Enteric Nervous System-Intestinal Lumen Communication: Role of an Epithelial Neural Network

Victor Salazar[1], Pura Bolanos[2], Jesus R. del Castillo[3*]

[1]*Light Microscopy Service, Instituto Venezolano de Investigaciones Científicas (IVIC), Venezuela*

[2]*Laboratory of Cell Physiology, Instituto Venezolano de Investigaciones Científicas (IVIC), Venezuela*

[3]*Laboratory of Molecular Physiology, Biophysics and Biochemistry Center, Instituto Venezolano de Investigaciones Científicas (IVIC), Venezuela*

Corresponding Author*

Jesús R. del Castillo,

Laboratory of Molecular Physiology, Biophysics and Biochemistry Center

Instituto Venezolano de Investigaciones Científicas (IVIC)

Caracas, Venezuela

E-mail: jrdelcas@gmail.com, jrdelcas@correo.ivic.gob.ve.

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Abstract

The intestinal lumen is constantly exposed to multiple challenges induced by changes in pH, mechanical and osmotic forces, chemicals, commensal bacteria and diet-derived products, which must be detected by specific epithelial sensors that induce a local response to regulate those changes and maintain intestinal homeostasis. It is generally accepted that communication between the lumen and the enteric nervous system is accomplished *via* paracrine through the release of serotonin (5-hydroxytryptamine, 5-HT) and intestinal peptides, by enteroendocrine cells located in the intestinal epithelium, which senses luminal stimuli through receptors exposed to the intestinal lumen. The serotonin would activate receptors in afferent nerve terminals in the intestinal mucosa's sub-epithelial region and transmit the information to the intestinal plexuses and the CNS. Nevertheless, we recently reported histological and functional evidence demonstrating the existence of a complete epithelial neural network that reaches the epithelial surface and connects the duodenal lumen with the enteric nervous system. This finding is novel and significant in existing research, as it challenges the current understanding of the communication between the lumen and the enteric nervous system. The network reaches glia, nerve terminals from the vagus or the enteric nervous system, enterochromaffin cells and sub-epithelial lymphoid follicles at the lamina propria through axon extensions. Here, we review the experimental evidence that led us to the identification of this neuronal complex in the duodenal epithelium and discuss, based on histological evidence, some possible implications that this system would have for the intestinal sensory and regulatory functions at the epithelial level, as secretion, endocrine regulation in the mucosa, and likely the local innate immune response.

Keywords: Duodenal epithelium • Epithelial neuron • Enteric Nervous System

Introduction

For years, scientists have been intrigued by the transmission of sensory information from the intestinal epithelial barrier to the Central Nervous System (CNS). A crucial step in this mechanism is the communication between the luminal space, in direct contact with the intestinal epithelium, and the enteric nervous system. This system, located within the gastrointestinal tract's wall, comprises enteric ganglia, which contain neurons, and glial cells connected by nerve fiber bundles. The axons of these nerve cells innervate other ganglia and tissues of the digestive organs, as well as the muscle layers and the mucosa. This structural nervous network supports multiple reflex circuits that modulate the digestive tract, which can function without control from the CNS. However, the enteric nervous system also interacts with the spinal cord and brain, ensuring information exchange between the enteric and central nervous systems [1-3].

Literature Review

Enteric nervous system and intestinal lumen communication

It is generally accepted that communication between the lumen and the enteric nervous system, as an interface between the gut and the brain, is accomplished through the release of serotonin (5-hydroxytryptamine, 5-HT). According to this model, enteroendocrine cells in the intestinal epithelium sense chemical, osmotic and mechanical stimuli through receptors exposed to the intestinal lumen, liberate serotonin and activate receptors in the intrinsic and extrinsic afferent nerve terminals that reach the sub-epithelial region of the intestinal mucosa and transmit information to the intestinal plexuses, intestinal ganglia and spinal cord [4,5].

From the functional point of view, there are three essential elements in this model:

Enteroendocrine sensory cells: The epithelial cells that express receptors for chemical, mechanical, or osmotic stimuli in direct contact with the intestinal lumen are the keystone of this model. Their crucial role in the initial steps of the communication process, as the first responders to various stimuli in the luminal environment, emphasizes their significance.

Nerve terminals: That reaches the sub-epithelial space without penetrating the epithelium and express serotonin receptors.

A neuronal network: Communicating epithelium with the ENS includes afferent neurons not associated with ganglionic structures, interneurons, glia, the submucosal and myenteric plexuses.

Epithelial enterochromaffin cells

Serotonin-containing Enterochromaffin (EC) cells, the most abundant enteroendocrine cells in the gut, are distributed through the gastrointestinal tract, mainly in the small intestine and rectum [6,7]. EC cells contain serotonin (5-HT) stored in large cytoplasmic granules [8]. Release of 5-HT from the EC cells is a Ca^{2+}-dependent process, mediated by mucosal movement, chemical stimulation with nutrients (e.g., pH, glucose), toxins (e.g., cholera toxin, chemotherapeutical drugs), bile salts, amines, tastants, odorants and endogenous substances such as adenosine [9,10]. Although endogenous 5-HT release from the mucosa is not required for normal gastrointestinal motility or transit in healthy bowel, there is evidence that endogenous 5-HT release from the mucosa can modulate ENS activity and gastrointestinal motility *via* chemosensory stimulants applied to the lumen [11,12].

