Korean J. Zool. 36:116-122, 1993

Immunocytochemical Localization of Serotonergic Neurons in Suboesophageal Ganglion of Cabbage Worm *Pieris rapae* (Insecta, Lepidoptera)

Jae-Weon Shim and Bong-Hee Lee

Department of Biology, Soonchunhyang University, P.O. Box 97, Onyang, Choongnam 336-600, Korea

An immunocytochemical investigation has been carried out to localize serotonin-immunoreactive (5-HTi) neurons in suboesophageal ganglion of fifth instar larva of cabbage worm *Pieris rapae*. The 28 5-HTi cell bodies were identified in the rind of suboesophageal ganglion. The four 5-HTi cell bodies of them are large in size (about 35 μm), while the remaining cell bodies are medium-sized (about 15 μm). The 5-HTi nerve processes are abundantly located in central large neuropil, circumoesophageal connectives which join the suboesophageal ganglion to the tritocerebrum of the brain, and connectives between the suboesophageal and the first thoracic ganglia. These results indicate that the 5-HTi nerve fibers, which constitute the central large neuropil, have structural connections with the above two connectives. Especially in central large neuropil, many 5-HTi nerve fibers form a large circular bundle, in which a 5-HTi nerve fiber bundle is crossing.

KEY WORDS: Immunocytochemical mapping, Serotonergic neurons, Suboesophageal ganglion, Cabbage worm

The suboesophageal ganglion of the insect controls the function of mandible, maxilla and labium of the head. In locust some of the neurons in the suboesophageal ganglion have been described to be involved in head movement and feeding (Altman and Kien, 1979). Altman and Kien (1979) have also reported that some suboesophageal neurons innervate the salivary gland of the locust. According to Park and Yoshitake (1971) the diapause of the silkworm was reported to be controlled by some neurosecretory cells located in the suboesophageal ganglion.

The recent publications have often mentioned the similarities between the neuroactive substances in vertebrate and invertebrate (Rémy *et al.*, 1979; Bishop and O'Shea, 1982, Könings *et al.*, 1988; Lee *et al.*, 1992). With the usage of immunocytochemical method Rémy *et al.* (1979) have demonstrated that some neurosecretory cells in the suboesophageal ganglia of the locust and the silkworm contain neuropeptide-like substances present in vertebrates.

Serotonin (5-hydroxytryptamine, 5-HT) has been described to play the roles as a neurotransmitter in the central nervous system of cockroach (Bishop and O'Shea, 1983), desert locust (Klemm and Sundler, 1983) and blowfly (Nässel *et al.* 1983), or neurohormone in the brain of *Locusta migratoria* (Könings *et al.* 1988). Lee *et al.* (1992) have also demonstrated that 5-HT play the role as a neurotransmitter in the neural networks of the postembryonic brains from cabbage butterfly.

Bishop and O'Shea (1982) have described the

This work was supported by Basic Science Research Fund in 1991 from the Ministry of Education, Republic of Korea.

localization of 5-HTi neurons in suboesophageal ganglion of the cockroach. Thereafter, the 5-HTi neurons were identified also in the suboesophageal ganglion of desert locust (Klemm and Sundler, 1983) and blowfly(Nässel *et al.*, 1983).

We were much interested in difference of localization of 5-HTi neurons in the suboesophageal ganglia of the various insects. Using the suboesophageal ganglion of the fifth instar larva of cabbage worm *Pieris rapae*, we investigated the localization of 5-HTi cell bodies and processes, and then discussed the difference of localization of 5-HTi neurons in comparison with the results described already in other insects.

Materials and Methods

The fifth instar larvae of cabbage worms, *Pieris rapae*, were collected from stock colony of Soonchunhyang University and used in this experiment. At temperatures of 28-30°C and relative humidities of 50-60%, the fifth instar larvae became adults about ten days later.

The heads and necks of the fifth instar larvae were together cut off with the sharp scissors, and fixed in 4% paraformaldehyde at 4°C for about 1 hr. The suboesophageal ganglia were dissected under the stereoscope. The isolated suboesophageal ganglia were continually put in new fixative solution at 4°C to complete the fixation for 2 hrs. They were washed three times in 0.01M phosphate buffer saline (PBS, 0.85%, pH 7.2), dehydrated in graded alcohols, and embedded in paraffin mixed with bee's wax. Thereafter, the embedded brains were cut transversely or horizontally into the serial sections of about 10 μm in thickness. All the sections were serially mounted on the gelatin-coated slides, and dried within an incubat or at 50°C.

