Myenteric Enkephalin Neurons around the Laser-Photocoagulation Necrosis: An Immunocytochemical Investigation in the Guinea Pig Jejunum and Proximal Colon

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Summary. Laser irradiation-caused features of neuronal regrowth containing enkephalin-like immunoreactivity in the myenteric plexus around the necrosis were investigated in the guinea pig jejunum and proximal colon using an antiserum to methionine-enkephalin-Arg6-Gly2-Leu4. The power level and irradiation time of the argon laser beam were adjusted to make a coagulation spot of about 0.5 mm in diameter.

A distinct accumulation of the immunoreactivity occurred in the laser photocoagulated ends of the enkephalin neuron processes. Regeneration of the severed axon-like process took place in the following order: 1) swelling and budding at the severed proximal end; 2) elongation and branching of newly-formed nerve fibers; and 3) formation of a tangled overgrowth. Approximately 43-90 hrs after the laser microsurgery, immunoreaction of the enkephalin neuron perikarya was stronger in the first several rows of ganglia, on both the oral and anal sides of the laser necrosis. In the jejunum, an increase in the intensity of the immunoreaction was more prominent on the oral side than on the anal side, whereas the proximal colon displayed an opposite state. In the jejunum, the anally-directed axon-like process of the enkephalin neurons transported a larger amount of the immunoreactivity as compared with the orally-directed wing-ramuli.

This paper seeks to prove that the laser irradiation combined with immunocytochemistry may provide a simple and reliable methodology for the study of the projections of enteric neurons.

In recent years many neuropeptides have been localized within mammalian enteric nervous system using immunocytochemical methods (Furness and Costa, 1980; Schultzberg et al., 1980; Jessen et al., 1980; Leander et al., 1981; Furness et al., 1981; Tange, 1983; Llewellyn-Smith et al., 1983; Keast et al., 1984). It has been established that a significant proportion of myenteric plexus neurons contain an opioid peptide-like immunoreactivity (Alumets et al., 1978; Larsson et al., 1979; Bu’Lock et al., 1983; Furness et al., 1983b; Domoto et al., 1984; Kobayashi et al., 1984, 1985; Nihei and Iwanaga, 1985). These neurons are referred to as enkephalin neurons, because both methionine-enkephalin and leucine-enkephalin, which were found by Hughes et al. (1975), are the best known opioid peptides.
The categorization of various kinds of cytoplasmic projections is necessary for understanding the enkephalin neurons. Furness, Costa and coworkers examined the effects of microsurgical lesions, such as in vitro nerve crush and in vivo myotomy and myectomy, on the distribution and projections of various enteric neurons. Based on their observations in the guinea pig intestine, they deduced the shapes of the enteric neurons containing immunoreactivities for vasoactive intestinal polypeptide (Costa et al., 1980b), somatostatin (Costa et al., 1980c), substance P (Costa et al., 1981), serotonin (Furness and Costa, 1982; Costa et al., 1982), enkephalin (Furness et al., 1983b), pancreatic polypeptide/neuropeptide Y (Furness et al., 1983a) and gastrin-releasing peptide/bombesin (Costa et al., 1984). However, skillful surgical techniques and careful in vitro manipulations were required to obtain tissue preparations adequate for detailed examination. We anticipated that laser microsurgery would easily cause a precise lesion to a desired site in the enteric neuron network, and that it would provide a suitable substitute for the microsurgical methods used by Furness, Costa and coworkers.

The role of the enkephalin neurons in the nervous control of the intestinal activity is unknown. In the guinea pig small intestine, where peristalsis occurs, there are enkephalin neurons with a long axon-like process running in the anal direction (Furness et al., 1983b; Kobayashi et al., 1984), whereas in the proximal colon where antiperistalsis takes place (Elliott and Barclay-Smith, 1904; Hukuhara and Neya, 1968) a considerable number of enkephalin neurons possess an orally-directed axon-like process (Kobayashi et al., 1985). Thus, the present study of the regrowth structures of laser photo-coagulated enkephalin neurons in the myenteric plexus was performed with the aim of further examining the significance of the cytoplasmic projections of the enkephalin neurons under the control of the intestinal motility. We immunostained whole-mount preparations of perfusion-fixed intestinal tissues with the recently improved cytochemical procedures (Kobayashi et al., 1984, 1985). Preservation of the immunoreactivity and the fine structure of the enkephalin neurons seemed better than in the samples prepared by the previous methods (Costa et al., 1980a). Therefore, we could visualize a series of regrowth structures of the enkephalin neurons around the laser-photo coagulation necrosis (laser necrosis) in detail.