The intestinal EC cell population is heterogeneous [13]. For its part, integrating single-cell transcriptomics and spatial image analysis, identified fourteen EC cell clusters topographically organized along the gut [14]. They identified some subtypes predicted to be sensitive to the chemical environment and mechanical forces that express distinct

transcription factors and hormones. These findings provide evidence of EC cell molecular and cellular heterogeneity.

Enterochromaffin cells have been proposed as chemo-sensors. Bellono et al., using cultured intestinal organoids and single-cell measurements, showed that EC cell expresses specific chemosensory receptors and voltage-gated ion channels [15]. In addition, they showed the modulation of serotonin-sensitive primary afferent nerve fibers *via* synaptic connections, suggesting that EECs can directly detect and transduce environmental, metabolic and homeostatic information from the gut to the nervous system. However, the local control of serotonin secretion by the enterochromaffin cells in the intestine has yet to be elucidated. It is necessary to remark that the newly described epithelial neuron contains serotonin storage in small cytoplasmic vesicles, as identified in some other neurons [16]. In this sense, by using formaldehyde-induced fluorescence and immune labeling, we showed that 39% of the total 5-HT-containing cells in the guinea pig duodenal epithelium were NKAIN4-positive cells, the neuronal protein used to identify the epithelial neurons [17]. Interestingly found, in thick (20 µm) sections of rat duodenum, serotonin-containing cells with similar distribution and shape to the recently described epithelial neurons [18,19].

It has been reported that any vagal afferent endings made close contact with EC cells. The absence of physical contact between 5-HT-containing EC cells and vagal afferent nerve endings in the mucosa leads to the inescapable conclusion that the mechanism by which 5-HT release from ECs in the colonic mucosa occurs in a paracrine fashion to activate vagal afferents [7]. However, Bellono et al., report that close contacts occur between enteroendocrine and neuron dishes in culture cells, but strong evidence of synapses *in situ* is lacking [15]. Recent anterograde tracing studies found no evidence of close contact between EC cells and spinal afferent endings in the mucosa, contrasting the hypothesis of synaptic activation from EC cells [7,20]. Recently, Bohórquez et al., reported synaptic links between epithelial sensory cells and nerves in the colon, using co-culture of CCK-GFP cells purified by cell sorting and sensory neurons dissociated from sensory ganglia by enzymatic digestion [21]. Furthermore, they reported intestinal enteroendocrine cells with cytoplasmic processes that they named neuropods, providing a putative direct connection between enteroendocrine cells and neurons innervating the small intestine and colon [22,23].

Sub-epithelial neurons and nerve terminals

The enteric nervous system, a remarkably intricate network within the gastrointestinal tract, is a wonder of biological architecture. Comprising of ganglia housing neurons, this system innervates other ganglia, intestinal muscle layers, and the mucosa, creating a complex web of communication. While it can function autonomously, it relies on innervation from the autonomic nervous system, specifically through the vagus nerve and sympathetic ganglia, for specific functions [24]. This network, essential in regulating the function of the gastrointestinal tract, is the backbone of numerous reflex circuits.

At the mucosa level, an extensive innervation comes from intrinsic intestinal neurons through nerve endings in the connective tissue of the lamina propria. These nerve terminals do not directly contact the luminal contents since they do not cross the epithelium. In addition, until recently, there was no conclusive evidence for the presence of neuronal bodies in the intestinal epithelium. Some small groups of neurons lie in the lamina propria of the mucosa, close to the muscularis mucosae. Thus, no evidence existed that the enteric nervous system directly contacts the intestinal lumen. A true celebrity in the field used silver impregnation and methylene blue staining in the intestinal tissues of rabbits and guinea pigs to meticulously map out the general arrangement of the plexuses and the structure of the cells forming their ganglia [25].

Her innovative work revealed that the myenteric and sub-mucous plexuses are nerve cells connected by intrinsic and extrinsic fiber bundles. The extrinsic fibers run in fiber bundles end with the nerve cells, and the processes of these latter, either as axons, convey the impulses to the muscle and glands, or as dendrites, terminate as receptive endings related to nerve cells in other plexuses ganglia. Hill's model of gut innervation was a cornerstone in histological education, as evidenced by its inclusion in histology texts, and a framework for the development of a model of hormonal and paracrine signaling and nerve control between the intestinal epithelium and the nervous plexi [26,27].

Most intestinal intrinsic neurons are in the myenteric and submucosal plexi. In the myenteric plexus (Auerbach's plexus), there is a continuous net along the intestine, with single neurons occasionally located outside this plexus. In the submucosal plexus (Meissner's plexus), ganglia contain secretomotor but not motor neurons. However, in larger mammals (pigs, monkeys and man), some motor neurons have cell bodies in the submucosal ganglia [28].

In this model, the communication between the intestinal lumen and the enteric nervous system is an indirect process mediated by the local secretion of serotonin from the EC cells. There has been a dogma regarding neuroepithelial interactions, seen exclusively from the perspective of soluble and spreading mediators, as proposed for the skin [29]. This creed has also been maintained for the gut, mainly based on histological evidence.

Are there any epithelial neurons in the gut?