The immunocytochemical staining of 5-HTi neurons was performed according to avidin-biotin-peroxidase (ABC) method of Hsu *et al.* (1981). Prior to the immunocytochemical staining, the paraffin on the sections was initially removed by treatment with xylene. They were hydrated in distilled water, treated in methanol containing 0.3% H_2O_2, and washed three times in PBS. Thereafter, the serial sections were reacted in goat normal serum (1:70, in PBS) for 20 min. They were then incubated in rabbit anti-serotonin antiserum (Incstar Co.) diluted to 1:1000 in PBS for 1hr at room temperature. After washes in PBS the incubation of the sections in goat secondary antibody associated with biotin (Vecta stain Lab.) were performed for 30 min at room temperature. They were then reincubated with avidin-biotin-peroxidase complex (Vecta stain Lab.) for 40 min. The reactions of the sections with 0.06% diaminobenzidine and 0.015% H_2O_2 were continued. Finally, all the serial sections were dehydrated, mounted in Euparal, and examined under the Normaski microscope.

Results

The 5-HTi cell bodies are located in the rind of suboesophageal ganglion (Fig. 1). The 5-HTi occurs in six pairs of cell groups composed of bilaterally symmetric cell bodies. These 5-HTi cell bodies can be divided into two kinds in size. The four 5-HTi cell bodies of the two most anterior pairs are large (about 35 μm) (Fig. 2). The remaining 5-HTi cell bodies in the suboesophageal ganglion are found to be medium-sized (about 15 μm).

The 5-HTi cell bodies are distributed in clusters (Fig. 2). The 6 5-HTi cell bodies are located in the anterior surface, while approximately half numbers (14) of 5-HTi cell bodies are present in the lateral surfaces. The remainings of all the 5-HTi soma present in suboesophageal ganglion have localizations in the dorsal or ventral surfaces. Hence, the total number of 5-HTi cell bodies in the suboesophageal ganglion of fifth instar larva is ca.28 (Fig. 2). As shown in lower left part of Fig. 1a, some of 5-HTi cell bodies project their axons into central large neuropil.

Numerous 5-HTi nerve processes are found in central large neuropil (Figs. 3). Most of 5-HTi nerve fibers show varicose arborizations in the neuropil, as shown in Fig. 1b (arrow). Many 5-HTi nerve fibers form a large circular bundle in the neuropil (Fig. 4). Also, within the circular bundle a large 5-HTi nerve fiber bundle is crossing between the left and the right (Fig. 4f). Some 5-HTi axon-