MATERIALS AND METHODS

Laser irradiation: The findings of a computer simulation experiment (Nishisaka et al., 1983), suggested that either the carbon dioxide (CO₂) laser or argon (Ar) laser produces the necrosis of whole layers of guinea pig intestine, without post-operative perforations. Therefore, we first performed a pilot study using both the Cavitron AO-300-G CO₂ surgical laser system and the Spectra-physics model 770-10 Ar photocoagulator. The results of this pilot study confirmed our expectations concerning the extent of the laser necrosis. However, in the present investigation, we only used the argon-laser for the purpose of making the experimental plan as simple as possible. The choice between using the CO₂ laser or the argon laser was an arbitrary decision. The laser power level and irradiation time were adjusted to make a coagulation spot of about 0.5 mm in diameter.

Experiment: Loops of the intestinal canal were withdrawn from the opened abdomen
Laser-Photocoagulated Myenteric Enkephalin Neurons

of eleven anesthetized male guinea pigs of the Hartley strain weighing 300–360 g in body weight and fed ad libitum. Several circumferential photocoagulation lines (at least 2–4 cm apart) were made on the jejunum (10–30 cm anal of the sphincter pylori), and the proximal colon (antimesenteric border, 2–15 cm anal of the ileocaecal junction), in order to sever the myenteric plexus of these intestinal segments. At different time intervals, which include: 5 (n = 2), 15 (n = 1), 19 (n = 2), 22 (n = 1), 25 (n = 1), 43 (n = 2) and 90 hrs (n = 2) after the operation, the animals were anesthetized with an intraperitoneal injection of a sodium pentobarbital solution (50 mg/kg b.w.). Next, their intestinal canal was perfusion-fixed with Bouin’s fluid through a polyethylene tube inserted into the thoracic aorta. Whole-mount preparations of the myenteric plexus layer, including the laser-irradiated area of the jejunum and proximal colon were prepared under a dissection microscope. Using an anti-methionine-enkephalin-Arg6-Gly7-Leu8 (Met-Enk-Arg-Gly-Leu) serum (R-0171), the tissue preparations were immunostained by the peroxidase-antiperoxidase method, as described previously (KOBAYASHI et al., 1984, 1985).

RESULTS

Distribution and structure of myenteric enkephalin neurons in the intact area

In both the jejunum and proximal colon, Met-Enk-Arg-Gly-Leu-like immunoreactivity was demonstrated in the perikarya of many ganglionic cells of the myenteric plexus. However, the exact proportion of the immunopositive and immunonegative neurons was undeterminable, because the intensity of the immunoreaction varied widely from cell to cell. There were many immunopositive nerve fibers in the myenteric ganglia and in the primary, secondary and tertiary fasciculi of the myenteric plexus. Varicosities of these immunopositive nerve fibers seemed to make direct contact with both the immunopositive and immunonegative ganglionic cells in the myenteric ganglia. Immunopositive nerve fibers in the internodal fasciculi were usually non-varicose. Large swellings of immunopositive nerve fibers were occasionally found which measured up to 10 μm in diameter (not illustrated).

Enkephalin neurons around the laser necrosis

A series of various tissue reactions occurred in and around the site of laser irradiation. Structural changes took place on both proximal and distal segments of enkephalin neurons which were injured by laser photocoagulation. However, the following description will focus on the regrowth structures of the proximal segments. The abrupt transition from the intact area to the necrotic area was one of the most characteristic features of the laser necrosis.

As shown in Figures 1 and 2, the laser irradiation-severed area contained no distinctly shaped immunopositive neurons. The general architecture of the ganglia and internodal fasciculi was preserved, though no cellular details of the necrotic myenteric plexus layer remained.