Identifying neurons and nerve endings in the intestinal epithelium has been elusive. Lundberg et al., found catecholamine-containing nerve terminals near the basement membrane of EC cells, suggesting adrenal innervation of enterochromaffin cells in the intestine of guinea pigs and Newson et al., observed neuron-like cells beneath the epithelium of the crypts of rat ileum [30-32]. Their processes were often separated from the crypt cells only by a basal lamina. Dahlström et al., identified, by electron microscopy and immunofluorescence, using some neuronal markers available for the time, sub-epithelial neuron-like cells located just beneath the basal lamina of rat ileal crypts, but the evidence was non-conclusive [33]. However, there are no reports about sensory neurons in the intestinal epithelium, as identified in other epithelia.

The gut can sense messages from the external (luminal medium) and internal environment and can be considered a sensory organ [34]. This sensing function is carried out by specialized cells that can elicit electrical activity in response to an external stimulus [35]. For a cell to be classified as a sensor, it must possess molecular receptors to sense or detect an input (e.g., nutrients, bacteria). Once these receptors are activated, they trigger an amplifying intracellular signaling cascade, resulting in the release of a secreted signaling molecule (e.g., neurotransmitter).

Indeed, the sensory cells in the intestinal epithelium, with their receptors in contact with the external environment, play an essential role. These receptors identify molecules that act as stimuli, triggering an intestinal response. Among these, the mammalian sweet and umami taste T1R receptor family stands out as a significant group, spanning the entire gastrointestinal epithelium. Stimulation of taste receptors activates a signaling cascade that ends in the release of intracellular Ca^{2+}, which in turn activates the Transient Receptor Potential Channel M5 (TRPM5) to trigger membrane depolarization and induces an additional influx of Ca^{2+} from voltage-gated channels [36-38]. Transporters or channels can also sense stimuli. Absorbed nutrients can be detected at the transport site or during subsequent metabolism. Absorption of nutrients is frequently coupled with ion uptake, which generates a small depolarizing current, allowing the sensory cells to identify the stimulus.

Contrasting with the existing evidence, we recently reported histological and functional evidence demonstrating the existence of a complete epithelial neural network that reaches the epithelial surface and connects the duodenal lumen with the enteric nervous system [17]. The neuron reaches the lumen through the neuronal apical extension (including the knob-like terminal) and the sub-epithelial space through axon extensions, connecting with glia, nerve terminals, enterochromaffin cells and sub-epithelial lymphoid follicles at the lamina propria. In addition, the epithelial neuron sends basal extensions toward the epithelial cells, surrounding them, projecting the network toward the epithelial surface (Figure 1).

