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Nerves Versus Hormones in the Regulation of an Organism

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Abstract The pendulum of concepts concerning the regulatory mechanism of the gut dramatically swung from the nervism of Pavlov to the hormonism of Bayliss and Starling, and recently to a compromise on the prerequisite of sensory nerves recognizing food chemical information and transmitting it to endocrine cells. Our studies have settled this problem by neglecting this intervention of nerves and demonstrating the endocrine cells as being open to the lumen to recognize luminal stimuli. The cells thus are half nervous and half secretory in nature; this recognition has motivated us to propose a cell family of paraneurons.

Advances in studies have indicated that neurons and paraneurons comprise a continuous and unseparable entity, their roles regulating the organs being also unseparable. The signal substances (peptides and in some cells also amines) are common to neurons and paraneurons; the routes and distances they travel to their targets show every gradation without regard to their source cell, whether neuron or paraneuron. These data indicate that transmitters and hormones are overlapping and unseparable.

Phylogenetical studies have clarified that the peptide producing regulatory cells are derived from the intraepithelial bipolar paraneurons and subepithelial multipolar neurons occurring in Hydra (Coelenterata). These prototype cells are inherited, without any large modification, in our gut wall, to continue their ancient task, i.e., to recognize food information, to respond to it adequately for effective nutrition uptake, and to protect the body against toxic invasions.

Key Words : Nerves, Hormones, Paraneurons, Gut endocrine cells

Concepts on the regulation of the gut

The regulation of an organism essentially comprises the mechanism of recognizing and adequately responding to external and internal stumuli so that the homeostasis both in different organs of the body and in the whole body is maintained. The process of this regulation of the body was first believed to be accounted for exclusively by the functions of nerves. This nervism proclaimed by the great Russian physiologist I. P. Pavlov, was overwhelmingly powerful at the end of the last century.

Hormonal regulation, on the other hand, was discovered by Bayliss and Starling[1] in 1902 in their experiment on the intestine.

It had been known since the last century that perfusion of the canine small intestine with 0.1N HCl causes an increased output of pancreatic juice, which neutralizes acid in the gut. Under the influence of nervism, this

This paper is dedicated to Prof. Dr. Kazuhiko Awaya, the former President of Yamaguchi Univercity, on the occasion of his retirement.

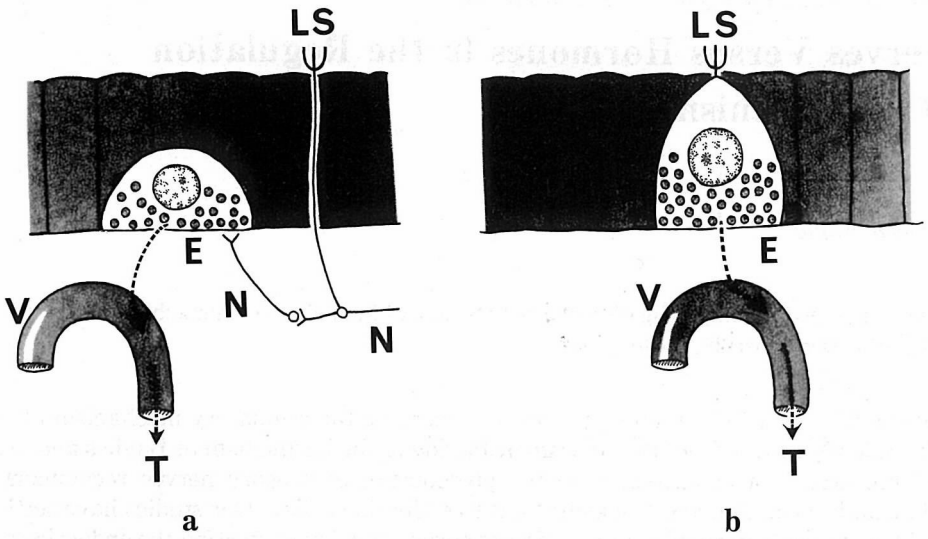

Fig. 1 Concepts as to how luminal information is transmitted to the endocrine cell in the gut. **a** The view which prevailed until recently. The luminal stimuli (LS) are received and transmitted by nerves (N) to the endocrine cell (E), which releases its secretions and regulates a target (T) via blood vessels (V). **b** Our opinion, which neglects the intervention of the nerves. The endocrine cell, with its apical microvilli, can directly respond to the luminal stimuli.

phenomenon also had been ascribed to nervous reflex, as it was believed that nerves reaching the mucosal surface would recognize the chemical stimuli in the gut lumen and conduct excitation to the pancreas.

Bayliss and Starling[1] skillfully demonstrated, using a single dog, that this phenomenon was not inhibited even after severing all nerves which might be involved. They further removed a piece of the mucosa of the small intestine, poured 0.1N HCl on it, and prepared an extract of the mucosal tissue. When they injected this into the dog intravenously, the output of pancreatic juice was markedly increased. They thus concluded that an agent enhancing the output of pancreatic juice, which they designated secretin, was formed in the intestinal mucosa by the chemical action of HCl and then transferred to the pancreas to stimulate its secretion.