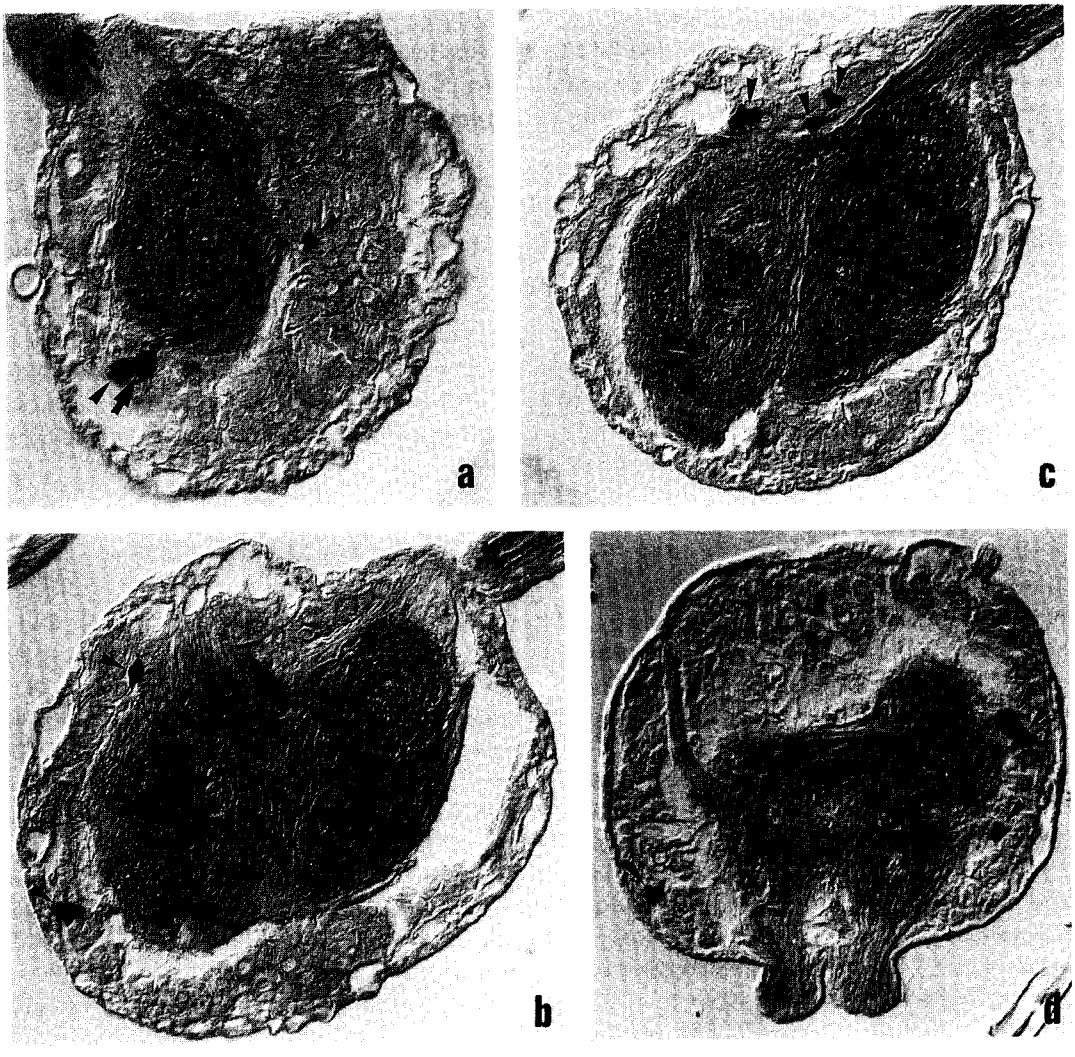
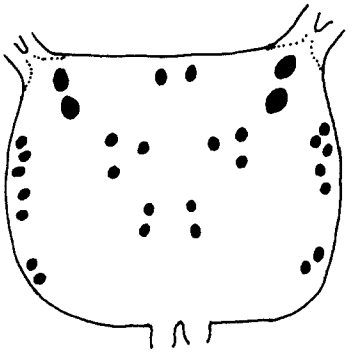


Fig. 1. Transverse or horizontal sections of suboesophageal ganglia containing serotonin-immunoreactive cell bodies (small arrows). (a-c) Transverse sections. A 5-HTi neuron (arrow) project into central large neuropil (np) (a). (d) Horizontal section. (a-c), × 560; (d), × 290.

like processes are found in the circumoesophageal connectives which join the suboesophageal ganglion to the tritocerebral lobes of the anterior cerebral ganglion (Figs. 1a, b, c, 2d). Some of these appear to contribute to the dense matrix of 5-HTi fibers found in the suboesophageal ganglion. This dense matrix is highly concentrated on the dorsal surface in the anterior region (Fig. 4a). Also, several 5-HTi axon-like processes are located in both connectives between the suboesophageal and the first thoracic ganglia(Figs. 1d, 4f).

Discussion

The 28 nerve cell bodies which react to anti-serotonin are located in the suboesophageal ganglion of the fifth instar larva. This number is very similar to that described in cockroach suboesophageal ganglion, which has 27 5-HTi cell bodies (Bishop and O'Shea, 1983). However, in the suboesophageal ganglion of locust (Tyrer *et al.*,



1984) and blowfly (Nässel and Cantera, 1985) there are smaller number of 5-HTi cell bodies than in cabbage worm and cockroach: 19 in locust and 4 in blowfly. One prominent feature of the 5-HTi

Fig. 2. Map of 5-HTi cell bodies in the suboesophageal ganglion of fifth instar larva (horizontal view). The total number of 5-HTi cell bodies are ca. 28. The four large (about 35 μm) 5-HTi cell bodies are located in the anterior surface, while 24 medium-sized 5-HTi soma are dispersed in clusters in suboesophageal ganglion.

