

THE ENTERIC NERVOUS SYSTEM OF ECHINODERMS: UNEXPECTED COMPLEXITY REVEALED BY NEUROCHEMICAL ANALYSIS

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Summary

Echinoderms are one of the most important groups of metazoans from the point of view of evolution, ecology and abundance. Nevertheless, their nervous system has been little studied. Particularly unexplored have been the components of the nervous system that lie outside the ectoneural and hyponeural divisions of the main nerve ring and radial nerve cords. We have gathered information on the nervous components of the digestive tract of echinoderms and demonstrate an unexpected level of complexity in terms of neurons, nerve plexi, their location and neurochemistry. The nervous elements within the

digestive system consist of a distinct component of the echinoderm nervous system, termed the enteric nervous system. However, the association between the enteric nervous system and the ectoneural and hyponeural components of the nervous system is not well established. Our findings also emphasize the importance of the large lacunae in the neurobiology of echinoderms, a feature that should be addressed in future studies.

Key words: echinoderm, nervous system, digestive tract, neuropeptide.

Introduction

It has been proposed that the echinoderm nervous system consists of three main components: the ectoneural, hyponeural and entoneural systems (Hyman, 1955; Smith, 1965; Pentreath and Cobb, 1972; Cobb, 1987). The ectoneural system is both sensory and motor and makes up the nerve ring and most of the radial nerve cords. The hyponeural system, exclusively motor, is present in the radial nerve cords and is associated with the skeletal muscle system. The third component, the entoneural system (also called the apical or aboral nervous system), occurs in asteroids and crinoids and is absent from the other groups. However, this simplified division of the echinoderm nervous system does not allow for numerous reports that have appeared during the last decade showing unexpected nervous system components that do not correspond clearly with the three divisions described above. Prominent among these are the neurons and nerve fibres present in the viscera. Visceral innervation has been poorly studied, and Cobb (1987), the leading authority on echinoderm nervous systems, in his review of the biology of Echinodermata, states that 'the viscera are also innervated by a nerve plexus that is not ectoneural but there appears to be no direct connection to the hyponeural nervous system', suggesting that the visceral component is a fourth division of the echinoderm nervous system.

We have focused our studies on the digestive system of *Holothuria glaberrima*, using a series of neurochemical and immunocytochemical markers to identify the neurons and plexi that form the enteric nervous system. Although this analysis provides little, if any, information on the physiological

functions of the nerves, it does provide a very detailed neuro-anatomical map of different neuronal and nerve fibre populations, a map that is essential for the future understanding of the physiology of the nervous system. The accumulated data demonstrate that the echinoderm digestive tract has a complex enteric nervous system formed by components with distinct morphological and neurochemical properties (García-Arrarás et al., 1991a; García-Arrarás et al., 1991b; García-Arrarás et al., 1999; García-Arrarás and Viruet, 1993; Díaz-Miranda et al., 1995). Some of these components have been mentioned briefly by several authors in their description of a particular organism or organ. The present article is the first detailed description of the holothurian enteric nervous system and also presents a comparative analysis of results from other groups of Echinodermata.

Anatomy

The histology of the echinoderm digestive tract has been well described by several investigators (see Hyman, 1955; Anderson, 1954; Fish, 1967; Schechter and Lucero, 1968; Farmanfarmaian, 1969). Although changes in the width of the layers of the tract are encountered within the different levels of the digestive tract, i.e. the oesophagus, stomach, intestine, etc., the echinoderm digestive tract consists, from lumen to coelom, of a pseudostratified luminal epithelium, a layer of internal connective tissue, a muscle layer and a layer of visceral coelomic epithelium. A basal lamina separates the internal

connective tissue layer from the luminal epithelium and from the muscle layer. The muscle layer is usually subdivided into circular (internal) and longitudinal (external) layers. Connective tissue (external) can sometimes be found associated with the coelomic epithelial layer. The term mesothelium is also currently used to describe the layer of cells between the muscle layers and the coelom. This layer is described as a pseudostratified epithelium made up of two cell layers, an external cell layer of ciliated peritoneocytes and a more internal layer of granular cells (Smiley, 1994; Vanden Spiegel and Jangoux, 1987). Electron microscopy, performed on the digestive tract of the sea star *Marthasterias glacialis*, has demonstrated that most of the cells that form the mucosal and coelomic epithelia are choanocytes containing true flagella (Martínez et al., 1991).

Our neurohistochemical analysis shows that the enteric nervous system of echinoderms can be divided into four components: the visceral plexus, the basiepithelial plexus, the mucosal neuroendocrine plexus and the connective tissue plexus.

Visceral plexus

This plexus is associated with the coelomic epithelium that surrounds the viscera and has also been termed the subcoelomic plexus (Martínez et al., 1993), intestinal plexus (García-Arrarás et al., 1991a; García-Arrarás et al., 1991b), coelothelial plexus (Heinzeller and Welsch, 1994), outer plexus (Heinzeller and Welsch, 1994) and coelomic lining/muscular plexus (García-Arrarás et al., 1999). It has been proposed that this plexus innervates all the viscera, and it is characterized by a dense system of fibres associated with the visceral musculature (Cobb, 1987). The plexus has been best described ultrastructurally in the haemal vessels of *Cucumaria frondosa* (Doyle, 1967). In addition, immunohistochemical studies with the peptide GFSKLYFamide have demonstrated the presence of cells and nerve fibres within the serosa of the haemal vessels and within the respiratory tree (Díaz-Miranda et al., 1995). The plexus is not, therefore, unique to the digestive tract but appears in many visceral systems including the coelomic epithelium associated with the mesentery.

