A Scanning Electron Microscope Study on the Autonomic Groundplexus in the Lamina Propria Mucosae of the Guinea-Pig Small Intestine

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Summary. The occurrence and fine structure of the autonomic groundplexus in the lamina propria mucosae of the guinea-pig small intestine were studied by scanning electron microscopy after removing connective tissue elements by maceration in hydrochloric acid. Transmission electron microscopy was also performed to investigate the fine structure of the neuronal and glial elements in the autonomic groundplexus. Nerve fasciculi consisting of neuronal processes and enteroglial cell sheaths formed a three-dimensional network intercalated between blood and lymph vessels. Processes of enteric neurons ran within the enteroglial cell framework. No blind ends of nerve fasciculi were found. Terminal vari-cosities of neuronal processes were frequently exposed on the surface of the nerve fasciculi. The locations of these naked varicosities may represent the sites of interaction between the enteric neurons and their target tissues.

Although many studies on the neuronal and glial elements of the enteric nervous system have been performed by transmission electron microscopy (TEM) (see GABELLA, 1979), the three-dimensional structure of the peripheral innervation apparatus in the gut is yet to be fully defined. Previous authors who studied the enteric nervous system by scanning electron microscopy (SEM) (FUJIWARA and UEHARA, 1980; KOMURO, 1982) have illustrated and discussed the fine structure and nature of some neuronal somata, their projections and “interstitial cells of Cajal”. However, these authors did not refer to the significance of each cellular element in the formation of the autonomic groundplexus (HILLARP, 1946, 1959).

The purpose of the present study is to clarify the architecture of the autonomic groundplexus in the enteric nervous system. The surface view of the nerve fasciculi in the lamina propria mucosae of the guinea-pig small intestine was examined by SEM using the HCl-maceration method. It was expected that this methodology would be useful in disclosing the features of various cellular elements in the autonomic groundplexus.
MATERIALS AND METHODS

Transmission electron microscopy
Two male guinea-pigs (Hartley strain, 300 and 450 g in body weight, respectively) were used. Animals anesthetized by an intraperitoneal injection of sodium pentobarbital solution (50mg/kg in body weight) were perfused with 2.5% glutaraldehyde buffered with 0.09 M phosphate at pH 7.1 from the descending aorta by means of a thin polyethylene tube. Pieces of the jejunum were removed and further fixed for several hours in the same glutaraldehyde solution followed by OsO₄ post-fixation. The fixed tissues were treated with 1.0% uranyl acetate solution for 1 hr, dehydrated with a graded series of ethanol, transferred to propylene oxide and embedded in Araldite epoxy resin. Thin sections were stained with Luft's lead solution and examined using a Hitachi H-700 electron microscope at 75 kV.

Scanning electron microscopy
Two male guinea-pigs (800 and 1,000 g in body weight, respectively) were anesthetized by an intraperitoneal injection of sodium pentobarbital and then decapitated. The small intestine was removed, cut open and immersed in a fixative containing 2% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 4°C for 16hr. After rinsing in the buffer, the mucosa was removed with forceps and placed in 8N HCl at 60°C for 30-50 min. After rinsing in warm distilled water (about 60°C), the specimens were conductive-stained by the tannin-osmium method of Murakami (1974), dehydrated in an ethanol series, transferred to isoamyl acetate, critical point-dried using liquid CO₂, sputtering-coated with gold and examined with a Hitachi S-430 SEM at 10 or 15 kV.

RESULTS

1. Transmission electron microscopy
Two kinds of cellular elements were demonstrated in the nerve fasciculi of the autonomic groundplexus in the lamina propria mucosae of the guinea-pig small intestine: 1) neuronal processes, and 2) enteroglial cells (Fig. 1).

Neuronal processes containing mitochondria, smooth-surfaced endoplasmic reticulum, microtubules and neurofilaments were grouped into bundles incompletely enclosed by an enteroglial cell sheath. The thickness of these neuronal processes was variable within a range of 0.5 to 2 μm in diameter. There were profiles of nerve terminal varicosities which contained large cored vesicles about 80 nm in diameter and/or small synaptic type vesicles about 40 nm in diameter.

Enteroglial cells possessed a chromatin-rich nucleus. Their cytoplasm was irregular in shape and enclosed neuronal processes. In the cytoplasm of enteroglial cells, were centrioles, Golgi complexes, mitochondria, granular endoplasmic reticulum and lysosomes. Microtubules and fine filamentous structures were also present.

It was frequently noted that the neuronal processes were only partially enclosed by the enteroglial cell sheath. There were neuronal processes which were covered only by the basal lamina. The latter covered the surface of the autonomic groundplexus and formed the boundary between the domain of the enteric nervous system and surrounding tissues.
2. Scanning electron microscopy

Nerve fasciculi formed an irregular network in the lamina propria of the guinea-pig small intestine (Fig. 2). Although there were severance-ends of nerve fasciculi apparently caused during tissue preparation, no “true” blind ends of nerve fasciculi were found. Small nerve fasciculi swelled at the sites where the enteroglial cell bodies were located (Fig. 3).