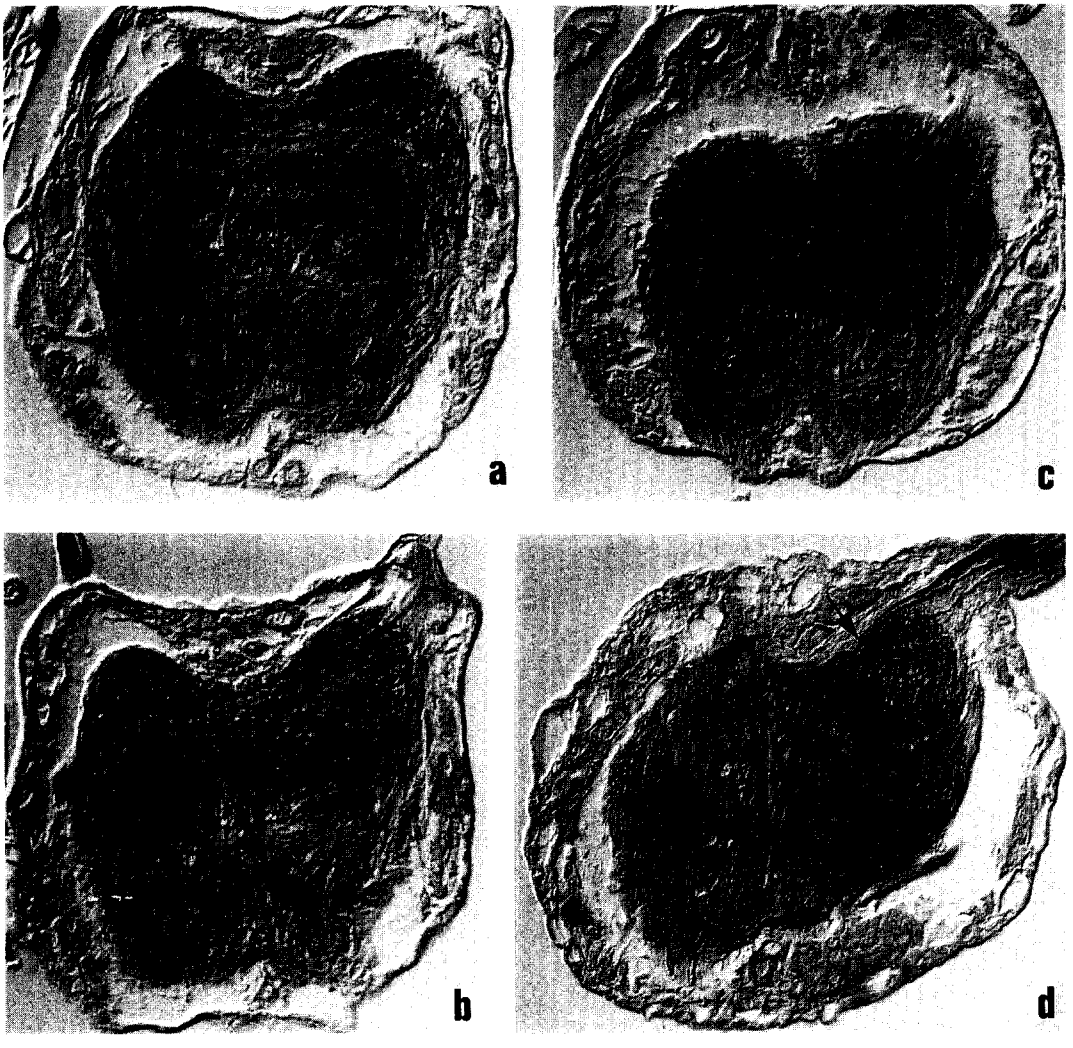


Fig. 3. Transverse or horizontal sections of suboesophageal ganglia containing many 5-HTi nerve processes in the central neuropils (np) and the nerve connective (nc). (a-c) Horizontal sections. A 5-HTi fiber shows rich arborization (arrow) (c). (d) Transverse section. A 5-HTi nerve fiber projected into the central large neuropil from the nerve connective is branched (arrow), indicating afferent 5-HTi nerve fiber (d). (a-d), × 280.