In the digestive tract, this plexus is present in the coelomic epithelium/muscular layer that extends from the oesophagus to the cloaca (Fig. 1). One can conclude that this plexus probably contains cholinergic cells and fibres on the basis of the following assumptions: (i) the gut contains acetylcholine (see Welsch, 1966; Pentreath and Cobb, 1972); (ii) physiological experiments demonstrate that the intestinal muscle is responsive to acetylcholine, contracting in a dose-dependent manner (Beauvallet, 1938; García-Arrarás et al., 1991b); and (iii) small clear vesicles, similar to those known to contain acetylcholine, described in ultrastructural studies (Smiley and Cloney, 1985; Jensen, 1975; Rivera-Bermúdez, 1992), are present. However, there is no study in which the presence of acetylcholine, choline acetyl transferase (its synthesizing enzyme) or cholinesterases have been localized to the enteric

nervous system of adult echinoderms. The distribution of cholinesterases has been studied in the sea urchin larva (Ryberg, 1973). Acetylcholine is the preferred candidate to be the contraction-inducing agent released from fibres within the visceral plexus. Whether the putative cholinergic fibres originate from neurons in the coelomic epithelia or from neurons extrinsic to the digestive tract remains to be determined. No biogenic amines have been found to be associated with this plexus; in fact, the biogenic amines of the digestive tract appear to be restricted to the basiepithelial nerve plexus described below.

In the digestive tract of *Holothuria glaberrima* and *Holothuria mexicana*, the visceral plexus is made of isolated neurons that appear to innervate the muscular layer and to extend fibres into other tissue layers (Fig. 2). Our initial description of the plexus was made using an antibody against mammalian cholecystokinin (García-Arrarás et al., 1991b). The cells are small in size and are isolated or occur in small groups throughout the digestive tract. The presence and extent of this plexus were confirmed by immunoreactivity to various neuropeptides, including GFSKLYFamide, galanin, proctolin and bradykinin (García-Arrarás et al., 1991a; García-Arrarás et al., 1991b; García-Arrarás et al., 1999; Díaz-Miranda et al.,

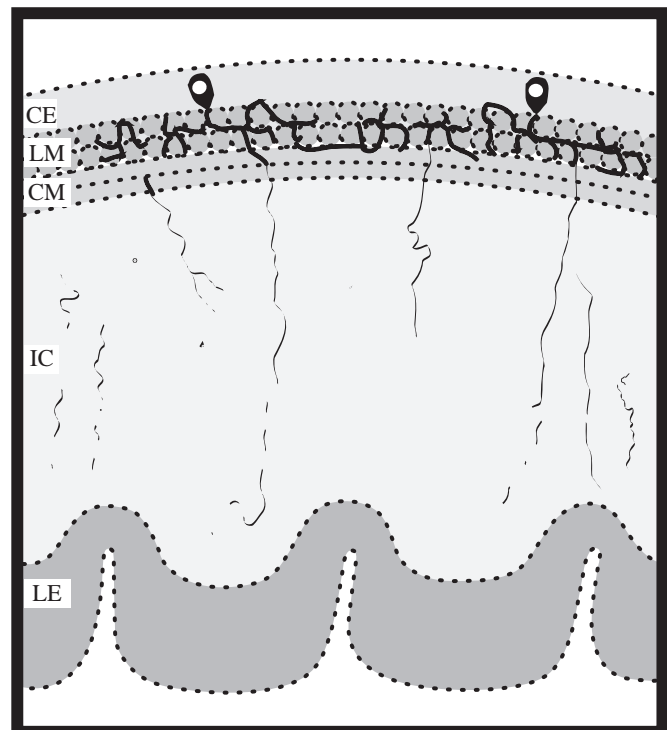


Fig. 1. Schematic diagram of the holothurian visceral plexus component of the enteric nervous system. Neurons are associated with the coelomic epithelium (CE) and with a dense fibre plexus associated with the longitudinal (LM) and circular (CM) muscle layers. Neuronal fibres that appear to originate from these neurons are found within the internal connective tissue layer (IC), where they are mostly observed to be perpendicular to the circular muscle layer. Some of the fibres may extend to the luminal epithelium (LE) layer.