Some of the nerve fasciculi ran along the blood vessels (Fig. 3). Others ran beneath the network of flattened fibroblast-like cells which ensheathed the intestinal crypts (Fig. 2). A considerable number of thin nerve fasciculi were inserted into the space between the fibroblast-like cells and epithelial cells (Fig. 2).

At a higher magnification, neuronal and glial elements of nerve fasciculi could be identified. For convenience of description, we classified the patterns in the surface view of nerve fasciculi into two types: 1) the open, and 2) closed area (Fig. 4). The open area represented the region occupied by naked neuronal processes where bundles of varicose nerve terminals were directly seen. The closed area showed a smooth surfaced structure corresponding to the region covered by processes or cell bodies of the enteroglial cells. The surface view of the nerve fasciculi of the autonomic groundplexus consisted of a patchwork of these two patterns of structures.

The results of the present study are summarized in Figure 5.
HILLARP (1946, 1959) proposed the concept of the autonomic groundplexus in which he mentioned that the autonomic innervation apparatus is a fine-meshed network of anastomosing protoplasmic strands. However, he could not show a detailed morphology of the autonomic groundplexus by the techniques available at that time. In the present study we confirmed the existence of the autonomic groundplexus in the lamina propria mucosae of the guinea-pig small intestine by TEM and SEM. To our knowledge, the present paper is the first to describe the three-dimensional construction of

**Fig. 2.** A scanning electron micrograph of a part of the lamina propria mucosae of the guinea-pig small intestine. Nerve fasciculi (arrows) from a network around the crypt (C). F fibroblast-like cells, L leucocytes (probably granulocytes). $\times 3,400$
the autonomic groundplexus by SEM. It was demonstrated that the nerve fasciculi of the autonomic groundplexus in the lamina propria mucosae form an endless network. The framework of this autonomic groundplexus coincides with that previously demonstrated in light microscopic immunocytochemistry using an antiserum to S-100 protein (KOBAYASHI et al., 1986).

In the present study we demonstrated that the varicose nerve "terminals" were exposed in many places on the surfaces of the nerve fasciculi of the autonomic groundplexus. Here the enteroglial cell sheath is disrupted and openings of various sizes occur. It is likely that messenger substances released from the nerve terminal varicosities reach the targets more easily through these enteroglial discontinuities than otherwise. With their extensive varicose portions, the bundles of the neuronal processes diffusely innervate various kinds of cells in the epithelium and connective tissues of the mucous membrane. It is also likely that various stimuli from the surrounding tissues reach the nerve terminals through these openings of the enteroglial cell sheath.

The construction of the innervation apparatus in the enteric nervous system has long been a matter of dispute. KOBAYASHI et al. (1986) pointed out that the enteroglial cells containing S-100 protein resemble the special type of dendritic cells described by CAJAL (1893, 1911) under the name of the interstitial cells. CAJAL himself proposed that they may be primitive neurons such as those seen in Hydra, because they apparently

Fig. 3. Detailed view of a nerve fasciculus running along a blood vessel (B). The swollen part of the nerve fasciculus seems to correspond to the location of a glial cell body. Varicose neuronal processes (arrows) are seen on the surface of the nerve fasciculus. ×6,800
have long cytoplasmic processes which are identical with those of authentic neurons. However, KOBAYASHI et al. (1986) suggested that the theory proposed by CAJAL (1893, 1911)—stating that the interstitial cells are intercalated between the intramural neurons and the effector cells—must be discarded, since CAJAL’s concept of the interstitial cells was constructed on a misinterpretation of the products of Golgi’s silver impregnation method. The results of the present study showed no such existence of interstitial cells as described by CAJAL (1893, 1911). We conclude that, in place of the interstitial cells of Cajal, there is the autonomic groundplexus (HILLARP, 1946, 1959); ramifying processes of enteric neurons run within the enteroglial cell cord. This conclusion is based on the interpretation that the “anastomosing Schwann plasmodium” proposed by HILLARP (1946, 1959) correspond to the “framework consisting of enteroglial cells” proposed by KOBAYASHI et al. (1986).

We regard the basic structure of the enteric nerve plexus as a three-dimensional network consisting of the ganglia, nerve strands and autonomic groundplexuses. In the autonomic groundplexus, projections from different neurons converge into a bundle as proposed by HILLARP (1946, 1959). TAXI (1965) also recognized that the terminalplexuses in the gastro-intestinal tract consisted of bundles of neuronal processes; he used the term “innervation fasciculée” to describe this type of nerve terminal arrangement.

Concerning the distribution of neuronal processes, previous immunohistochemical studies of the enteric nervous system have indicated that each kind of peptide immunopositive neuron (peptide neuron) has a highly ordered pattern (SCHULTZBERG et al., 1980; FURNESS and COSTA, 1980; KOBAYASHI and NISHISAKA, 1985; ENDO et al., 1986; DANIEL et al., 1987). However, how each process of the peptide neuron terminates
in the gastrointestinal tissues remains unknown. In the present study we found no true end of any neuronal process. Further studies are required to identify the end apparatus of each neuronal process in or around the autonomic groundplexus.

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