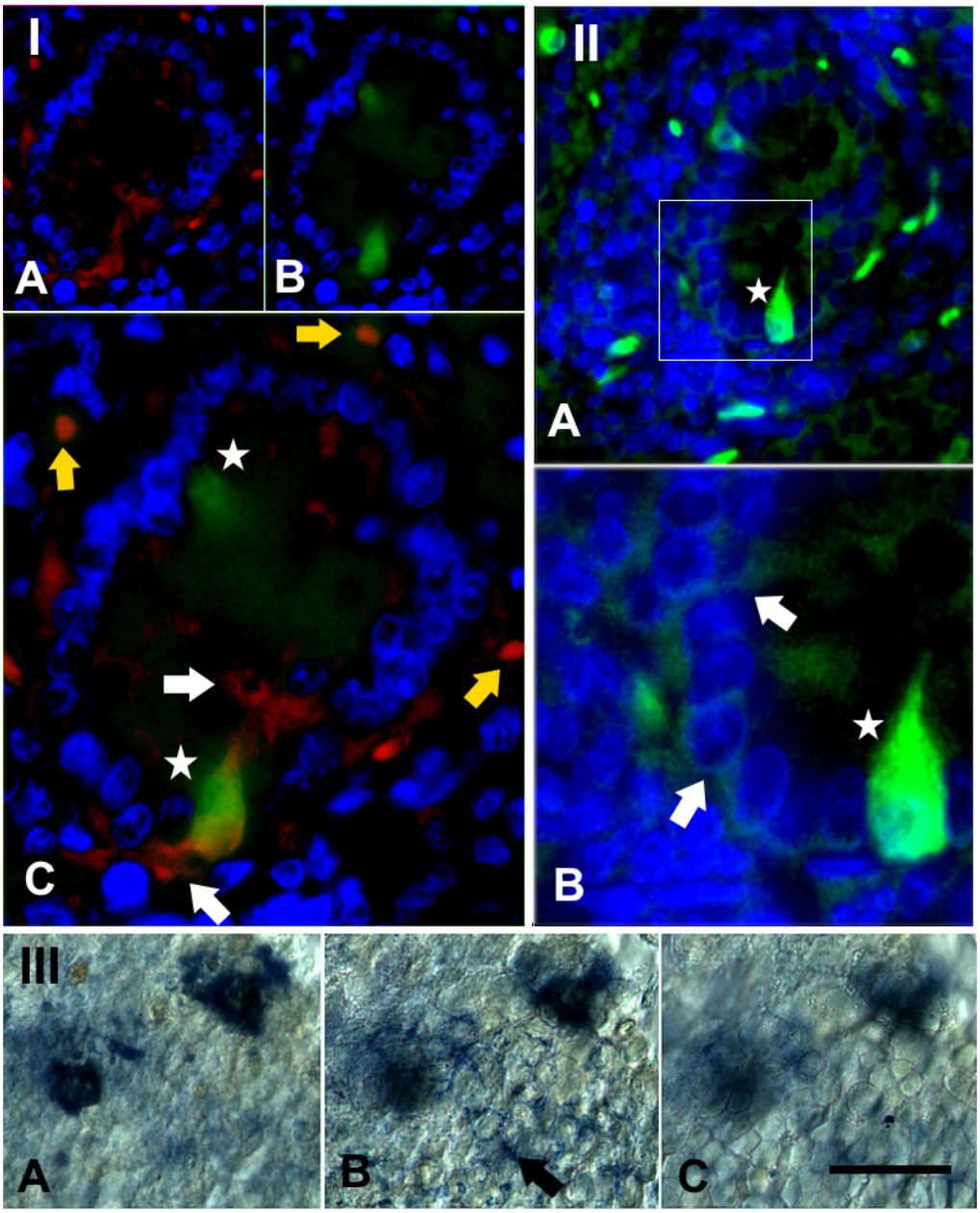

Figure 1. Epithelial neurons send projections to the epithelial surface, surrounding the epithelial cell base and forming a network of nodular endings very close to the luminal surface of the epithelium without penetrating it.

I. Image presents a transverse histological section (5µm) at the level of the middle portion of the intestinal crypts in the duodenum stained by immunofluorescence using antibodies again NKAIN-4 (A, in red), COX1 (B, in green), and DAPI (blue). Image in C presents the composite of A and B.

   Two epithelial neurons (stars), their basal extensions towards the base of epithelial cells and sub-epithelial structures, and their projections towards the surface of the epithelium are identified (white arrows). Nerve terminals (yellow arrows,) were also observed around the crypt.

II. Serial section (5µm) of the material presented in the panel I, stained by immunofluorescence using antibodies again COX1 (green) and DAPI (blue), of an intestinal crypt in the duodenum.

   In (A), we observe an epithelial neuron, and its basal extensions directed toward the epithelial cell bases surrounding them and sending projection to the epithelial surface. The image in (B) presents a magnification of the area marked by the rectangle at the base of epithelial cells. White arrows indicate the inter-epithelial cell projections of the epithelial neuron (star).

III. Duodenal epithelium in thick sections (50µm) cleared and stained with methylene blue. At 20µm from the surface of the epithelium (A), well-defined epithelial neurons are observed, but their extensions are not visible. At 10µm from the surface (B), the nodular extensions of the epithelial neurons are observed (black arrow), and the neurons are now less defined (located in another plane). Finally, the neurons and extensions are not defined on the epithelial surface (C), which indicates that the neuronal projections do not cross the epithelium, probably reaching the intercellular junctions of the epithelial cells.

Bar corresponds to: 30 µm (I-A and I-B); 19 µm (I-C); 30 µm (II-A); 12 µm (II-B) and 80 µm (III).

Interestingly identified a richly developed plexus composed of intrinsic varicose fibers present directly under the epithelium of the villi. These fibers penetrate between the epithelial cells at their base to form a network at their basal halves. Still, she could not find a connection between these epithelial neural fibers and the intestinal plexuses.

The network distribution formed by the epithelial neurons, glial cells and neural fibers is non-alary. The complex forms patches in the epithelium, frequently surrounded by enterochromaffin cells, as shown in Figure 2.

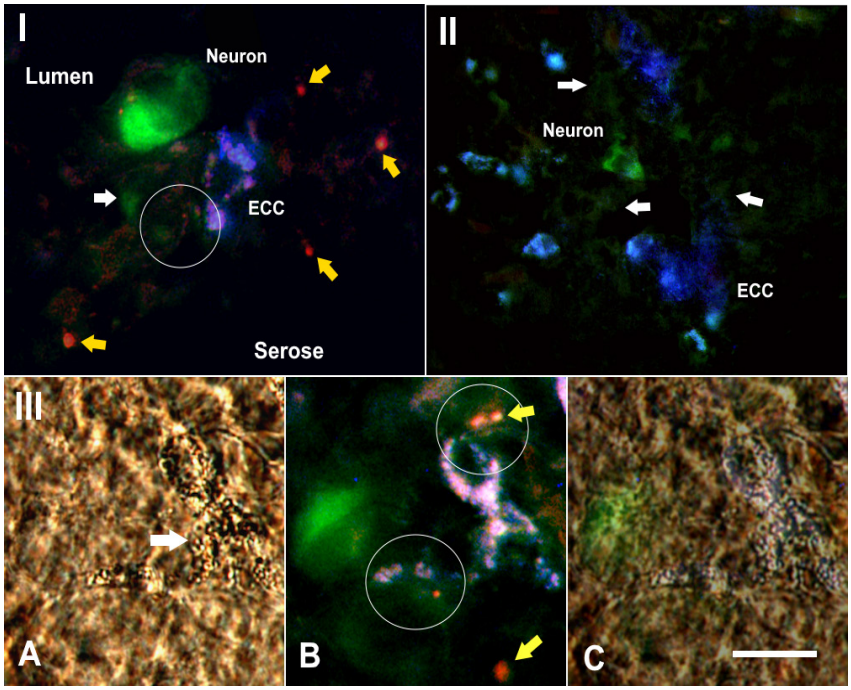

Figure 2. Epithelial neurons extend to the enterochromaffin cells and vagus nerve terminals of the mucosa, establishing direct contact.