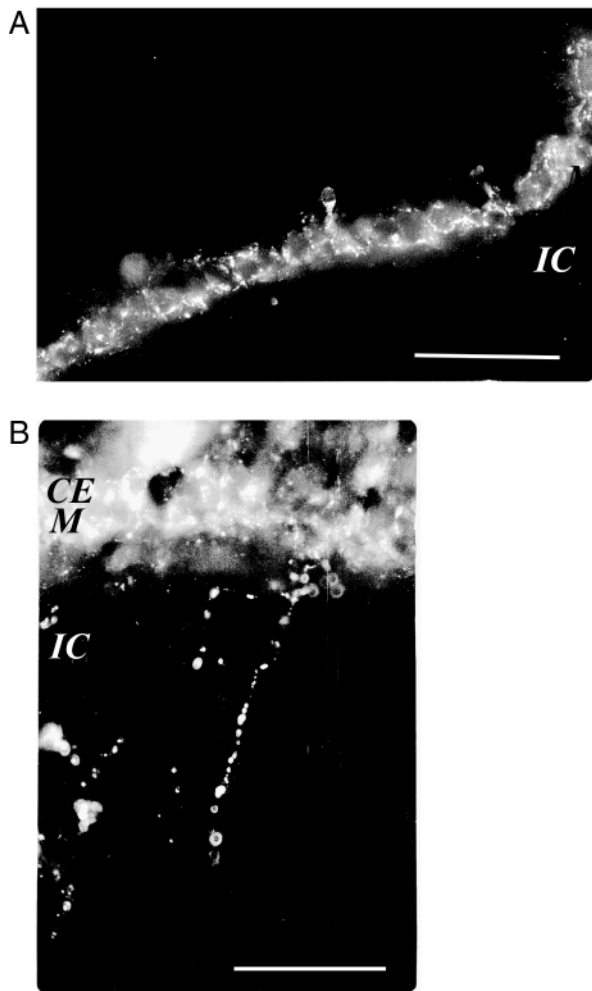


Fig. 2. Transverse sections of the digestive tract of *Holothuria glaberrima* labelled for galanin (A) and GFSKLYFamide neuropeptide (B) immunoreactivity. (A) An immunoreactive neuron in the coelomic epithelium and numerous fibres associated with the muscle layer of the intestine. (B) Immunoreactive fibres originating from the visceral plexus within the coelomic epithelium (CE) and muscle layers (M) are observed to continue into the internal connective tissue (IC). Scale bars, 50 μ m.

1995; Díaz-Miranda et al., 1996; J. E. García-Arrarás, J. Serrano, L. Díaz-Miranda and M. Rojas-Soto, unpublished) and to acetylated α -tubulin, a marker of nerve fibres (García-Arrarás and Viruet, 1993). The plexus is also found in the coelomic epithelium/muscle layers of the mesenteries, where it is continuous with the visceral plexus of the digestive tract (Díaz-Miranda et al., 1995). However, the plexus within the mesenteries consists mainly of fibres; no neuronal bodies have been detected.

The visceral plexus of the intestine has also been studied ultrastructurally, confirming some of the results obtained by immunocytochemistry (Rivera-Bermúdez, 1992; Díaz-Miranda et al., 1995). The neuron–neuron and neuron–muscle synapses characteristic of the echinoderm nervous system (Cobb, 1987) are present. These are formed by vesicle-

containing varicose fibres in close apposition to the postsynaptic cell, but lack the membrane ultrastructural specializations that are characteristic of vertebrate nerve–muscle synapses. In fact, many of the echinoderm nerves ending on muscles have been compared with the ‘*en passant*’ synapses characteristic of the vertebrate autonomic nervous system (Pentreath and Cobb, 1972). A second characteristic of the echinoderm synapse is also found within the visceral plexus: processes originating from the muscle cells that elongate towards the site of innervation (Cobb, 1969b; Pentreath and Cobb, 1972; Rivera-Bermúdez, 1992; Díaz-Miranda et al., 1995). In some cases, these processes can be found at a considerable distance from the main cell body.

The presence of the visceral plexus in the digestive tract of other echinoderm species has been detected mainly by immunoreactivity to various neuropeptides. In the sea cucumber *Apostichopus japonicus*, fibres and cells within the intestinal and mesentery portion of the plexus are immunoreactive to an antibody to the peptide NGIWAYamide (Inoue et al., 1999) (although in that study the visceral plexus was erroneously identified as the basiepithelial plexus). Similarly, other studies have demonstrated that fibres in the visceral plexus of the sea stars *Asterias rubens* (Moore and Thorndyke, 1993) and *Marthasterias glacialis* (Martínez et al., 1993) express the neuropeptide S1, a member of the SALMFamide neuropeptide family (Elphick et al., 1991; Elphick et al., 1995). In addition, immunoreactivity to the neuropeptides PYY and α MSH has also been found in the visceral plexus of the digestive tract of *Marthasterias glacialis* (Martínez et al., 1993).

The visceral plexus, present in all echinoderms, may provide the functional connectivity between the viscera and the other divisions of the nervous system, particularly the ectoneural and hyponeural divisions. Nothing is known about how these connections occur, although it is highly probable that the extension of the visceral plexus into the mesenteries plays an important functional role in the neuronal circuitry.

Basiepithelial plexus

The basiepithelial plexus is probably the best-described nervous component within the echinoderm enteric nervous system. This plexus is prominent in asteroids and echinoids, where it forms an extensive nervous layer adjacent to the luminal epithelium (Hyman, 1955) (Fig. 3A). It is formed by abundant fibres and a few scattered neuronal cell bodies associated with the basal parts of the secretory luminal epithelium (Schechter and Lucero, 1968; Chia and Koss, 1994). This plexus contains catecholaminergic cells and fibres described, using histochemistry, in the oesophagus of the sea urchin *Heliocidaris erythrogramma* (Cobb, 1969a; Cobb and Raymond, 1979) and in the stomach of the sea star *Asterias rubens* (Cottrell and Pentreath, 1970). Immunoreactivity to γ -aminobutyric acid (GABA) has also been detected in this plexus, mainly in fibres and unipolar neurons in the oesophagus and stomach (Newman and Thorndyke, 1994). Cells and fibres expressing neuropeptides are also abundant within this plexus, but the possibility that these are co-