In 1905, the existence of gastrin, another agent enhancing the acid output from the gastric body was proposed by Edkins,[2] who stated that it was released into the bloodstream from the pyloric antrum when a certain substance in foodstuff reached the site.

It was in the same year that the term "hormone" was coined for such blood-conveyed chemical messengers like secretin and gastrin.[3] Although a few other hormones, including cholecystokinin (CCK),[4] pancreozymin[5] and enterogastrone,[6] were later added to the category of gut hormones, studies in this field lapsed to a standstill, until the polypeptide nature and subsequently the amino acid sequences of gastrin,[7] secretin[8] and CCK (which turned out to be chemically identical to pancreozymin)[9] were revealed in the 1960s.

One of the reasons for this decline in interest for gut endocrinology is found in the concept of a gut hormone being the chemical product born in the mucosal tissue by the interaction of a precursor substance and an extrinsic agent including, in the case of secretin, HCl. This erronious idea was derived from the original study by Bayliss and Starling.[1]

This view of "tissue hormones", however, became incompatible with the modern doctrine of endocrinology that every hormone is

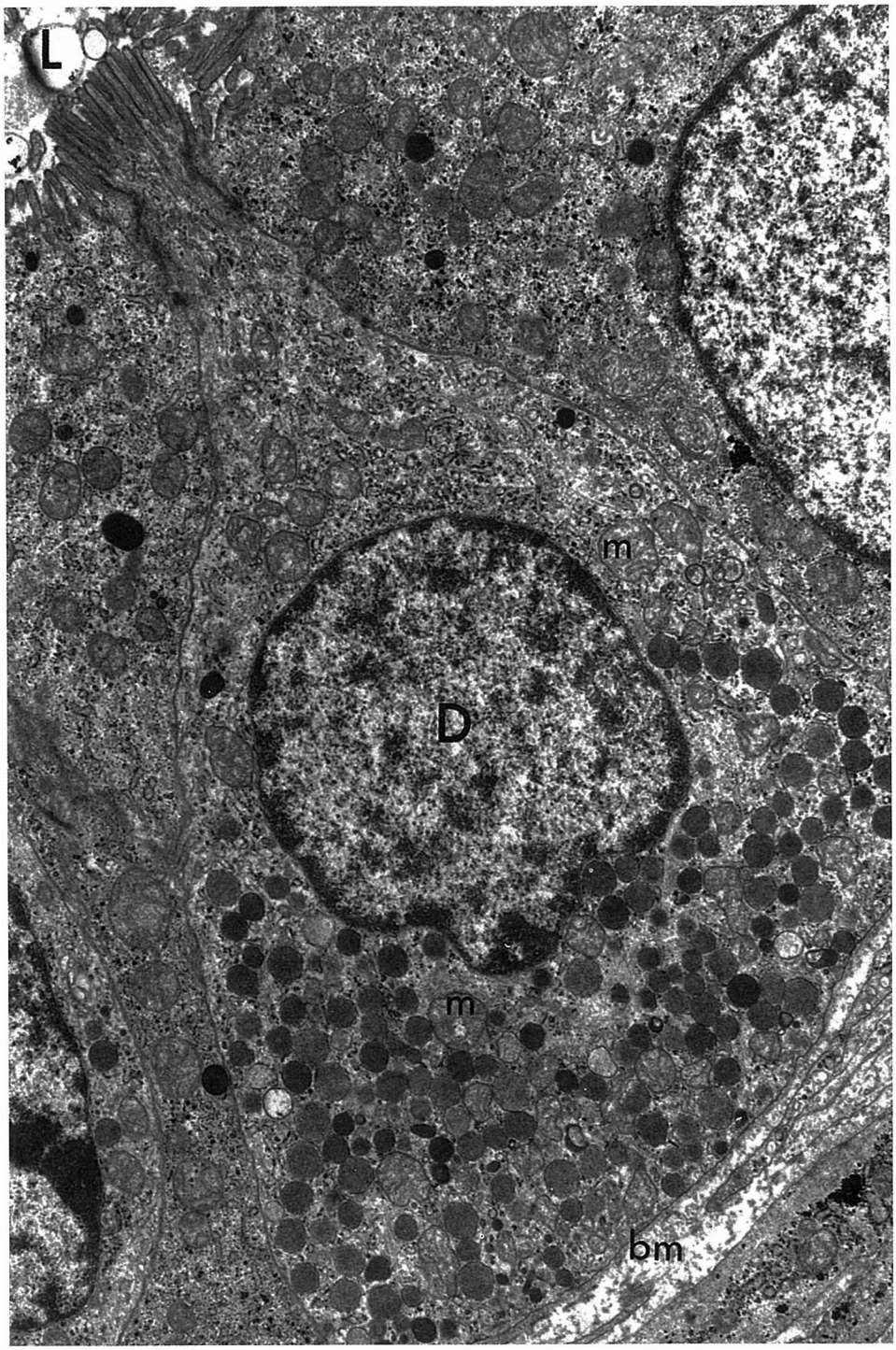

Fig. 2 A typical basal-granulated cell (D cell) in the duodenal mucosa of a 7-month human fetus. In this fetal stage, the shape of the cell as a receptor is exaggerated, as its microvilli protruding into the intestinal lumen (L) are strikingly longer than the microvilli of neighboring absorptive cells. m: Mitohchondria, bm: basement membrane. ×12,000 (Electron micrograph by M. Osaka; reproduced under permission from T. Fujita: Gastro-Entero-Pancreatic Endocrine System—A cell-biological approach, Igaku-Shoin, 1973).