In this figure, our methodology involved a combination of Immunofluorescence for NKAIN-4 (green), retrograde labeling with DII (red), formaldehyde-induced fluorescence (white/blue), and Differential Contrast Interference (DIC) microscopy in thick duodenal sections (50 µm). This approach allowed us to make the following observations:

I. Image shows epithelial neurons (green) in direct contact with enterochromaffin cells (EC). The EC cells have a white/blue color caused by the formaldehyde-induced fluorescence due to their bioamine content, generating fluorescence in red, green, and blue channels. Nerve terminals from the vagus (yellow arrows) were stained by retrograde labeling with DII. The epithelial neuron reaches the EC cells and nerve terminals in the lamina propria through its projections (white arrows). These connections were previously demonstrated using confocal microscopy in isolated villous-crypt units loaded with Fluo4-AM to measure ACh-induced intracellular Ca^{2+} changes. The stimulation of epithelial neurons induces serotonin secretion in EC cells (Salazar et al., 2023). The circle indicates a contact area between nerve terminals, EC cells, and epithelial neuron extensions.

II. This image presents an epithelial neuron surrounded by several EC cells at the duodenal mucosa. White arrows show epithelial neuron extensions.

III. A composition of DIC and immunofluorescence is shown. In the DIC image (A), a cell with pseudopodia containing large granules is observed. In (B), the epithelial neurons and their projections were labeled by immunofluorescence with anti-NKAIN4 (green) The circles surround areas where the epithelial neurons' extensions, the EC cells pseudopodia, and nerve endings from the vagus (yellow arrows) make contact. Image in C presents a composite of A and B.

Bar corresponds to: 17 µm (I); 47 µm (II) and 13 µm (III-A, III-B and III-C).

The recently identified epithelial neurons have morphological characteristics of sensory neurons, as defined by Heiman [39]. They are polarized cells with a thin and long apical process that finishes in a knob-like terminal protrusion in contact with the luminal space. At the base of the apical extension in the supra-nuclear region of these cells, we also identified nodular elements rich in γ-tubulin. Similar structures have been identified as primary cilium and basal bodies, based on a non-motile microtubule structure, present in neurons of many sensory systems [40,41]. These cellular elements could be part of a sensory apparatus in these epithelial cells. At their basal region, the epithelial neurons present projections toward the lamina propria that contact afferent nerve terminals from the vagus, particularly those at the base of epithelial crypt cells (Figure 3).

Previous research has hinted at a close relationship between cyclooxygenase, duodenal motility and mucosal alkaline secretion. In addition to the neurotransmitter ACh, arachidonic acid-derivates are also involved in regulating intestinal secretion. In this context, the cyclooxygenases (COX-1 and COX-2) are involved in the synthesis of Prostaglandins (PG) and Thromboxane (TX). COX-1, abundantly expressed in this cholinergic neuron (17), is constitutively expressed in most tissues and participates in the basal synthesis of eicosanoids. COX-1 is necessary to maintain the integrity of the gastrointestinal mucosa under physiologic conditions. In contrast, the expression of COX-2 is induced by stimuli such as inflammation [42,43].

Preceding findings suggest a close relationship between cyclooxygenase, duodenal motility and mucosal alkaline secretion. The administration of indomethacin, a cyclooxygenase inhibitor, induces duodenal contractions and increases mucosal alkaline secretion, effects that are abolished by the nicotinic receptor antagonist hexamethonium. In addition, luminal perfusion of the duodenum with lidocaine reduced mucosal alkaline secretion but did not affect motility, whereas serosal application of the drug reduced both motility and mucosal alkaline secretion. Authors speculated that serosal application of lidocaine might inhibit extrinsic autonomic neurons as well. It has also been shown that indomethacin stimulates mucosal alkaline secretion in vagotomized animals [44]. The relation observed between the epithelial neuron extensions and the vagus nerve terminals that reach the intestinal crypts in Figure 3 suggests that cholinergic systems and COX-1 activity could modulate the cryptal function.

Along with the expression of a cholinergic system, the epithelial neuron expresses Glutamate Decarboxylase (GAD), particularly GAD65 [17]. The GAD is the rate-limiting enzyme in synthesizing γ-Amine-Butyric Acid (GABA). GAD has two isoforms, GAD67 (also named GAD1) and GAD65 (also named GAD2), with different molecular weights encoded by different genes. The expression of GAD65 is confined to neuron membranes and nerve terminals and is transiently activated to produce GABA in response to the extra demand for GABA in neurotransmission [45,46]. GABA and GABA receptors have been identified along the gastrointestinal tract and implicated in the immune-mediated inflammatory processes [47-49]. The GABA-A receptor is recruited at low GABA concentrations to increase acetylcholine release and propulsive activities.

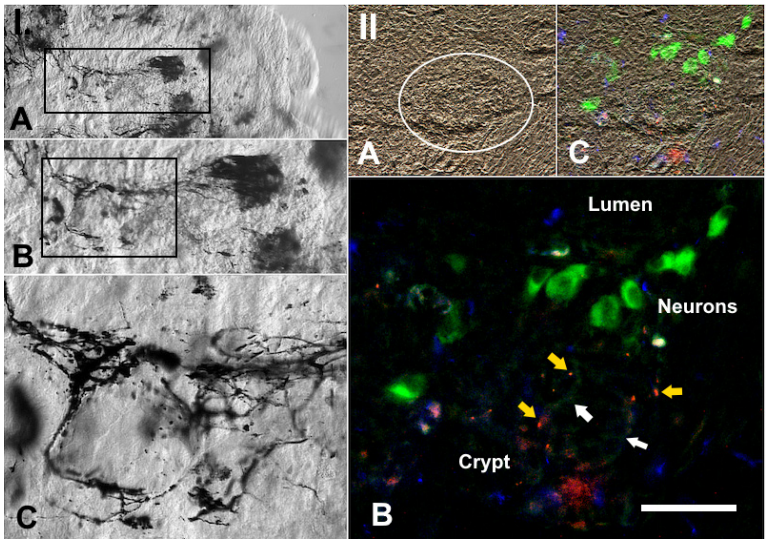

Figure 3. Epithelial neurons send extensions to the intestinal crypts in the duodenum.