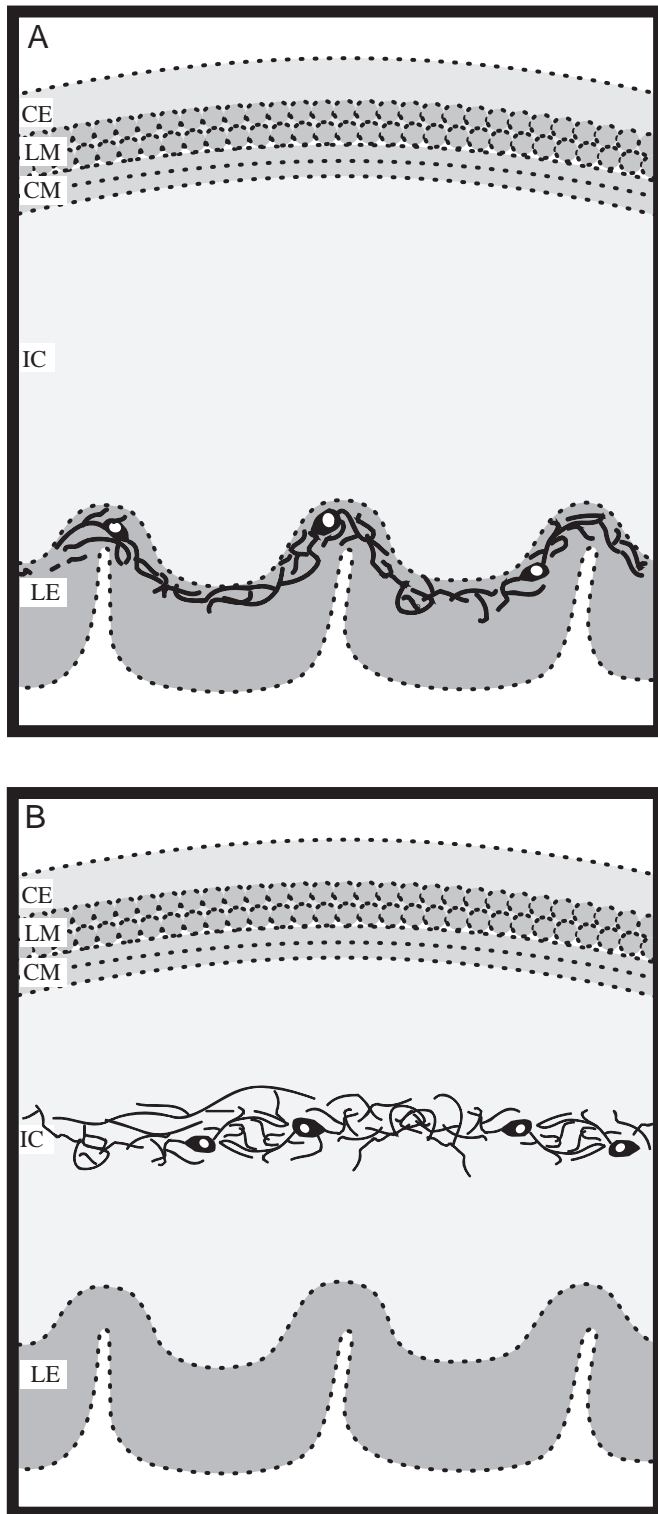


Fig. 3. Schematic diagram of the asteroid and echninoid (A) and holothurian (B) basiepithelial nerve plexus component of the enteric nervous system. In the anterior part of the intestine, a catecholaminergic nerve plexus is present in the internal connective tissue layer (IC). The plexus in most echinoderms is found within the base of the luminal epithelium (LE) layer. In holothurians, however, the plexus is found adjacent to the muscle layer. CE, coelomic epithelium; CM, circular muscle layer; LM, longitudinal muscle layer.

expressed by the catecholaminergic cells has not been studied. Moore and Thorndyke (Moore and Thorndyke, 1993) provide an extensive description of the expression of the peptide S1 in the basiepithelial plexus of *Asterias rubens*, while GFSKLYFamide, a member of the same neuropeptide family, has been described in *Holothuria glaberrima* (Díaz-Miranda et al., 1995). In *Marthasterias glacialis*, the basiepithelial plexus has been found to express immunoreactivity to the neuropeptide adrenomedullin (Martínez et al., 1996). The immunoreactivity is found in both the cardiac and pyloric stomach and in the pyloric caeca and is absent from the intestine and rectal caeca, a distribution that correlates with the proposed localization of the basiepithelial plexus presented here. Similarly, immunoreactivity to several neuropeptides, including pancreatic polypeptide, PYY and α MSH, and to the enzyme nitric oxide synthase has been found in fibres of the basiepithelial plexus of *Marthasterias glacialis* (Martínez et al., 1989; Martínez et al., 1993; Martínez et al., 1994).

The basiepithelial plexus is considered to be similar to the plexus in the epidermis and, therefore, to be part of the ectoneural nervous system division (Cobb and Raymond, 1979). In sea stars, the fibres form unspecialized contacts with the luminal cells or choanocytes (Bargmann and Behrens, 1968; Martínez et al., 1991). The basiepithelial plexus is separated from the muscle layer and from the visceral plexus by a basement membrane, with no apparent direct contact between the two plexi. In this respect, it follows the proposed separation by basement membranes found for other ectoneural-hyponeural nervous system divisions in echinoderms (Cobb, 1987). The function of the catecholaminergic plexus remains a mystery. It has been suggested that the catecholaminergic neurons and fibres of the basiepithelial plexus are involved in the control of ciliary or flagellar beating (Cobb and Raymond, 1979) and in the secretory activity of the luminal endothelial cells (Cobb, 1987), but experimental evidence is lacking.