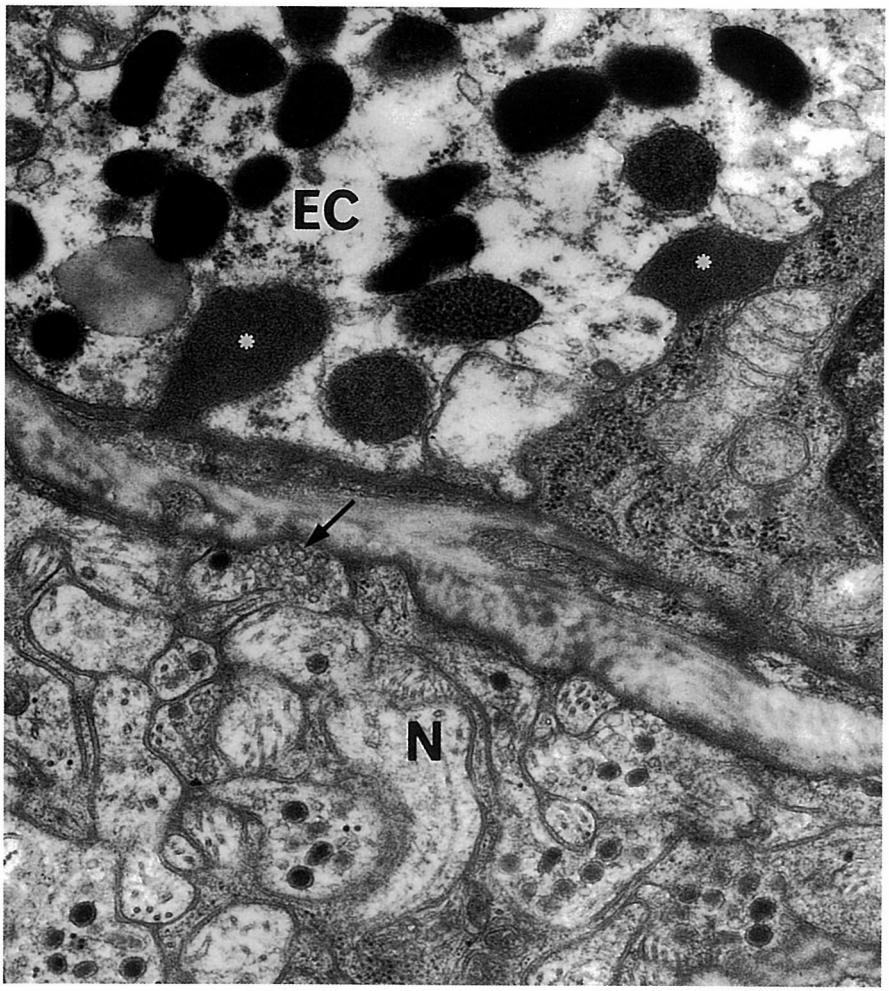

Fig. 3 Basal portion of a rabbit EC cell after luminal stimulation with cholera toxin. Exocytotic granules are indicated with asterisks. It is conceivable that serotonin and possibly other granule contents released from the EC cell may stimulate the nerves (N) approaching the cell. This cell type, also in humans, is in closer topographical relation to nerve fibers than any other type of endocrine cell. The accumulation of synaptic vesicles of a small clear type (arrow) close to the EC cell base suggests a cholinergic nerve influence on this cell. ×25,000 (Reproduced under permission form T. Fujita and S. Kobayashi: Int. Rev. Cytol. Suppl. 6 : 187-233, 1977).

produced by a specific cell.

Although in the 1950s it became known that a type of basal-granulated cell, the enterochromaffin or EC cell was the source of serotonin or 5-hydroxytryptamine (5HT) in the gut epithelium,[10] it was only in 1967 that gastrin was shown by Solcia to be produced in another specific cell type, the G cell.[11]

With the advances of immunocytochemistry, the source cells of other gut hormones were then identified one after another. In 1970's, biochemists isolated and characterized several new peptide hormones from

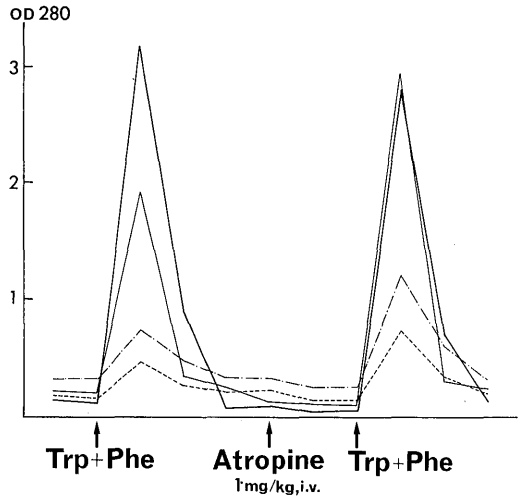

Fig. 4 Changes in every 15 min of the protein output from the pancreas of the anesthetized dog as measured by spectrophotometry. Results obtained in four different dogs are shown. The output is increased (first peak) after infusion of the amino acid solution (50 mM tryptophan and 50 mM phenylalanine in saline) into the duodenal loop. This response is not influenced by atropine administered intravenously at 1 mg/kg 30 min before the amino acid infusion (second peak).