I. Duodenal mucosa (50µm slices) stained with Methylene Blue was observed with Differential Interference Contrast (DIC) microscopy. Epithelial neurons are very close to the epithelial surface, and their basal extensions towards epithelial and sub-epithelial structures are observed. In this case, the neuron projections reach a crypt, surrounding and penetrating it, as seen in the enlargement (C). The rectangles indicate the magnified areas.

II. Image in (A) shows an intestinal crypt (circle) observed with DIC microscopy. Photo in (B) presents immunofluorescence for NKAIN4 (green), retrograde labeling with DII (red), and formaldehyde-induced fluorescence (white/blue). (C) is a composite of (A) and (B).

A group of epithelial neurons (in green) was identified. They send basal extensions (white arrows) that surround and penetrate the crypt structure. Note the large number of nerve endings from the vagus (yellow arrows) that reach the crypt and contact the epithelial neuron extensions.

These images demonstrate the interaction of the vagus and the epithelial neuron at the intestinal crypts in the duodenum, suggesting a close relationship between vagal innervation and epithelial neurons in controlling intestinal secretion.

Bar corresponds to: 160 µm (I-A); 76 µm I-B); 33 µm (I-C); 100 µm (II-A and II-C) and 43 µm (II-B).

At higher concentrations of GABA, the GABA-B receptor is activated to decrease acetylcholine release and peristaltic activities. The GABAergic system plays an essential role in the anti-inflammatory process by inhibiting the expression of pro-inflammatory cytokines and inducible Nitric Oxide Synthase (iNOS) [48]. In addition, the GABAergic system has also been found in immune cells, i.e. monocytes, macrophages, and T cells.

Functional co-transmission of ACh and GABA has been reported in the retina and other neuroepithelia. Anatomical studies suggest that ACh/GABA co-transmission is a common feature. This functional fact has been characterized in retinal starburst amacrine cells [50].

ACh activates the epithelial neuron

The epithelial neurons expressed neuronal markers such as NeuN, ChAT, AChE, α3-AChR, and α7-AChR, suggesting that they are cholinergic neurons. Additionally, we showed that ACh activates the epithelial neurons [17].

The activation was evaluated using two probes: Neutral red, a highly lipophilic dye that permit to follow rapid changes in membrane potential. Depolarization alters the association of the neutral red with the plasma membrane, inducing changes in fluorescence [51].

Fluo-4 to detect free intracellular calcium concentration by using fluorescence and confocal microscopy. Ca^{2+}-imaging identifies neurons and their circuits [52-54]. Using these techniques, we identified the activation of the epithelial neuron by ACh, its associated neuronal circuit, and (with Ca^{2+} imaging) the triggering of remote enterochromaffin cells inducing 5-HT release. Serotonin secretion and the intracellular Ca^{2+} dynamic are intimately linked. EC cells release 5-HT from large dense core vesicles in a calcium-dependent process with kinetics resembling release from synaptic vesicles [55].

The activating ACh effect on the epithelial neuron was reversibly blocked by added serotonin, suggesting a negative feedback mechanism to control serotonin secretion from enterochromaffin cells, modulating the activity of the epithelial neuron [17].

Enterochromaffin cells are scattered throughout the intestine and exposed to different agents that induce serotonin secretion. In the face of an external stimulus maintained over time (osmolality, pH, or chemicals in the intestinal lumen), the secretion of serotonin would be permanent or exhausted if the stimulus exists. However, this phenomenon is not observed under physiological conditions, so there must be modulating mechanisms to avoid the constant release of serotonin when the EC cells confront an external stimulus. Therefore, regulating serotonin synthesis, release and inactivation should be essential factors in this process. Serotonin is synthesized from tryptophan by the action of the rate-limiting tryptophan hydroxylase and decarboxylation by the aromatic amino acid decarboxylase that can adapt to either short or long-term demands on activity. Reuptake is a significant means of terminating the action of serotonin. A plasma membrane Na^{+}-dependent transporter (SERT), the serotonin carrier, takes up 5-HT. Serotonin is also eliminated by enzymatic degradation. The primary catabolic pathway for serotonin is oxidative deamination by the enzyme monoamine oxidase [56,57]. These mechanisms seem appropriate for restricted diffusion spaces such as synaptic clefts but do not seem efficient in highly vascularized regions such as the lamina propria of the intestinal mucosa. A control through the epithelial neural network appears more appropriate.