In contrast to most echinoderm groups, in which the basiepithelial plexus is localized at the base of the luminal epithelium, this plexus is found within the internal connective tissue or submucosal layer in holothurians, lying adjacent to the muscular layer (Fig. 3B). In *Holothuria glaberrima* and *Holothuria mexicana*, the plexus contains catecholaminergic fibre bundles densely packed and running mainly in a longitudinal direction (Fig. 4). However, the pattern is not entirely linear, so that the fibres intertwine, creating a network. This oesophageal catecholaminergic nerve layer is continuous with the central nerve ring in the anterior part of the organism, providing support for the hypothesis that it is part of the ectoneural nervous system division. Isolated neurons or small groups of cells are intermingled along the fibre tracts. Smaller nerve bundles are occasionally seen to diverge towards more internal layers of the tract, ending within the connective tissue layer, but are never observed to reach the mucosal layer. As mentioned above, we have described the distribution of GFSKLYFamide expression in this plexus (Díaz-Miranda et al., 1995) (Fig. 5A,B). In addition, unpublished results from

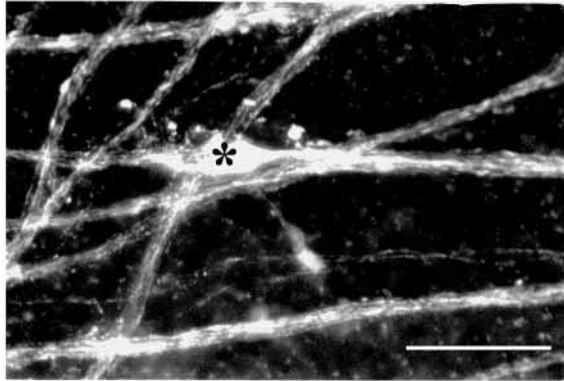


Fig. 4. The catecholaminergic nerve plexus in the oesophageal-intestinal region. Glyoxylic-acid-induced fluorescence was used to detect the presence of catecholamines in nervous elements of the digestive tract. In whole mounts of oesophageal tissue from *Holothuria glaberrima*, both the fibre plexus and one cell body (asterisk) can be clearly observed. Scale bar, 60 μ m.

our group have demonstrated immunoreactivity to other neuropeptides (galanin and bradykinin) and to GABA within the fibres and cells of this plexus in *Holothuria glaberrima* (Fig. 5C).

In contrast to the visceral plexus, the basiepithelial plexus of holothurians appears to be limited in extent and is found only in the anterior part of the digestive tract, being most prominent in the oesophagus and stomach (Chia and Koss, 1994; Díaz-Miranda et al., 1995; García-Arrarás et al., 1998). Several reports show that the number of cells and the size of the plexus become progressively reduced as one moves in the oral to aboral direction (Chia and Koss, 1994). A comparison between echinoderm groups can be made upon examination of the various observations presented in the seminal volume of Hyman (Hyman, 1955) on the Echinodermata. For example, in sea stars, the plexus appears to be present in the oesophagus, stomach and radial caeca, but not in the posterior part of the digestive tract and, apparently, the rectum. Similarly, there is strong evidence for the presence of the basiepithelial plexus in the oesophagus of sea urchins and in the stomach of ophiuroids (Byrne, 1994). However, the plexus is not found in the intestine of sea urchins (Hyman, 1955). Finally, in crinoids, the basiepithelial plexus is present in the oesophagus but is absent from the rectum (Hyman, 1955; Heinzeller and Welsch, 1994). In holothurians, Hyman (Hyman, 1955) describes the basiepithelial plexus in the pharynx of *Cucumaria* and in the stomach of *Leptosynapta* but not in the stomach of *Cucumaria*, in the intestine of *Leptosynapta* and *Claudina* and in the cloaca of *Stichopus*. Thus, most of the available evidence supports a specific localization of the plexus to the anterior areas of the digestive tract. Nevertheless, other investigators have found components of the basiepithelial plexus in the posterior digestive tract of sea stars. Martínez et al. (Martínez et al., 1993) describe the presence of immunoreactivity to various neuropeptides in the basiepithelial plexus of the intestine and rectal caeca of the sea star *Marthasterias glacialis*, although