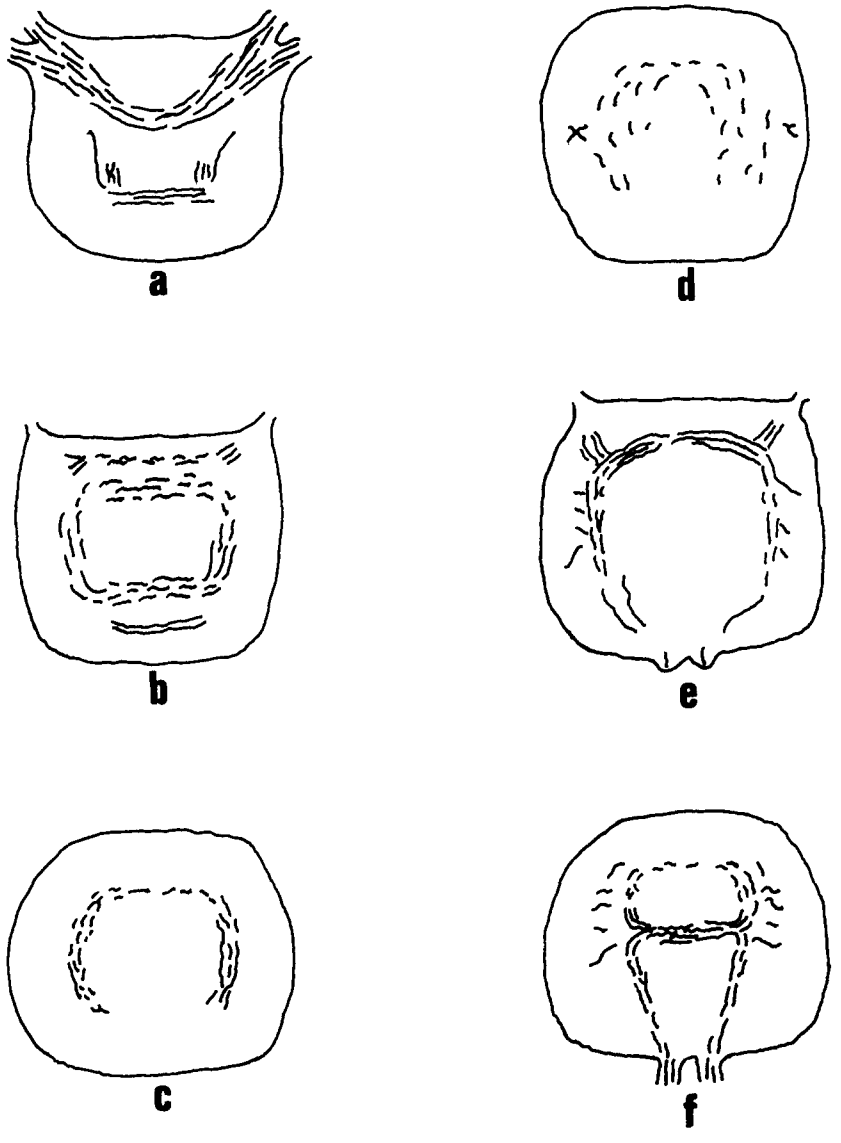


Fig. 4. Maps of 5-HTi nerve processes in the suboesophageal ganglion of fifth instar larva (serial horizontal views). The neuropils contain a large 5-HTi nerve fiber bundle which forms circular form. Also, the 5-HTi fiber bundles which are connected with the nerve comnective are located in the central large neuropils.

neurons is that they occur in relatively small numbers in the central nervous system of insects (Nässel, 1988). In the brain and suboesophageal ganglion(excluding the optic lobes) there are ca.64 5-HTi cell bodies in blowfly (Nässel and Cantera, 1985), ca.100 in cockroach (Bishop and O'Shea, 1983), and ca.75 in honey bee (Schürmann and Klemm, 1984). Including the optic lobes this would mean that ca. 0.03% of the blowfly neurons and ca. 0.01% of the honey bee neurons are 5-HTi.