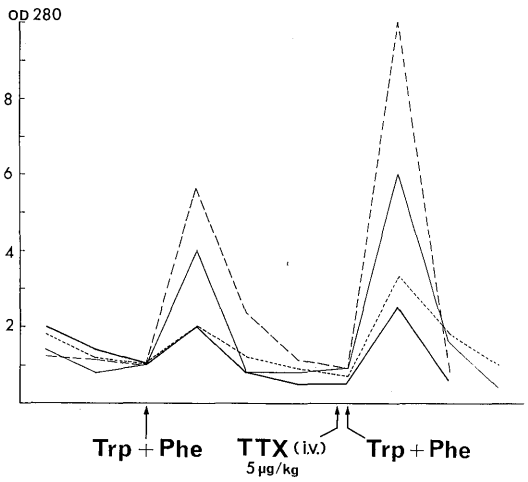

Fig. 5 Changes in every 15 min of the protein output from the canine pancreas. Results obtained in four different dogs are shown. Values are normalized, taking the amount of protein output at the starting point of amino acid infusion as 1. Infusion of the amino acid solution into the duodenal loop increases the pancratic protein output (first peak). Three minutes after the intravenous injection of tetrodotoxin (TTX) at 5μg/kg, the protein output in response to amino acid infusion is not inhibited, but rather enhanced for unknown reasons (second peak).
(Figs. 4 and 5: redrawn from the data reported in T. Fujita et al. Biomed. Res. 1: 59-65, 1980).

porcine guts, and in correspondence with them, the morphologically and immunocytochemically identifiable endocrine cell types were increased to ten or more.[12,13]

In spite of these advances, the central problem of gut physiology remained unanswered, i.e., how the gut mucosa recognizes acid and food substances in order that appropriate hormonal messengers can be sent to their target organs. To account for this mechanism, physiologists conceived of—and actually published schemes of—nerves which receive luminal chemical information at the epithelial surface and transmit it to the source of gut hormones— in their case, gas trin (Fig. 1). Our own observation of light microscopic preparations, especially silver impregnated, as well as electron micrographs, never revealed nerves coming to the mucosal surface. A new scheme, therefore, was required to account for chemical recognition by the gut.

Around 1970, we formulated the opinion that the basal-granulated cells were "open" to the gut lumen, extending an apical process, which was often very slender and could have been easily overlooked by previous researchers. Our morphological observations in humans and other mammals determined that the cells were regularly "open" in type (Figs. 1, 2) from the pyloric antrum down to the rectum, while the gastric body contained only "closed type" basal-granulated cells, i.e., cells not reaching the lumen. Electron-microscopic studies revealed a

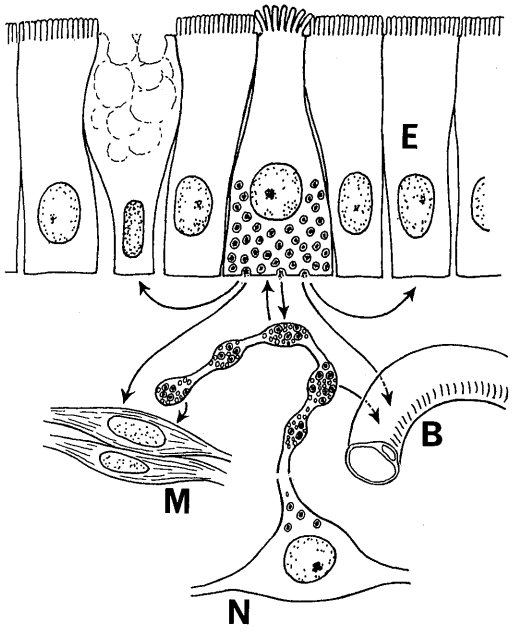

Fig. 6 Diagram showing different routes of transport and targets of actions of messenger substances released from a gut basal-granulated cell, as an example of endocrine and sensory paraneurons. The messenger substances partly enter blood vessels (B, hemocrinia) and partly are transported by diffusion (paracrinia) to regulate vicinal targets, including neurons (N), muscular cells (M), and epithelial or glandular cells (E).

more or less elaborate tuft of microvilli on the apical end of the cell. We thus proposed that this represented the chemoreceptive site of the cell.[16,17,18]

We made a series of experiments in humans and in dogs, stimulating the luminal side of the gut mucosa with HCl, certain amino acids or other chemical agents known to cause the release of a specific hormone. Electron-microscopic examination of the mucosa taken several minutes after indicated images of exocytotic granule release from the cell type which was expected to contain that hormone.[16,19,20] This result evidenced, for the first time, the previously vaguely conceived endocrine nature of the basal-granulated cells.