Finally, it is essential to remark that ACh is an essential component of the cholinergic anti-inflammatory pathway, which regulates various immune processes. The cholinergic anti-inflammatory pathway links the parasympathetic system and the local immune response. The parasympathetic terminals can release ACh that interacts with α7-AChR on macrophages' surfaces, involved in anti-inflammatory pathways (57). During inflammation, the α7-AChR anti-inflammatory action has been associated with Ca^{2+} influx and the reduction of the levels of the pro-inflammatory cytokines MIP-2 and TNF-α, and the activation of the nuclear factor kappa B [58-60]. In this sense, Figure 4 shows that epithelial neurons send extensions to the sub-epithelial lymphoid follicles, providing histological evidence of the contact between the epithelial neuron and the local immune elements, suggesting a possible role of the cholinergic epithelial neural network in the mucosal immune response (Figures 4 and 5).

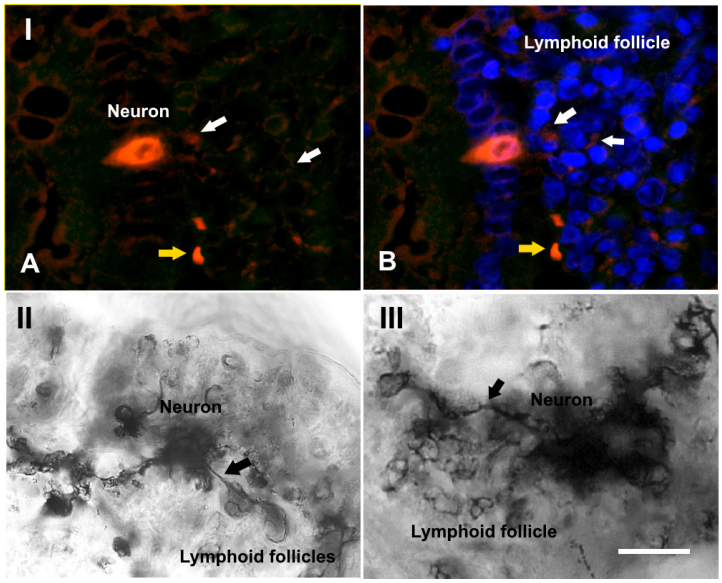

Figure 4. The epithelial neurons send extensions to the sub-epithelial lymphoid follicles.

I. Images show Immunofluorescence images of double-staining with NKAIN in red, COX1 in green (A) and DAPI, in blue (B). The epithelial neurons send projections (white arrows) to subepithelial lymphoid nodules, surrounding and penetrating them. The yellow arrows show some nerve terminals.

II. Silver impregnation allows identifying the connection between the epithelial neurons and the sub-epithelial lymphoid nodules in the duodenum. The neuronal basal projections (black arrows) reach, surround, and penetrate the sub-epithelial lymphoid nodes, suggesting the epithelial neurons could modulate part of the duodenum's epithelial immune activity.

III. Image presents a different neuron processed as described in Panel II.

The bar corresponds to: 18 µm (I-A and I-B); 40 µm (II and III).

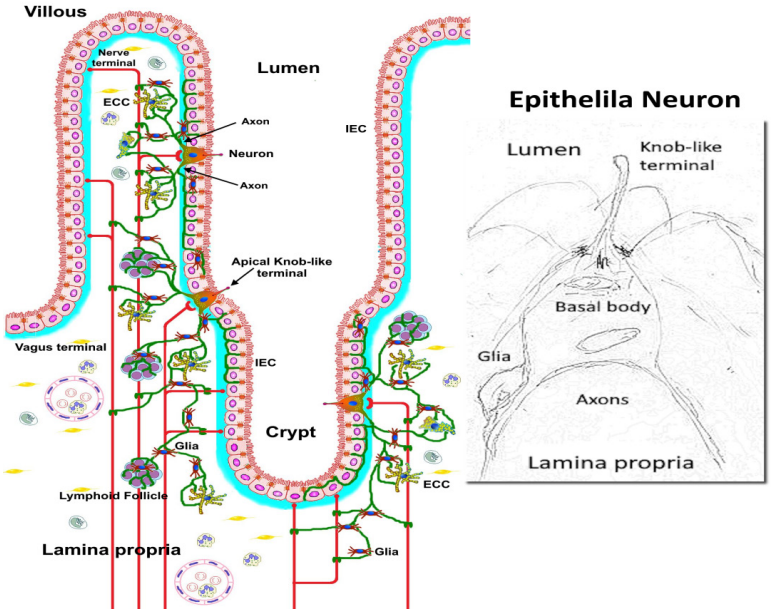

Figure 5. The diagram represents the Villous-Crypt axis, showing the localization of the epithelial neuron between intestinal epithelial cells and its apical extension that reaches the intestinal lumen.

The axons of the epithelial neuron and their extensions (drawn in green) surround the base of the intestinal epithelial cell, forming a dense epithelial neuronal web. This net also surrounds the intestinal crypts and contacts vagus nerve terminals (drawn in red) that reach the lamina propria. The neuron also contacts two other essential elements at the lamina propria, the Enterochromaffin (EC) cells and the sub-epithelial lymphoid follicles. Salazar et al., showed, using Ca^{2+}-imaging, that ACh activates the epithelial neuron and induces serotonin secretion from EC cells [17]. The neuronal extensions also reach the isolated lymphoid follicles, suggesting a possible neuronal modulation of the mucosal innate immune response. On the right, we present a schematic drawing of the epithelial neuron. It highlights some relevant structures identified on this cell, such as the Knob-like luminal terminal, a basal body, axons, and connections with other epithelial cells through tight junctions and glia. The potential implications of our findings open exciting avenues for future research and clinical applications.