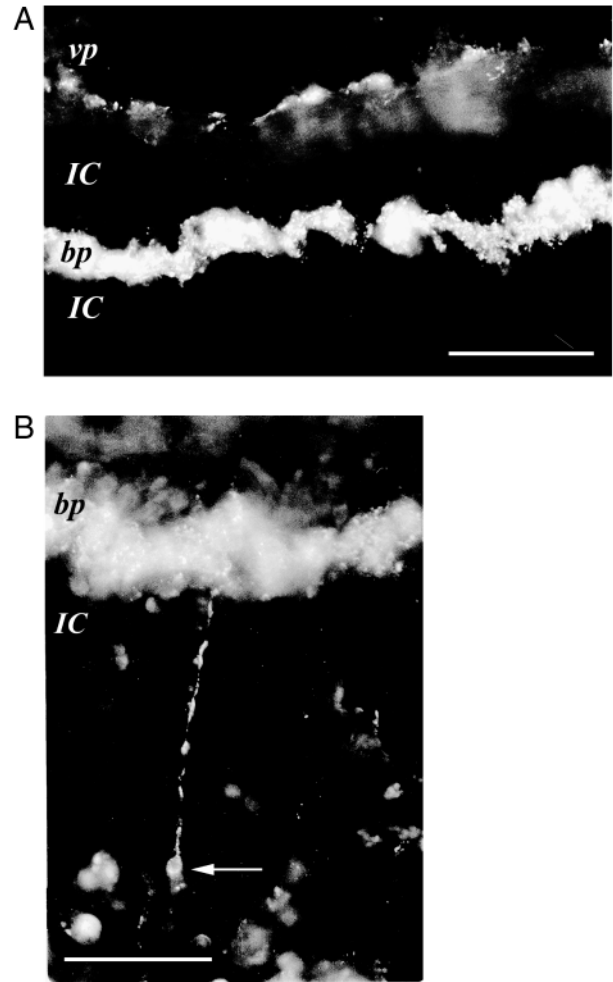


Fig. 5. Neuropeptide and γ -amino butyric acid (GABA) immunoreactivity in the basiepithelial nerve plexus (bp). Transverse sections of the oesophagus of *Holothuria glaberrima* showing immunoreactivity to GFSKLYFamide (A,B) and to GABA (C) within the internal connective tissue (IC). For both neuropeptide and GABA, the immunoreactive cells (arrows) associated with the plexus are located close to the luminal epithelium, with fibres extending to the main plexus. The visceral plexus (vp) can also be observed in A. M, muscle layer. Scale bars: A, 60 μ m; B,C, 50 μ m.

they report that the plexus is reduced in size and extent in these sites. Similarly, Cobb and Raymond (Cobb and Raymond, 1979) found catecholamine-induced fluorescence in fibres of the rectal caeca of *Asterias rubens*. They also emphasize that, at this site, the fibres form a fine plexus, not the nerve bundles found in the anterior part of the digestive tract, that can be best observed in sagittal sections. This may indicate that the basiepithelial plexus can extend to the posterior sections of the digestive tract in some species or that, because of a reduction in its size, the plexus can only be detected in these posterior structures using high-sensitivity probes and has not been detected in studies in which the antibodies or other labelling techniques used lack sufficient sensitivity.

In a study directed at examining these apparent anatomical differences in *Holothuria glaberrima*, we have also found that

detection of the presence of the plexus depends on the part of the digestive tract examined (Fig. 6A,B). We have used histochemical labelling for catecholamines and immunoreactivity to various neuropeptides (Díaz-Miranda et al., 1995; L. Jimenez, W. Mejías and J. E. García-Arrarás, unpublished) to show that the plexus is prominent in the oesophagus of this species. However, at the posterior end of the oesophagus, where it joins the small intestine, the nerve plexus gradually changes from a continuous plexus into fibre tracts. These tracts eventually disappear, ending within the small intestine. Thus, neither the catecholaminergic fluorescence nor the peptide immunoreactivity associated with

this plexus is detected in the internal connective tissue layer of most of the holothurian digestive tract. Therefore, the available information leads us to conclude that the basiepithelial plexus is found in most, if not all, echinoderm groups and that this plexus is particularly associated with the oesophagus and stomach, but is absent from most of the intestine and posterior digestive tract.

The probability that the basiepithelial plexus of holothuroids is not homologous to that of echinoids and asteroids must be considered. Nevertheless, the fact that in all groups this plexus contains the only catecholaminergic neurons in the digestive tract, is continuous with the ectodermal system of the nerve ring and appears to be prominent in the anterior portion of the digestive tract, provides strong evidence for it being a homologous structure whose location has endured an evolutionary change in holothurians.

Mucosal neuroendocrine plexus

Cells that resemble vertebrate intestine neuroendocrine cells are found within the luminal epithelium of the digestive tract (Fig. 7). These cells are present along most of the digestive tract, including the oesophagus, stomach, intestinal system and rectum. Most studies have been made in sea stars, in which cells expressing immunoreactivity to the

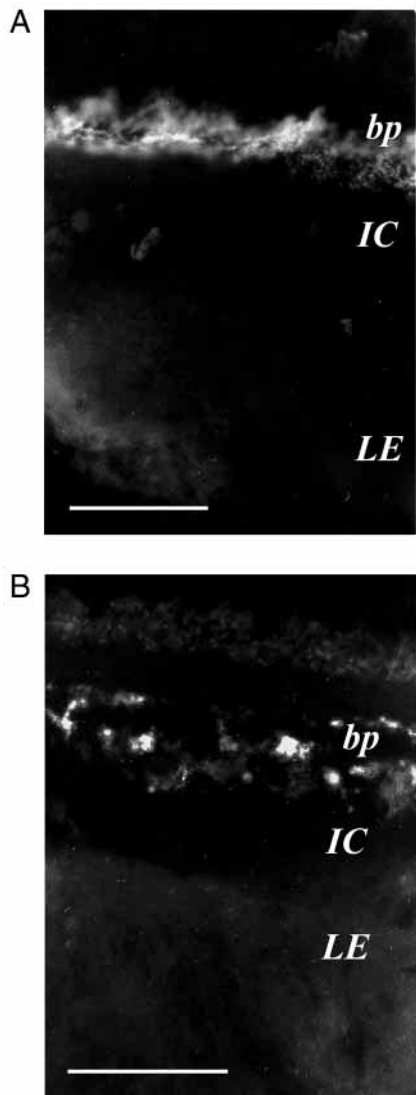


Fig. 6. Glyoxylic-acid-induced fluorescence of the basiepithelial nerve plexus at two levels of the holothurian oesophagus. (A) Catecholaminergic fibres occur as a continuous and extended layer of fluorescence within the internal connective tissue layer (IC) in the most anterior part of the oesophagus. (B) At more posterior levels, the catecholaminergic plexus is divided into smaller tracts that will eventually end within the anterior part of the small intestine. bp, basiepithelial plexus; LE, luminal epithelium. Scale bars, 50 μ m.