Diarrheagenic agents, i.e., hypertonic (50%) glucose solution and cholera toxin introduced into the small intestine of the dog and young rabbit, respectively, were shown to cause severe and selective degranulation of EC cells as demonstrated by our electron-microscopic observation (Fig. 3).[19,21,22] We suggested that serotonin, probably in collaboration with an unknown peptide hormone coexisting with the amine, might cause the diarrhea.

The absence of nerves intervening between the luminal stimuli and the endocrine cell was later evidenced by our experiments in the dog by the use of nerve-blocking agents, atropine and tetrodotoxin. Under anesthesia with neuroleptoanalgesia, dogs were laparatomized and a duodenal loop was made. Perfusion into this loop of a solution of 50mM tryptophan and 50mM phenylalanine caused CCK release from the loop, which was measured in terms of the protein output from the pancreas. An intravenous administration of atropine (1 mg/kg) or tetrodotoxin (5 μg/kg) did not inhibit the increase of pancreatic protein output after the stimulation with the amino acids (Figs. 4, 5).[23,24]

The gut endocrine cell as a paraneuron

As reviewed above, the swing of the pendulum of concepts concerning the gut response to luminal stimuli from Pavlov's nervism to the so-called hormonism by Bayliss and Starling has brought us back to a middle point. It can be said that the nerve terminal which recognizes the luminal stimuli actually stands at the top of the endocrine cell. Luminal administration of local anesthetics can inhibit the gut hormone release by luminal stimuli, though this is not because nerves are involved in the reaction but rather beause the receptive site of the gut endocrine cell is sensitive to anesthesia. As the gut endocrine cell further shares many cell-biological features with neurons, which have been dealt with elsewhere,[25] it is regarded as a typical member of the paraneuron family.[18,25,26]

The functional significance of the gut endocrine cell is most fundamental for the regulation of the organism. The central problem concerning the gut mentioned above is

