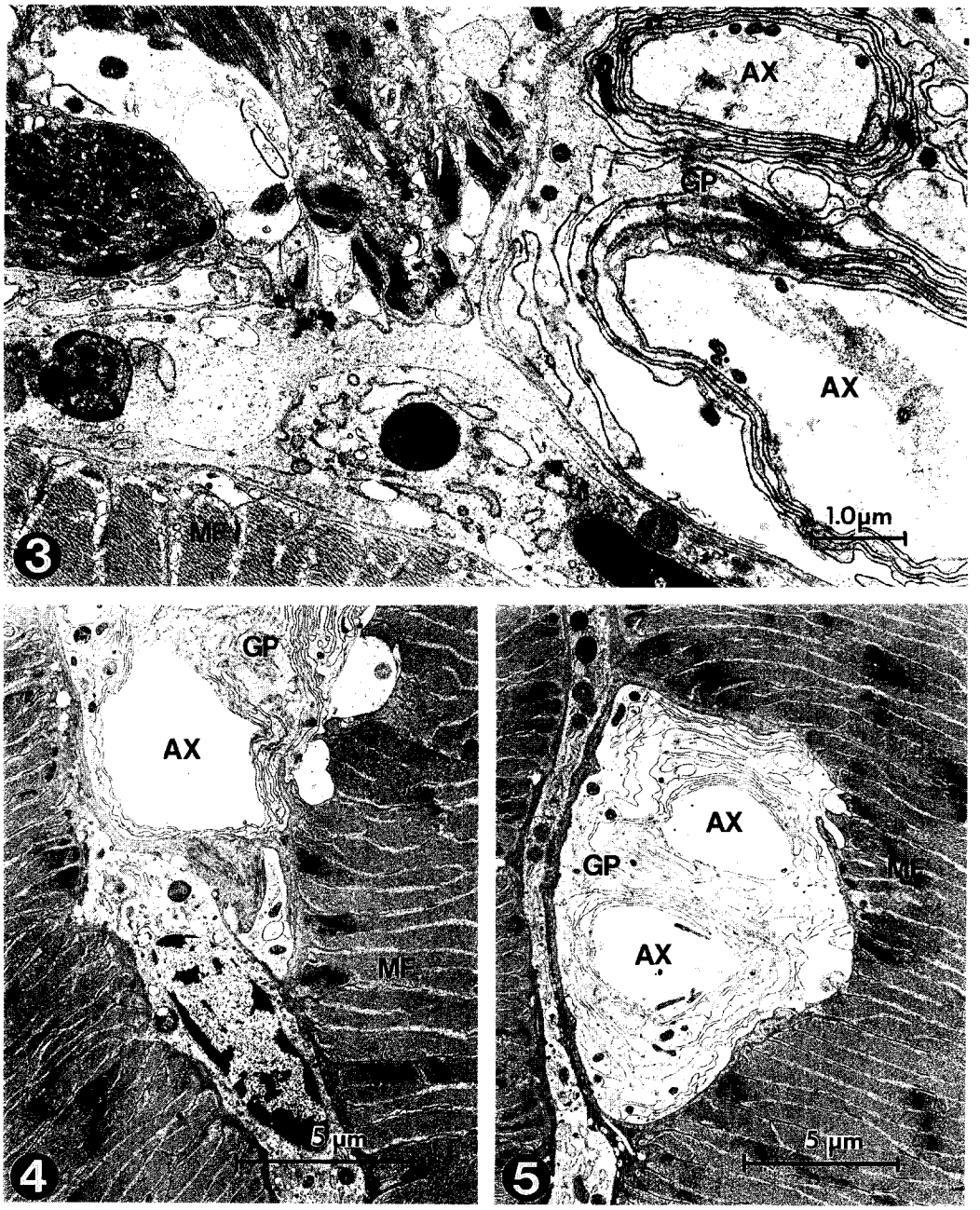
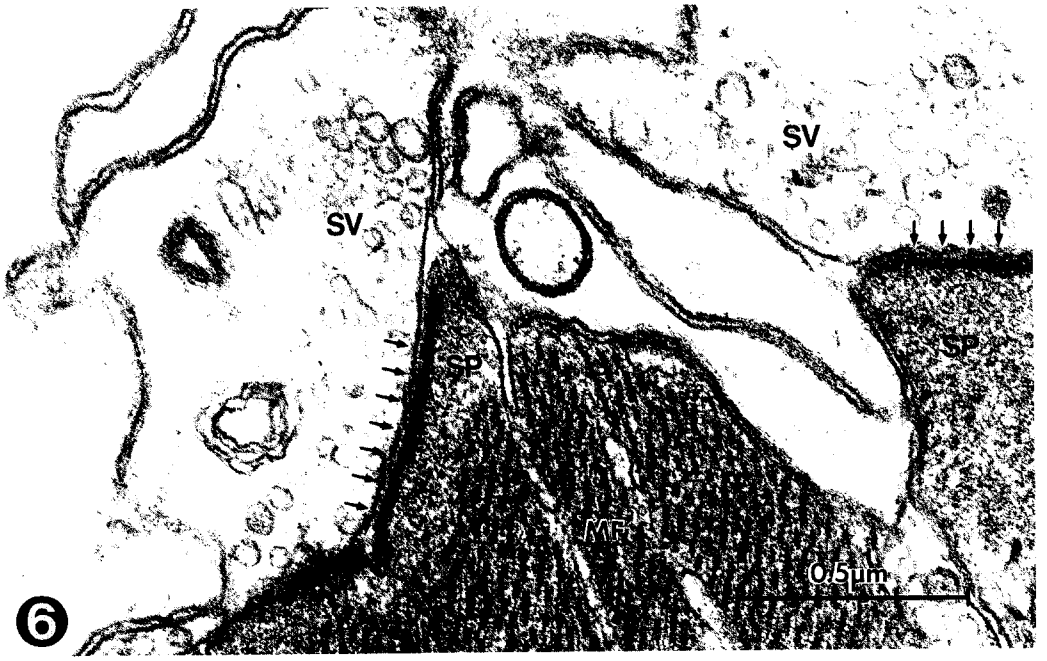