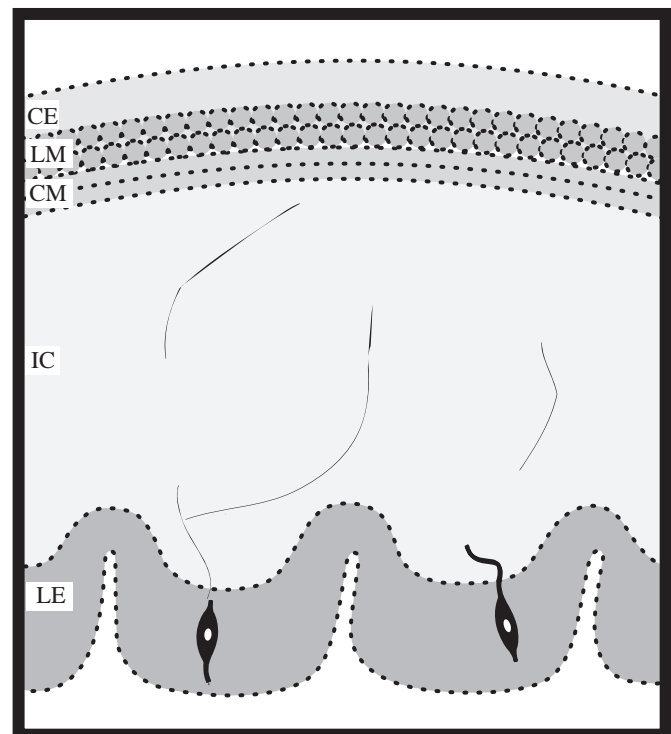


Fig. 7. Schematic diagram of the holothurian mucosal endocrine plexus component of the enteric nervous system. Neuroendocrine cells are found associated with the luminal epithelium (LE). Some of these cells extend fibres into the internal connective tissue layer (IC), some of which may contact the muscle or coelomic epithelial cells. CE, coelomic epithelium; CM, circular muscle layer; LM, longitudinal muscle layer.

neuropeptides somatostatin, glucagon, pancreatic polypeptide, S1, FMRFamide, PYY and α MSH (Martínez et al., 1989; Martínez et al., 1993; Martínez et al., 1996; Moore and Thorndyke, 1993) have been reported. Some of the cells have also been reported to express immunoreactivity to nitric oxide synthase (Martínez et al., 1996). In *Holothuria glaberrima*, some of the cells in the mucosal epithelium of the small and large intestine express immunoreactivity to GFSKLYFamide (Díaz-Miranda et al., 1995; García-Arrarás et al., 1999) (Fig. 8). Similarly, in *Apostichopus japonicus*, NGIWYamide-containing cells have been found within the digestive epithelium (Inoue et al., 1999).

Some of these cells are endocrine-like cells expressing immunoreactivity to vertebrate peptides. Examples of these are the cells expressing immunoreactivity to somatostatin, glucagon, pancreatic polypeptide and α MSH in the pyloric caeca of the gut of sea stars (Martínez et al., 1989; Martínez et al., 1993). In contrast to some of the neuroendocrine cells that have been described in sea stars, the holothurian GFSKLYFamide-expressing cells have long neurite-like fibres that, in some cases, can be followed for long distances away from the luminal epithelial layer (Díaz-Miranda et al., 1995). In fact, in some cases, the fibres arising from the mucosal cells can be traced to the visceral muscle layer, suggesting that some of the fibres within the visceral plexus may originate in the luminal mucosal cells. Moore and Thorndyke (Moore and Thorndyke, 1993) have also suggested the neuronal nature of the neuropeptide-expressing cells within the digestive mucosa, proposing that they may be the perikarya of the neurons that provide the fibres of the basiepithelial plexus. However, two facts argue against this possibility: (i) the neuropeptide-expressing mucosal cells can be found in areas of the digestive

tract in which there is no basiepithelial–epithelial plexus; and (ii) some of the neuropeptide markers found within these cells, such as insulin and glucagon, are not found within the nerve plexus. Nevertheless, the hypothesis presented by Moore and Thorndyke (Moore and Thorndyke, 1993) may be partially correct, and at least some of the cell bodies within the mucosal epithelium may correspond to the perykarya of the basiepithelial plexus. Particular examples are presented in the photographs of Martínez et al. (Martínez et al., 1993; Martínez et al., 1994); the spherical cell soma of some of the cells within the luminal epithelia is more typical of a neuronal cell than of the elongated cell body that characterizes neuroendocrine cells in the digestive tract of many species. An alternative explanation is that the holothurian mucosal neuroendocrine plexus represents a reduced basiepithelial remnant in this highly evolved group and that what we have named the basiepithelial plexus is a new nervous structure. However, the facts that catecholaminergic cells and fibres are not found associated with the mucosal plexus and that it is the basiepithelial plexus that is continuous with the nerve ring argue against this possibility. Thus, future experiments are needed to determine the complex structural and evolutionary relationship between the basiepithelial and mucosal plexi.

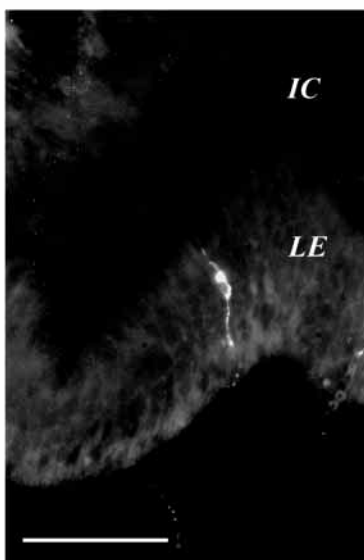


Fig. 8. A neuroendocrine cell expressing the neuropeptide GFSKLYamide in a transverse section of the intestine of *Holothuria glaberrima* within the luminal epithelium (LE). IC, internal connective tissue. Scale bar, 60 μ m.