It is essential to note that epithelial neurons in the duodenal epithelium could be inaccurately identified as enterochromaffin cells due to their serotonin content. Table 1 presents some morphological and functional differences between EC cells and epithelial neurons to address this point. This distinction is essential and intriguing in understanding the gut/lumen's complex communication system (Table 1).

Finally, it is essential to note that epithelial neurons in the duodenal epithelium could be inaccurately identified as enterochromaffin cells due to their serotonin content. Table 1 presents some morphological and functional differences between EC cells and epithelial neurons to address this point. This distinction is essential and intriguing in understanding the gut/lumen's complex communication system [61-64].

Table 1. Some morphological and functional differences between Enterochromaffin cells (EC cells) and epithelial neurons in the gut.

Characteristics	Enterochromaffin cell	Epithelial neuron [17]
Intestinal location	In the duodenum and throughout the rest of the small intestine.	In the duodenum
Epithelial location	Yes (open EC cells)	Yes
Extra-epithelial location	Yes (close EC cells)	No
Serotonin content	Into large granules	Into small granules
Extension to apical surface	Through microvilli [62]	By a knob-like terminal
Extension to Lamina Propria (LP)	No (It has short basal extensions)	Yes, by basal extensions
Cell size	<10µm [15]	~30 µm, without extensions
Direct contact with structures and cells	No*	Yes, with EC cells in the lamina propria, sub-epithelial lymphoid follicles, afferent nerve terminals, and epithelial cells at the basal halves.
Demonstrated markers	Tryptophan hydroxylase (Tph1), (Tph2 is only present in neurons)**	*NKAIN4*, NeUN, ChAT, AChE, α3-subunit AChR, α7-subunit AChR, GAD65, Ser7-R. Na- and Na/K-ATPases over-expression.
Activation mechanism	G-protein-linked receptors/in Ca^{2+}. Only a small subset of duodenal EC cells presents spontaneous Action Potential [15].	Action potential neurons use voltage changes across the plasma membrane for intercellular communication (Yamashita.)

Note: * Except the Neuropod identified in cultured organoid experiments (Bohorquez).

* * Serotonergic neurons express Tph2 in the Central Nervous System (CNS) and enteric nervous system [61,62,64].

Conclusion

The physiological functioning of an organ or tissue is founded on its cytoarchitecture. In this sense, wrote, "Every activity of a cell, tissue, or organ has a structural basis; the salient point is whether appropriate techniques are available to reveal the underlying structure".

Identifying an epithelial neural network associated with the enteric nervous system represents a significant advance in understanding intestinal homeostasis, particularly in the communication between the luminal external medium, ENS, and the CNS. An epithelial neural network guarantees a rapid and efficient means to transfer signals from the intestinal lumen to their effectors in the organ and regulate or modulate their responses. Like other sensory epithelia, the newly described epithelial neuronal complex in the duodenum induces and probably regulates the release of local serotonin and other amines and peptides that participate in gastrointestinal functions.

The epithelial neuron has all the characteristics of a sensory neuron. It is a polarized cell with an apical pole in contact with the luminal medium through a knob-like terminal, forming a sensorial network, as demonstrated in other neuroepithelia. It also has a basal pole with two axonal extensions, which innerve the epithelial surface with an extended superficial net, the peri-glandular space, probably regulating intestinal secretion, and sub-epithelial follicles, indicating its interaction with the local immune system. It is known that neurons regulate intestinal immune responses through local axon reflexes or neuronal circuits *via* the gut-brain axis. Thus, different immune cells respond to neural signals in host defense and inflammation. The neuro-immune crosstalk is essential for homeostasis and disease resolution in the intestine.

Like other sensory neurons, it is cholinergic, GABAergic and serotoninergic, given its capacity to synthesize ACh, GABA and serotonin. In addition, the epithelial neuron can induce remote serotonin secretion. ACh activation induces serotonin secretion from EC cells. A negative feedback mechanism modulates this serotonin secretory mechanism.

At the duodenal level, we have proposed that communication between the intestinal lumen and the enteric nervous system is conducted through a neuronal complex. This network includes the cholinergic neuron located in the intestinal epithelium, which serves as a sensor of luminal stimuli. These stimuli are then transmitted to the ENS, epithelial cells of the intestinal villi and crypts, glia, and subepithelial elements located in the lamina propria (lymphoid nodules and afferent nerve fibers).

The epithelial neuron, an essential component of this complex, contains serotonin in small granules, as reported for serotonergic neurons, and Glutamate Decarboxylase (GAD-65), an enzyme necessary for synthesizing γ-Aminobutyric Acid (GABA). On the other hand, EC cells are distinguished by their biogenic amine content, particularly serotonin in large granules, the presence of the Tph1 (Tryptophan hydroxylase 1), an enzyme involved specifically in serotonin synthesis, or Chromogranin A (ChgA), an acidic protein associated with secretory granules in enterochromaffin cells. Identifying this new sensory system in the duodenal epithelium is very promising. Although it is only the beginning, the prospect for studies related to the response to multiple external factors (nutrients, irritants, local microbiota), homeostasis, and the immunological balance of the intestinal mucosa is encouraging.

Author Contributions

All authors contributed to the conception, design, acquisition, analysis, interpretation of the data, and the critical revision of the manuscript for relevant intellectual content.

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Note: Salazar et al. (2023) extensively describe the methodological procedures utilized in the experiments shown in Figures 1-4.

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