Fig. 3. Electron micrographs of neuromuscular system of the venom gland in the spider, *Agelena limbata*. Numerous synaptic contacts are formed by the axon, and glial processes occur between adjacent synapses. Each motor axon gives off numerous branches which extend over the muscle fiber surface, and each branch forms multiple synaptic contacts. (× 13,000)

Figs. 4, 5. Each efferent nerve is formed by three components which are axons, glial cells, and a basal lamina. These nerves reach the secretory epithelial cells traversing the extracellular gland sheath. The surface of the glial cells is covered with a prominent basal lamina, which often contains distinct collagen fibrils. The axon branches are enveloped by glial cell processes except at the point of synaptic contact between axon and muscle fiber (× 5,000) (× 5,000).



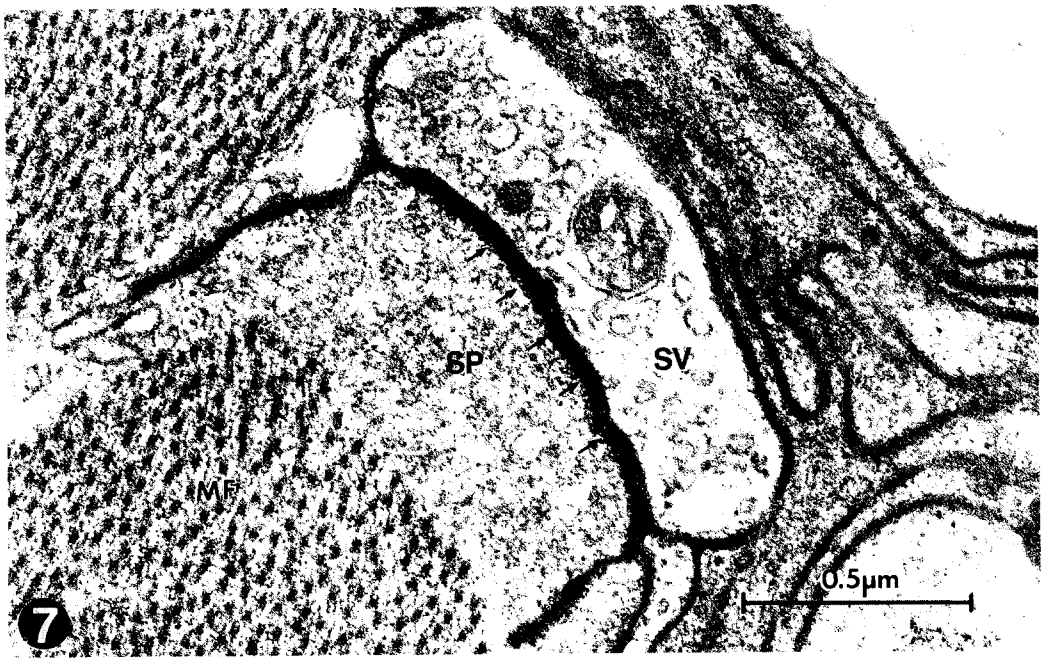


Fig. 6. Enlarged view of an area of synaptic contact between a motor axon and a muscle fiber in the musculature of the venom gland. Numerous spherical synaptic vesicles are prominent in the nerve terminal. The sarcoplasm beneath the synapse has a granular appearance and lacks myofilaments. Gap junctions formed by glial and muscle fiber membranes appeared. (× 62,000)

Fig. 7. Cross section of a nerve terminals embedded in muscle granular sarcoplasm. Synaptic contacts are recognized by dense staining of regularly aligned presynaptic and postsynaptic membranes. At this contact the presynaptic side has a dense body representing an active zone. (× 62,000)

synaptic cleft. The motor nerve terminals are characteristically populated with clear synaptic vesicles and make synaptic contact with the muscle granular sarcoplasm. At these synaptic contacts the membranes are densely stained and a dense body is found on the presynaptic membrane, denoting an active site for transmitter release (Fig. 7).