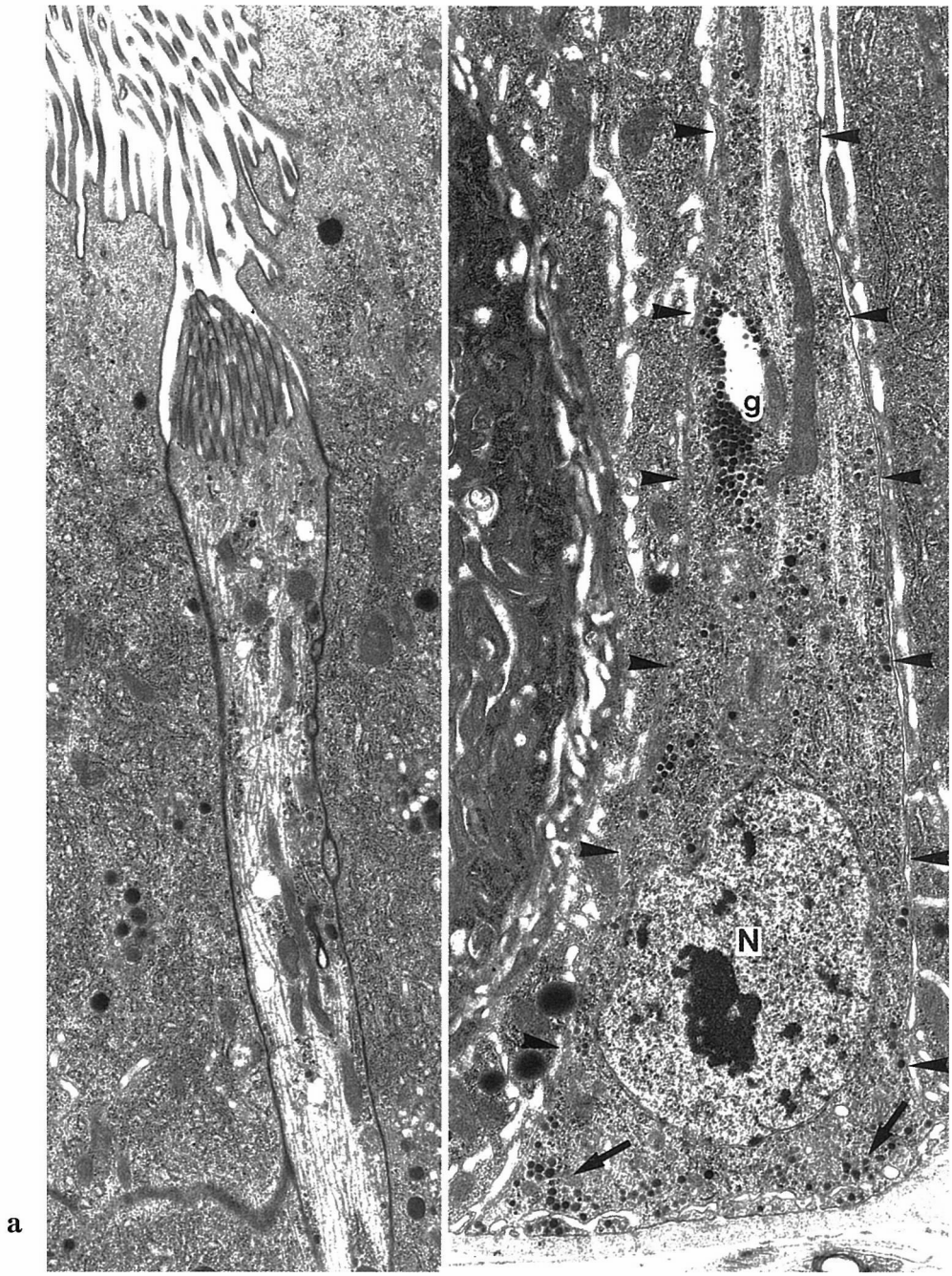

Fig. 7 Electron micrographs showing the apical and basal parts of an endocrine cell in the midgut of a butterfly larva, *Papilio xuthus*. **a** The cell apex is provided with a tuft of microvilli, the probable receptive site of the cell. A marked neurotubule-like system and some mitochondria are seen. **b** The basal part of the cell contains a nucleus (N) and secretory granules (arrows) mainly gathered in the infranuclear portion. g Glycogen particles. The cell boundary is indicated with arrowheads. Courtesy of Y. Endo and J. Nishiitsutsuji-Uwo; reproduced, with permission, from Biomed. Res. 2: 270-280 (1981).

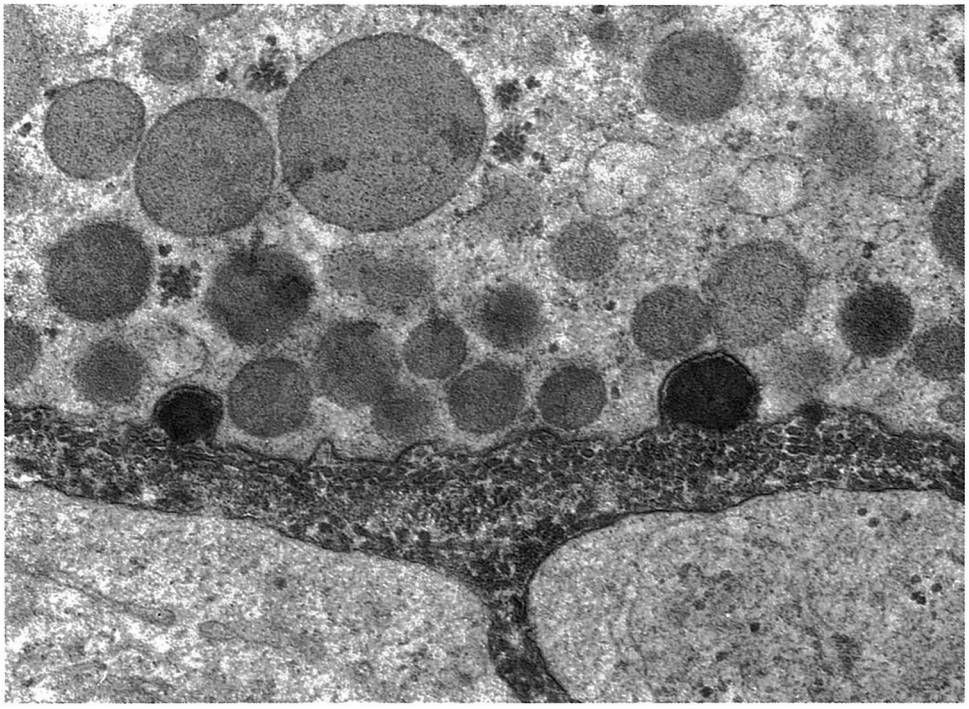

Fig. 8 Basal portion of an endocrine cell in the midgut of the cockroach *Periplaneta americana* showing exocytotic images. In this specimen prepared by the tannic acid-glutaraldehyde-osmium method, the contents in the two open granules and the intercellular substances are stained dark with tannic acid. Courtesy by Y. Endo and J. Nishiitsutsuji-Uwo; reproduced under permission from Peptides, Suppl. 2: 123-131 (1981).

believed to have been solved by the recepto-secretory nature of this cell. The simple bipolar cell is open to the external milieu with its apical end and faces the internal milieu with its basal aspect. It transduces the stimuli from the external milieu into chemical signals which are represented by amine/peptide messengers released by the exocytotic opening of individual granules, i.e., in a digital fashion according to the intensity of the excitation of the cell.

The chemical signals released from the gut endocrine cells can be called hormones as they partly enter the blood stream to be transferred to remote targets; they can also be regarded as transmitters as they partly act upon vicinal nerves, smooth muscles and other target cells. The chemical transmission, in the latter case, occurs by paracrine mechanism, in other words, via synapse à distance. In the case of EC cells (vide supra), the cell base is closely approached by a bundle of nerve terminals over the basement membrane.[27] In this and in the cases of other cell types, the synapse, either close or at a distance, seems reciprocal, as both endocrine and nervous elements contain numerous granules or vesicles gathered at the synaptic site (Fig. 6).[25]

The above discussion supports the view that, firstly, the gut endocrine cell apparently plays the role of the primary sensory neuron in the autonomic sensory system and, secondly, transmitters and hormones comprise a continuum in their function.[25]