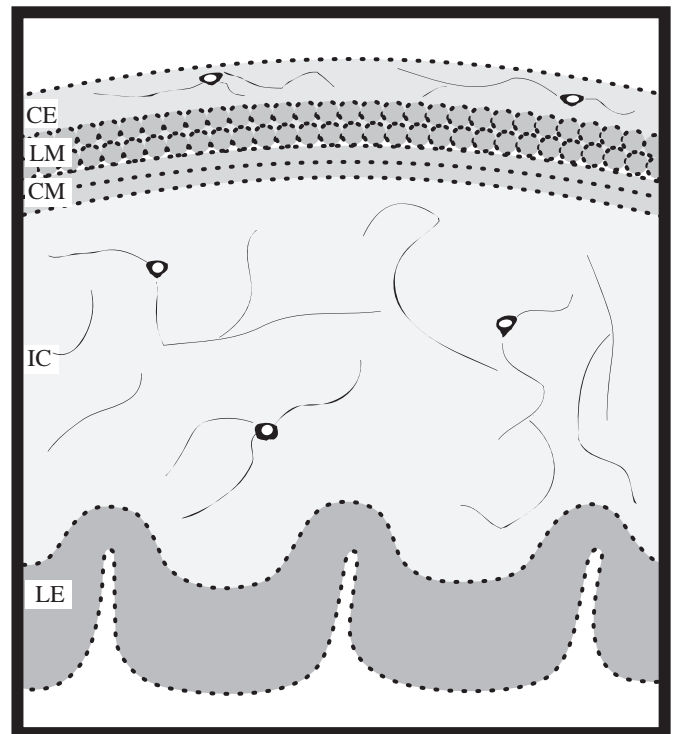


Fig. 9. Schematic diagram of the holothurian connective tissue plexus. This plexus consists of cells within the internal connective tissue (IC) layer that extend multiple, fine, varicose fibres that create a network within the connective tissue. A smaller number of cells and fibres can sometimes be found within the connective tissue associated with the coelomic epithelium (CE) and with the mesentery. CM, circular muscle; LE luminal epithelium; LM, longitudinal muscle.

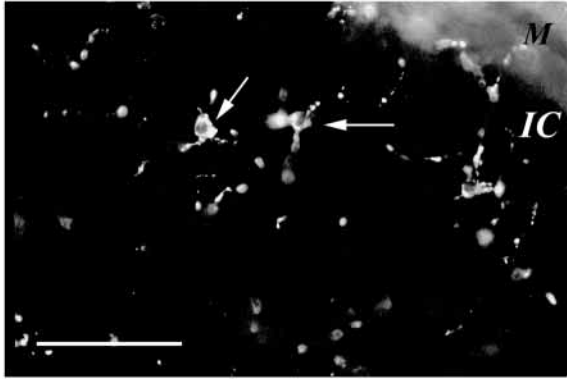


Fig. 10. A transverse section of a holothurian intestine showing immunoreactivity to the monoclonal antibody F6. The connective tissue plexus component of the enteric nervous system is recognized by this monoclonal antibody, which labels the neuronal perikarya (arrows) and the fine varicose fibres that extend throughout the internal connective tissue (IC). M, muscle layer. Scale bar, 65 μ m.

Connective tissue plexus

A putative neuronal network associated with the intestinal connective tissue has been described in our laboratory (García-Arrarás et al., 1999) (Fig. 9). Although the characterization of this plexus is incomplete at present, it is important to describe the available information so that it may be taken into account in future studies. The cells and fibres of this plexus have been identified solely by immunoreactivity using a monoclonal antibody (F6) generated against holothurian intestine (Fig. 10). The network is made up of small neurons with long fine varicose fibres that extend throughout the submucosal connective tissue. Although fewer in number, neurons and fibres are also found within the connective tissue of the mesentery and the serosa. The characterization of this network, however, has proved difficult because it is labelled only by a monoclonal antibody that appears to be species-specific and its neurochemical phenotype has not been determined. The possibility exists, however, that this plexus is associated with the changes in connective tissue viscosity characteristic of echinoderms and that the cells recognized by this antibody are similar to the juxtaligamental cells described by earlier investigators (see Wilkie, 1979; Cobb, 1988).

There is very little information about the role of the echinoderm enteric nervous system in digestive and other physiological processes. Although it has been suggested that neuroendocrine cells are involved in the control of digestive metabolism and that the visceral plexus is associated with the contraction of the digestive tract, experimental data are scarce. Information about other possible roles of the enteric nervous system in processes such as evisceration of the digestive tract in holothurians or stomach eversion in sea stars is limited. Moreover, the neuroanatomical data reviewed here, particularly the presence of nerve fibres that traverse the internal connective tissue, present some challenges to the proposed separation of echinoderm nervous components by basal laminae that need to be closely examined in future

studies. In summary, although our understanding of the echinoderm nervous system has improved over the last decade, several key issues are still unresolved. Basic information is still needed about the anatomical location of neurons and fibres expressing putative neurochemicals and about the neurochemical nature of identified neuronal elements. In addition, new information is essential to explain the circuitry of the enteric nervous system and its connection to the hyponeural and ectoneural components of the echinoderm nervous system.

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