Discussion

In arachnids the venomous material is produced at the venom glands in the cephalothorax, and secretion of the venom is regulated by the neural control, especially at the region of neuromuscular junction (Watanabe and Meldolesi, 1983; Love *et al.*, 1986). Thus there were some published reports dealing with the ultrastructural features of neuromuscular synapses in arachnid skeletal muscles (Mealamed and Trujillo-Cenoz, 1971; Sherman and Luff, 1971; Fourtner and Sherman, 1972, '73). The results of these studies indicate that except for the number of axon terminals, the structural details of the synaptic regions are essentially the same of all of the arachnid muscles examined.

The gross morphology of the venomous apparatus of the spider is investigated by several workers (Russell *et al.*, 1973; Moon *et al.*, 1987; Moon, 1992a,'b). The musculature of the venom gland is covered by a extracellular sheath and a thin adventitia (Smith *et al.*, 1969). During the act of biting, the venom is ejected from the venom glands by contraction of the muscular walls (Maretić, 1987). The venomous apparatus of the spider, *Agelena limbata* is situated in the cephalothorax, and basically composed of chelicera and paired venom glands. The gland musculature is covered by a thin adventitia which contains cross-banded collagen-like fibrils oriented in a circular pattern.

The muscle fibers observed in this spider are transversely striated, contain thick and thin myofilaments, and have from 7 to 12 thin myofilaments surrounding each thick myofilament. Moreover a sarcoplasmic reticulum and a transverse tubular system are very abundant. This arrangement appears to be common to the chelicerates, for it also has been observed in horseshoe crabs (Fourtner and Sherman, 1972), spiders (Sherman and Luff, 1971), and scorpions (Gilai and Parnas, 1972).

Parnas *et al.* (1968) investigated the neuromuscular physiology of a chelicerate muscles, the claw closer of the horseshoe crab *Limulus polyphemus*. They found two excitatory neurons innervating this muscle, a fast and a slow axon. Furthermore, intracellular recordings made during stimulation of the motor nerve revealed that most of the fibers were innervated by three of the six neurons. However the neuromuscular synaptic contacts of the venom gland in *Agelena limbata* were formed by a motor axon and the muscle fibers. Each motor axon gives off numerous branches which extend over the surface of the muscle fibers perpendicular to the long axis of the muscle. The axon branches are embedded beneath the basement membrane portion of the sarcolemma and are enveloped by glial cell processes except at the synapses.

According to Fourtner and Sherman (1973) two types of synaptic areas were found in the chelicerates. The main differences lies in the location of the synaptid region. In horseshoe crabs the synaptic region is formed in a large evagination of the sarcoplasm, and evaginations from more than one muscle fiber may combine into one synaptic region. In the arachnids the synaptic area is formed in a slight furrow in the muscle fiber or along the surface of the fiber. The synapses observed in the venom gland of the spider, *Agelena limbata* are characterized by the presynaptic clustering of agranular vesicles and by the presence of dense material in the synaptic cleft. One of the most interesting features of the presynaptic organization at the synapse is the linear alignment of the synaptic vesicles next to the presumed release site (Fourtner and Sherman, 1972, '73).

In the venom gland of this spider, the efferent nerves reach the secretory epithelial cells traversing the extracellular gland sheath. It appears that their axoplasmic content shows a variety of neurosecretory products and electron lucent vesicles. Hamilton (1972) reported the high level

of free gamma aminobutyric acid (GABA) in the venom may indicate that this is the transmitter substance from the synapses in the venom gland. And Järlfors *et al.* (1969) also suggest that the presence of axon terminals between the secretory cells provides strong evidence for neural control of some aspect of the cyclic function for the gland.

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거미(*Agelena limbata* Thorell) 독 분비기관의 신경근육간 연접장치의 미세구조적 분석

문명진(단국대학교 자연과학대학 생물학과)

거미의 독선에서 독(venom)의 생성과 분비를 조절하는 신경조절장치인 신경근육간 연접장치(neuromuscular junction)는 나선상의 횡문근 섬유로 이루어진 근육층과 기저막 사이에 신경원과 신경교세포로 이루어진 운동성 신경의 말단이 근섬유 주변에 분지된 형태로 관찰되었다. 신경의 축삭 말단이 근세포와 연접된 부분에서는 시냅스 간극이 형성되어 있었고, 이 부위의 근세포질에는 근섬유가 소실되어 있음이 확인되었다. 또한 축삭은 근세포사이에 형성된 공간을 통해 분지되어 있었고, 축삭돌기 말단에는 신경전달물질을 함유한 구형의 시냅스 과립들이 집적되어 있었다. 특히 독선의 신경근육간 연접부는 다른 지주강에서 보고된 것과는 달리 근섬유에 근접되어 위치하였고, 신경 연접부에서 함입된 원형질막과 시냅스과립에 의해 형성되는 후시냅스 복합구조도 결여되어 있음이 확인되었다.