Phylogeny of gut paraneurons

The simple and purposeful structure of the bipolar endocrine cell in the gut epithelium

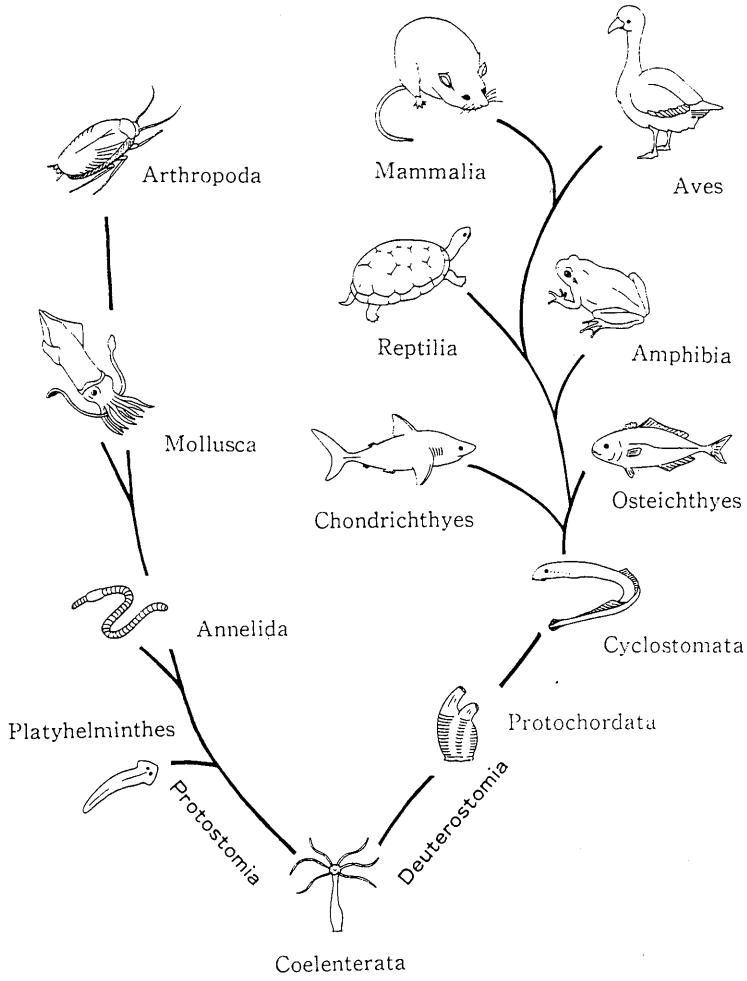

Fig. 9 Diagram showing a generally accepted view concerning the phylogeny of metazoan animals. (Redrawn under permission from T. Fujita, T. Kanno and S. Kobayashi: The paraneuron, Springer, 1989).

supported the idea, already at the beginning of our morphological study of this cell, that it must have a long phylogenetical history. This hypothesis was supported by our co-investigation with Drs. Nishiitsutsuji-Uwo and Endo on the midgut of insects.[28,29]

Electron microsopic studies revealed, in the cockroach and butterfly larvae, bipolar basal-granulated cells essentially of the same structure as those in mammals dispersed in the gut epithelium (Fig. 7). Feeding of the insect caused exocytotic granule release from the cell base (Fig. 8).[29,30] Immunocytochemical studies demonstrated cells containing immunoreactivities for PP, glicentin, somatostatin, motilin and urotensin I in the cockroach, for instance. The subepithelial muscular layer of the midgut revealed many nerves containing immunoreactivities for VIP, somatostatin and PP.[32,33]

These findings in insects encouraged us to propose that the bipolar, open-type paraneurons in the gut epithelium, together with relay neurons in the subepithelial layer, must occur in both the protostomian and deuterostomian series of multicellular animals, including the Coelenterata.[33,34] This view was based on the current concept of animal evolu-