Directions of transport of the immunoreactivity in the jejunum and proximal colon

As early as 15 hrs after the laser microsurgery, a series of structural changes in the photocoagulated stumps of enkephalin neuron processes started (Fig. 1, 2). The jejunal myenteric plexus showed bulbous terminal expansions of enkephalin neuron processes on the oral side of the necrosis; these terminal expansions were larger than those on
Fig. 1. **A**–**D**. Met-Enk-Arg-Gly-Leu immunopositive neurons in whole mounts of the guinea pig myenteric plexus after laser microsurgery. A series of low-power views of the myenteric plexus is shown. The top of each picture is arranged in the oral direction. ×40  
**A.** 5 hrs. Early expansions of the proximal end of the severed enkephalin neurons are seen. Accumulation of Met-Enk-Arg-Gly-Leu-like immunoreactivity on the oral margin of the necrosis is more substantial than that on the anal margin. **B.** 19 hrs. Each proximal end of the enkephalin neuron processes develops a club-shaped appearance, however the oral stumps are much larger. **C.** 43 hrs. Proliferative masses of the severed end of the primary fasciculus are bigger on the oral side than on the anal side of the necrotic area. Perikarya of the enkephalin neurons on both the oral and anal sides of the laser necrosis show an increased Met-Enk-Arg-Gly-Leu-like immunoreactivity. **D.** 90 hrs. Distribution pattern of regenerating fine fibers resembles that of the original myenteric plexus. *g* Myenteric ganglion, *n* necrotic area. Arrowheads indicate the approximate position of the original lesion. The pictures were separated at the center of the laser necrosis in order to cut the gap.
Laser-Photocoagulated Myenteric Enkephalin Neurons

In the proximal colon, similar terminal expansions of severed enkephalin neuron processes lay on both the oral and anal sides of the photocoagulated tissues (Fig. 2). However, contrary to the situation in the jejunum, larger swellings were seen on the anal side of the necrotic area.

In a guinea pig perfusion-fixed at 15 hrs after laser microsurgery, numbers of immunopositive nerve fibers with a distinct swelling were counted per internodal fasciculus. In the myenteric plexus of the jejunum, there were $12.8 \pm 4.3$ (mean $\pm$ S. D., $n=19$) swollen fibers per fasciculus on the oral side and $6.6 \pm 1.7$ ($n=21$) on the anal side.

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Fig. 2. A-D. Myenteric enkephalin neurons of the guinea pig proximal colon after laser microsurgery. $\times 50$. A. 5 hrs. Swellings of the severed end of enkephalin neuron processes accumulate the Met-Enk-Arg-Gly-Leu-like immunoreactivity. B. 15 hrs. Immunopositive regrowth structures are slightly more prominent on the anal side of the laser lesion than those on the oral side. The pattern of myenteric plexus is visible in the laser necrosis. C. 43 hrs. No formation of the amputation neuroma or pseudoneuroma takes place in the proximal colon. Regrowth of immunopositive fine fibers is more prominent on the anal side of the lesion than on the oral side. D. 90 hrs. Regrowth of the fine fibers on the anal margin of the laser necrosis is more prominent on the anal side than that on the oral side. Regeneration of the enkephalin neuron processes tends to take place along the pattern of the pre-existing myenteric plexus.
On the other hand, in the proximal colon, there were 7.9±3.1 (mean±S. D., n=24) swollen fibers per fasciculus on the oral side and 9.9±2.8 (n=20) swollen fibers on the anal side. However, there were many faint fibers with a small swelling. These fibers were not counted in this investigation, because discriminating between individual fibers was difficult. Therefore, the figures indicated above probably underestimate the numbers of immunopositive fibers projecting in each direction.

**Swelling and budding of the severed end of the enkephalin neuron processes**

Examples of immunopositive terminal expansions of the severed nerve fibers were illustrated in Figure 3 A, B. Their immunocoloration intensity was striking. They varied considerably in shape; many were oval or conical, and some were rod- or spindle-shaped.

Budding of the immunopositive terminal expansions of severed nerve fibers occurred within 5 hrs of laser microsurgery. Immunoreaction intensity tended to increase with the duration of time. A few sprouts emanated from some of the growing terminals (Fig. 3B). Twenty-two hours after laser microsurgery, many fine fibers originated from every immunopositive terminal expansion. These sprouting fibers grew in length as time passed, whereas the terminal expansions themselves rapidly decreased in size and became distorted in shape (Fig. 3 C, D).

**Overgrowth of regenerating enkephalin neuron processes**

One immunopositive terminal expansion extruded multiple fine fibers, and these newly-formed fibers produced multiple off-shoots (Fig. 3 C, D). Most of these regrowth fibers grew in the remnant of myenteric plexus in the direction of the original enkephalin neuron processes (Fig. 1, 2). However, there were also a few retrograde fibers which arranged themselves spirally and formed a tangled hank around the bundle of nerve fibers. A typical example of such an overgrowth was found 25 hrs after the laser microsurgery, illustrated in Figure 4 A.