Another feature of 5-HTi neurons is that their cell bodies tend to aggregate in clusters in the central nervous system including the suboesophageal ganglion, as in cabbage worm and many other insects. Clustering of cells with identical neuroac-

tive substances is common in the central nervous system of invertebrates and vertebrates (Klemm, 1976, 1985; Gardner and Walker, 1982; Steinbusch, 1981).

A few of 5-HTi cell bodies in the suboesophageal ganglion of fifth instar larva are large in size (about 35 μm), but most 5-HTi soma are medium-sized (about 15 μm) (Yang *et al.*, 1985). However, giant 5-HTi cell bodies (50 μm) cannot be found. In the suboesophageal ganglion of cockroach (Bishop and O'Shea, 1983) it has been demonstrated that one lateral pair of dorsal cell bodies are larger(ca. 50 μm in diameter) than the others.

Most of the 5-HTi neurons in the suboesophageal ganglion, as well as in other insect ganglia, are known to be interneurons (Nässel, 1988). Lee *et al.* (1992) have reported that some of 5-HTi neurons in postembryonic brains of cabbage butterfly are interneurons. Unfortunately, any 5-HTi neuron in suboesophageal ganglion of fifth instar larva could not be completely demonstrated to be interneurons.

The suboesophageal ganglia of cabbage worm contain afferent 5-HTi neurons (Figs. 1c, 3d), as described in locust (Tyrer *et al.*, 1984). In addition to the afferent 5-HTi neurons, efferent 5-HTi neurons are also located in the suboesophageal ganglion. These efferent 5-HTi neurons have been also mentioned in suboesophageal ganglion of the locust (Tyrer *et al.*, 1984). From these ganglia there are six to eight 5-HTi neurons projecting into the mandibular adductor muscle and four 5-HTi neurons innervating the salivary glands (Tyrer *et al.*, 1984; Klemm *et al.*, 1986). The 5-HTi neurons running to the salivary glands have been identified by means of cobalt infusion (Altman and Kien, 1979).

References

Altman, J.S. and J. Kien, 1979. Suboesophageal neurons involved in head movements and feeding in locusts. *Proc. Roy. Soc. Lond.* [Biol]. **205:** 209-227.

Bishop, C.A. and M. O'Shea, 1982. Neuropeptide proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH); immunocytochemical mapping of neurons in the central nervous system of the cockroach. *J. Comp. Neurol.* **207:** 223-238.

Bishop, C.A. and M. O'Shea, 1983. Serotonin immunoreactive neurons in the central nervous system of an insect (*Periplaneta americana*). *J. Neurobiol.* **14:** 251-269.

Gardner, C.R. and R.J. Walker, 1982. The roles of putative neurotransmitters and neuromodulators in annelids and related invertebrates. *Prog. Neurobiol.* **18:** 81-120.

Hsu, S.M., L. Ramine and H. Flanger, 1981. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.* **29:** 557-580.

Klemm, N., 1976. Histochemistry of putative neurotransmitters in the insect brain. *Prog. Neurobiol.* **7:** 99-169.

Klemm, N., 1985. The distribution of biogenic monoamines in invertebrates. *In*: Neurobiology. Comparative Aspects of Aminergic Neurons (Gilles, R. and J. Balthazer, eds.). Springer, Berlin.

Klemm, N. and F. Sundler, 1983. The organization of catecholamine-containing and serotonin-immunoreactive neurons in the corpora pedunculata in the desert locust *Schistocerca gregaria*. *Neurosci. Letts.* **36:** 13-17.

Klemm, N., R. Hustert, R. Cantera and D.R. Nässel, 1986. Neurons reactive to antibodies against serotonin in the stomatogastric nervous system and in the alimentary canal of locust and crickts (Orthoptera, Insecta). *Neuroscience.* **17:** 247-261.