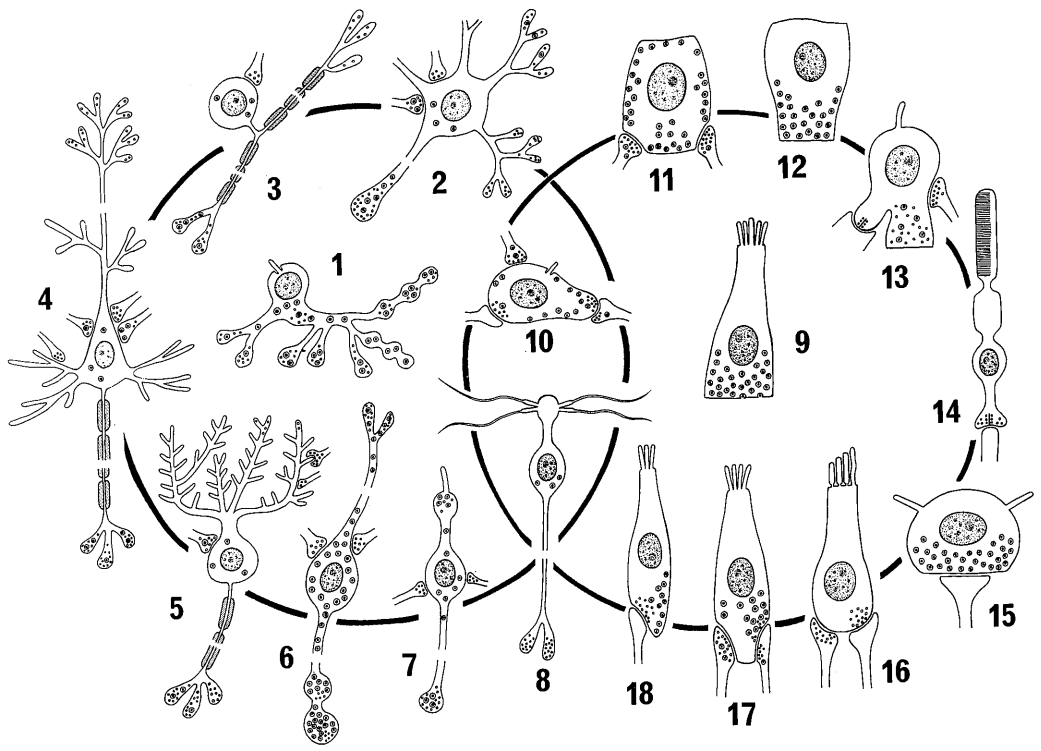

Fig. 10 Spectrum of neurons (left) and paraneurons (right) continuous and overlapping with each other. 1 Enteric intramural neuron, which represents the prototype of neurons; 2 multipolar autonomic neuron; 3 sensory neuron; 4 pyramidal neuron; 5 Purkinje cell; 6 "neurosecretory" or "peptidergic" neuron; 7 CSF-contacting neuron; 8 olfactory cell; 9 gut endocrine cell, which represents the prototype of paraneurons; 10 carotid body chief cell and SIF cell; 11 adrenal chromaffin cell; 12 adenohypophysial, parafollicular, or other endocrine cell; 13 pinealocyte; 14 visual cell; 15 Merkel cell; 16 inner ear hair cell; 17 bronchopulmonary paraneuron; 18 gustatory cell (Reproduced under permission from T. Fujita, T. Kanno and S. Kobayashi: The paraneuron, Springer, 1989).

tion comprising these two series dicotomically deriving from a common origin, Coelenterata (Fig. 9). If one finds cells and signal substances shared by mammals, the terminal descendants of the deuterostomian series, and by insects, those of the protostomian series, the cells and substances should be found in Coelenterata.

Electron microscopic observations by Westfall[35,36] and immunocytochemical studies by Grimmelikhuijzen[37,38] indicate that typical bipolar paraneurons in the epithelium, in addition to bipolar and multipolar neurons in the subepithelial layer, actually occur in Hydra, though they have realized them not as homologous with the gut endocrine cells of higher animals, but as a sensory type of neurons. Westfall[35,36] showed that both types of cells possess granules and vesicles similar to those in neurons and paraneurons in higher animals, and that some of them are gathered to synaptic sites while others to non-synaptic regions of the cell periphery. This implies that both neurons and paraneurons in Hydra are engaged in synaptocrine and endocrine release of amine / peptide messengers. The term "endocrine" signifies rather a paracrine release of humoral mes-

sengers by intercellular diffusion in such animals lacking in circulatory vessels.

Several research groups, meanwhile, have succeeded in demonstrating the occurrence of gut bipolar paraneurons in different protostomian and deuterostomian animals; the results have been reviewed elsewhere.[25,34]

Addition of more differentiated cell types

With the evolution of animals, both of the protostomian and deuterostomian series, increasingly variant types of neurons and paraneurons have been differentiated. The development of the brain, spinal cord and related nerves was accompanied by the appearance of particular-shaped neurons such as those equipped with elaborate dendrites or with an enormously long axon possessing rapid conductivity. The formation of the eye, ear and other sensory organs was coupled with the differentiation of sensory paraneurons with highly specified structures and functions. The development of endocrine glands was substantiated by the differentiation of endocrine paraneurons.

All these neurons and paraneurons, the main representatives of which are illustrated in Fig.10 are newly added elements in the course of animal evolution.

The original elements—the intraepithelial paraneurons recognizing stumuli from the external milieu and the subepithelial neurons relaying signals—remain in the gut of the highest animals of the protostomian and deuterostomian series. These cells, which will deserve the names of archiparaneurons and archineurons,[25] have not undergone essential modification, for several hundred million years of evolution from their prototypes in Hydra, either in cell structure or in signal substances. They continue to perform their task of recognizing food information for the effective uptake of nutrition and for the elimination of orally introduced toxic substances.

The present review, focussing on the history of concepts concerning the gut, has dealt with the overlapping and unseparable natures of the nervous and hormonal regulatory systems. The neurotransmitters (including modulators) and hormones (aminic and peptidic) are almost identical in chemical nature. They have been derived from the most primitive multicellular animals as secretions of two equivalent cell tyes—archineurons and archiparaneurons. Neurons are nothing but endocrine cells in nature, while paraneurons share cell-biological features with neurons.[25] It is no more tenable to evaluate the regulatory mechanisms in any organs of the body with the idea of their being "either nerve or hormone".

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