Fourty-three hrs after the laser microsurgery the bulbous terminal expansions of enkephalin neuron processes disappeared. Several fine fibers arose from one old fiber. They were long and complicated, and densely packed in the remnant of the myenteric plexus. Thus, the new fibers formed a tangled mass (amputation neuroma or pseudo-neuroma), as shown in Figure 4 B.

Ninety hours after the laser microsurgery the growth of the sprouts advanced further. Bundles of fine immunopositive fibers with varicosities tended to be packed in the remnant of the myenteric plexus. Unaligned bundles of fine immunopositive fibers were also found along the circular muscles. In the jejunum, these regrowth fibers were remarkably more prominent on the oral side, whereas, in the proximal colon, the situation was reversed (Fig. 2 C, D).

**Reaction of the perikaryon to the laser-photocoagulation**

One of the most striking features of the enkephalin neurons after laser microsurgery may be the increase in the immunoreactivity of the perikaryon (Fig. 5A, B). Approximately 43–90 hrs after laser microsurgery, immunoreaction of many enkephalin neuron perikarya was stronger in the nearest several of rows ganglia on both the oral and anal sides of the laser necrosis as compared with those at greater distance (ten rows or more). The increase in the intensity of immunoreaction demonstrated on the oral side of the necrotic area was more substantial than that on the anal side (Fig. 6 A, B). However, the situation was reversed in the proximal colon; perikarya of increased immunoreac-
Fig. 3. A-D. Examples of Met-Enk-Arg-Gly-Leu immunopositive terminal expansions of the proximal segment of the severed myenteric plexus neuron processes. All these pictures were taken from the oral margin of the necrotic area prepared in the jejunum. × 1,100. A. Early immunopositive terminal expansions (5 hrs). B. Typical club-shaped swellings (19 hrs). C. Several short sprouts lying on the immunopositive terminal expansion (15 hrs). D. Growing sprouts of the immunopositive terminal expansions of severed nerve fibers. It is already impossible to show the exact three-dimensional arrangement of the newly-formed nerve fibers.
S. Kobayashi and T. Nishisaka:

Activity were apparently more numerous on the anal side of the laser necrosis (Fig. 2 C, D).

As shown in Figures 5 A and 6 A, one myenteric ganglion of the nearest two or three rows on the oral side of the laser necrosis contained several strongly-immunopositive neurons with distinct winglets associated with wing-ramuli. The intensity of the immunoreaction of the anally-directed axon-like processes of these neurons was striking (Fig. 5 A).
Fig. 5. A and B. Enkephalin neurons whose immunoreactivity was intensified by the laser-photocoagulation. ×580. **A.** Strongly immunostained enkephalin neuron perikarya in a myenteric ganglion 1–2 rows oral to the laser necrosis (90 hrs). **B.** Myenteric ganglion 2 rows anal to the laser necrosis. It contains several immunopositive perikarya of ganglionic cells which are not sharply demarcated. Immunopositive wing-ramuli originating from the perikaryon (arrowheads) reach the tangled overgrowth at the anal margin of the laser necrosis. The thick arrow indicates the anal direction.
Fig. 6. A and B. Distribution and organization of the enkephalin neurons whose immunoreactivity was intensified by the laser-photocoagulation of their cytoplasmic projections (90 hrs). The top of the pictures is arranged in the oral direction. ×180 A. Regrowth features of the enkephalin neurons on the oral side of the laser necrosis (n) (jejunum). B. Regrowth features of the enkephalin neurons on the anal side of the laser necrosis (n) (jejunum). The immunoreactivity of some myenteric plexus neurons has increased; however, its intensity is lower than that on the oral side. Cells indicated by the arrowheads projects wing-ramuli running in the oral direction.
These strongly-immunopositive neurons decreased in number as the ganglion became more distant from the necrotic area. In the jejunum, the longest distance from the intensely-immunostained enkephalin neuron to the laser necrosis was eight rows of ganglia (3.5 mm).

On the anal side of the laser necrosis in the jejunum, as on the oral side, the number of immunopositive neurons remarkably increased in the first five rows of myenteric ganglia. However, the intensity of the immunoreaction was less prominent than on the oral side (Fig. 5B, 6B). These immunopositive neurons were not sharp in outline, and the winglets on their perikaryon usually remained indistinct (Fig. 5B). Many fine varicose processes, which probably represent wing-ramuli of some enkephalin neurons, apparently reached the tangled overgrowth at the anal margin of the laser necrosis (Fig. 5B, 6B). The axon-like process of the enkephalin neurons on the anal side of the laser necrosis remained difficult to detect.