Könings, P.N.M., H.G.B. Vullings, R. Siebinga, J.H.B. Diederen and W.F. Jansen, 1988. Serotonin-immunoreactive neurons in the brain of *Locusta migratoria* innervating the corpus cardiacum. *Cell Tissue Res.* **254:** 147-153.

Lee, B.H., J.W. Shim, K.S. Kim, C.H. Yu and J.W. Chung, 1992. Immunocytochemical mapping of serotonergic neurons in postembryonic brains of *Pieris rapae*. *Korean J. Zool.* **35:** 428-438.

Nässel, D.R., 1988. Serotonin and serotonin-immunoreactive neurons in the nervous system of insects. *In*: Progress in Neurobiology. Pergamon Journals Ltd. **30:** 1-85.

Nässel, D.R., M. Hagberg and H.S. Seyan, 1983. A new, possibly serotonergic neuron in the lamina of the blowfly optic lobe: an immunocytochemical and Golgi-EM study. *Brain Res.* **280:** 31-367.

Nässel, D.R. and R. Cantera, 1985. Mapping of serotonin-immunoreactive neurons in the larval nervous system of the flies *Calliphora erythrocephala* and *Sarcophaga bullata*. A comparison with ventral ganglia in adult animals. *Cell Tissue Res.* **239:** 423-434.

Park, K.E. and N. Yoshitake, 1971. Fine structure of the neurosecretory cell in the subesophageal ganglion of the silkworm, *Bombyx mori*. *J. Insect Physiol.* **17:** 1305-1313.

Rémy, C., J. Girardie and M. P. Dubois, 1979. Vertebrate neuropeptide-like substances in the suboesophageal ganglion of two insects: *Locusta migratoria* R and F (Orthoptera) and *Bombyx mori* (Lepidoptera). Immunocytochemical investigation. *Gen. Comp. Endocrinol.* **37:** 93-100.

Schürmann, F.W. and N. Klemm, 1984. Serotonin-immunoreactive neurons in the brain of the honey bee. *J. Comp. Neurol.* **225:** 570-580.

Steinbusch, H. W. M., 1981. Distribution of serotonin-immunoreactivity in the central nervous system of the rat. Cell bodies and terminals. *Neuroscience.* **6:** 557-618.

Tyrer, N.M., J.D. Turner and J.S. Altman, 1984. Identifiable neurons in the locust central nervous system that react with antibodies to serotonin. *J. Comp. Neurol.* **227:** 313-330.

Yang, H.Y., B.H. Lee and W.K. Kim, 1985. Morphological study on nerve cells in the subesophageal ganglion of adult cabbage butterfly (*Pieris rapae* L.). *J. Soonchunhyang Univ.* **8:** 157-160.

(Accepted December, 30)

배추벌레 5령유충의 식도하신경절에 분포하는 세로토닌 면역반응성 신경망의 구조

심재원 · 이봉희 (순천향대학교 생물학과)

배추벌레 5령유충의 식도하신경절에서 세로토닌 면역반응성 신경세포가 어떠한 신경연결망을 형성하고 있는지를 조사하였든바 그 결과는 다음과 같았다. 5령유충의 식도하신경절은 모두 28개의 면역반응성 신경세포를 포함하고 있었다. 이 신경세포들은 세포체 크기가 약 35 μm이거나 약 15 μm이었으며 4개가 35 μm이었고 나머지가 15 μm이었다. 35 μm크기의 세로토닌 면역반응성 세포체는 식도하신경절의 前端에 위치하였고 15 μm의 신경세포체는 신경절 전체에 분산되어 있었다. 또한 식도하신경절의 중앙에 위치한 신경망에는 많은 세로토닌 면역반응성 신경돌기들이 일정한 방향으로 뻗어 있었고 뇌와 식도하신경절 사이의 신경삭과 식도하신경절과 제일 흉부신경절 사이의 신경삭에도 구심성 또는 원심성인 세로토닌 면역반응성 신경돌기들이 뻗어 있었다. 식도하신경절 중앙의 신경망에서 관찰되는 세로토닌 면역반응성 신경섬유들은 큰 원형의 신경섬유속을 형성하였고 그 중앙에는 다른 세로토닌 면역반응성 신경섬유속이 좌우를 연결하고 있었다.