**Nerve terminal varicosities**

There were many profiles of immunopositive nerve terminal varicosities in the myenteric ganglia. The myenteric ganglia near the necrotic area and those away from it were carefully compared. No differences were detected with regard to the number, shape, size, distribution and immunoreaction intensity of the immunopositive nerve terminal varicosities.

**DISCUSSION**

The results of the present investigation showed that laser irradiation precisely and effectively severed the myenteric enkephalin neurons. The flow of the Met-Enk-Arg-Gly-Leu-like immunoreactivity synthesized in the perikaryon stopped at the margin of the laser necrosis. Large immunopositive swellings of the enkephalin neuron processes occurred on both the oral and anal sides of the laser necrosis, indicating that the enkephalin neurons send processes in both the oral and anal directions.

Figure 7 represents a series of morphological changes of the enkephalin neurons in the jejunal myenteric plexus severed by laser beam irradiation. The budding of the immunopositive terminal expansion begins at a time when it is still expanding (5 hrs). One terminal expansion projects several sprouts which produce multiple offshoots, and then the terminal rapidly atrophies in size (15-22 hrs). A part of the growing fibers pass spirally around the longitudinally-running immunopositive fibers. Thus, a tangled overgrowth of the severed internodal fasciculus occurs (25-43 hrs). There is a tendency for the regenerating nerve fibers to spread out in the pattern of the former myenteric plexus (90 hrs). On the oral side of the laser necrosis, when the anally-directed axon-like process is severed, a large amount of the immunoreactivity rapidly accumulates in the perikaryon of the enkephalin neurons (43-90 hrs). The winglets and wing-ramuli probably become visible due to the reverse flow of the immunoreactivity. On the anal side of the laser necrosis, however, severing the orally-directed wing-ramuli slightly increases the immunoreactivity in the enkephalin neuron (43-90 hrs). In this case, the immunoreactivity of the axon-like process is apparently unchanged.

The immunocytochemical techniques which we used in the present study were apparently more sensitive than the previous method (Costa et al., 1980a). However, it was still impossible to demonstrate the weakest immunoreactivity in the thin
Therefore, the absence of the demonstrable projections does not necessarily mean the non-existence of enkephalin neuron processes. In the present study, the number and size of immunopositive expansions in the jejunum were remarkably larger on the oral side of the laser necrosis than on the anal side, whereas immunopositive nerve terminal expansions in the proximal colon were more conspicuous on the anal side as compared to those on the oral side. Furness et al. (1983b) showed that many enkephalin neurons in the guinea pig small intestine sent a single prominent long process (axon-like process) in the anal direction, an observation confirmed by us in the guinea pig duodenum (Kobayashi et al., 1984). Furthermore, in

![Fig. 7. Regrowth structures of laser-photocoagulated enkephalin neurons. A series of morphological changes of immunopositive myenteric plexus neurons on both the oral and anal sides of the laser necrosis is schematically represented. Intact myenteric plexus (Intact) contains enkephalin neurons with an anally-directed axon-like process. At 5 hrs after the laser microsurgery, terminal expansions of the laser-photocoagulated proximal segment of the enkephalin neuron processes are present. Approximately 15 hrs after the laser microsurgery, budding of the immunopositive terminal expansions occurs. At 43 hrs after the laser microsurgery, the formation of tangled overgrowth of nerve fibers takes place. The new sprouts produce multiple off-shoots. Immunoreactivity in the perikaryon of enkephalin neurons near the laser necrosis gradually increases with time. At approximately 90 hrs after the laser microsurgery, there is a remarkable increase in the immunoreactivity of enkephalin neurons whose anally-directed axon-like process was severed. The moderate increase of the immunoreactivity of enkephalin neurons whose wing-ramuli were severed is also illustrated. Regeneration of both axon-like processes and wing-ramuli follows the pre-existing pattern of the myenteric plexus. For detailed explanation see text.]
the proximal colon of the guinea pig, approximately 70% of the axon-like processes were orally-directed while about 10% ran anally (KOBAYASHI et al., 1985). Thus, the experimental data on the direction of the enkephalin neuron processes coincide with previous morphological findings; we demonstrated only the thickest population of the enkephalin neuron processes in the previous studies (KOBAYASHI et al., 1984, 1985). The functional significance of the direction of the enkephalin neuron processes in the different segments of the intestine, in regard to peristalsis and antiperistalsis (ELLIOTT and BARCLAY-SMITH, 1904; HUKUHARA and NEYA, 1968), needs further investigation.

DOGIEL (1899) described three neuron categories in the enteric ganglia: type 1 neurons characterized by numerous short dendrites and a single long axon are motor in function; type 2 neurons have many slender dendrites and a long axon, and are sensory in nature; and, type 3 neurons, possessing dendrites which terminate in the ganglion of origin, having an unknown function. FURNESSE et al. (1983b) demonstrated that a majority of enkephalin neurons in the guinea pig small intestine possessed many short processes and a single long process. BORNSTEIN et al. (1984) proposed that enkephalin neurons in the guinea pig ileum were all Dogiel's type 1 cells. On the other hand, we were able to show that there were many unclassifiable enkephalin neurons in the guinea pig duodenum and proximal colon (KOBAYASHI et al., 1984, 1985). We proposed that re-examination of Dogiel's criteria for the classification of enteric neurons was needed for the understanding of the features and functions of the enkephalin neurons (KOBAYASHI et al., 1985). Furthermore, concerning the cellular shape in the myenteric ganglia, our impression was that the difference was greater in the enkephalin neurons themselves than in the previously-studied neurons containing various neuropeptide/neuropeptides such as vasoactive intestinal polypeptide (COSTA et al., 1980b), somatostatin (COSTA et al., 1980c), substance P (COSTA et al., 1981), pancreatic polypeptide/neuropeptide Y (FURNESSE et al., 1983a) and gastrin-releasing peptide/bombesin (COSTA et al., 1984).

Recent immunocytochemical studies of the enteric enkephalin neurons have shown that there are at least four types of cytoplasmic projections (KOBAYASHI et al., 1985): 1) The axon-like process was the most prominent long cytoplasmic projection. It was too thick in caliber at the origin, too quickly tapering and too ramulous to be called axon in the ordinary sense. 2) The winglet or alula was a short broad process on the perikaryon, which was regarded as the site of synapse formation. Fine filamentous processes termed wing-ramuli emanated from the free margin of the winglet. 3) A dendrite-like process was the dendrite of Dogiel's type 3 neuron. All these processes contained an opioid peptide-like immunoreactivity. As far as morphology is concerned, various transitional forms were demonstrated between the axon-like process, wing-ramulus, long tapering process and dendrite-like process of the enkephalin neurons (KOBAYASHI et al., 1985). Thus, the question arose whether precise categories of enkephalin neuron processes even existed.

DOGIEL (1899) drew a clear distinction between axons (neurites) and dendrites. However, a re-examination of the drawings of his original paper (DOGIEL, 1899) gave us the impression that there are many cases where there is no morphological distinction between axons and dendrites. In particular, the distinction between these two kinds of projections in the type 2 and type 3 neurons seems confusing. FURNESSE et al. (1983b), who illustrated the shape of a typical enkephalin neuron from their lesion experiments in the guinea pig small intestine, regarded the anally or circumferentially-directed prominent process of the enkephalin neurons as an axon, and the orally-directed fine processes as possible dendrites. Although these authors detected accu-
mulations of enkephalin-like immunoreactivity at the anal margin of the experimented gap, no direct connection between the orally-directed processes and the enkephalin immunopositive perikarya was demonstrated. In the tissue preparations used in the present study, many enkephalin neurons on the anal side of the laser necrosis (43-90 hrs after laser microsurgery) possessed orally-directed immunopositive projections. We assume that the wing-ramulus is invisible in normal conditions, and that it accumulates opioid peptides when occluded, and then becomes visible through immunocytochemical treatment. We propose that the orally-directed fine processes which Furness et al. (1983) regarded as possible dendrites are nothing but the wing-ramuli. These processes are of an axonic nature, with regard to the direction of opioid peptide flow. We noticed that the amount of flowing Met-Enk-Arg-Gly-Leu-like immunoreactivity in the axon-like process was greater than that in the wing-ramuli. However, it is unknown whether the wing-ramuli carry information to the perikaryon.

The unique properties of the laser beam are being applied in a variety of fields of research. Laser microsurgery has many advantages such as noncontact manipulation, small spot size irradiation and easy control of the irradiation dose (Polanyi, 1979). So far as we know, the present study is the first successful application of laser photoocoagulation technique for the investigation of the morphology of myenteric enkephalin neurons. A laser irradiation combined with immunocytochemistry may provide a useful methodology for investigating the organization of enteric neurons containing various aminic and/or peptidergic transmitters and modulators.

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