Pathophysiology of Hirschsprung's Disease

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7.1 Introduction

The basic pathophysiological feature in Hirschsprung's disease (HD) is a functional obstruction caused by a narrowed distal aganglionic colonic segment that prevents the propagation of peristaltic waves. Despite extensive research, the pathophysiology of HD is not fully understood. There is no clear explanation for the occurrence of spastic or tonically contracted aganglionic segment of bowel.

The digestive tract is unique among internal organs because it is exposed to a large variety of physiochemical stimuli from the external world in the form of ingested food. As a consequence, the intestine has developed a rich repertoire of coordinated movements of its muscular apparatus to ensure the appropriate mixing and propulsion of contents during digestion, absorption and excretion. The normal motility of the gastrointestinal system is dependent on the interaction of the neural apparatus and the muscular apparatus.

7.2 Organization of the Gut

7.2.1 The Gut Wall

The gut wall comprises two layers of smooth muscles. An outer thin layer of cells arranged along the length of the gut forms the longitudinal smooth muscle layer. A perpendicular, thicker, layer of cells immediately inside the longitudinal muscle forms the circular smooth muscle layer. A well-developed, ganglionated nervous plexus is situated between the two muscle layers, the myenteric plexus. On the luminal side of the circular muscle layer is the submucosa, which contains connective tissue, glands, small vessels and a second ganglionated plexus, the submucous plexus. A thin muscle layer separates the submucosa from the mucosa. The mucosa is densely innervated by sensory nerve fibers from nerve cells in either of the plexuses. Enteroendocrine cells involved in the control of the gut functions are common in the mucosal lining [1–3].

7.2.2 Smooth Muscle Cells

The smooth muscle cells are long thin cells with a large central nucleus. They are interconnected via gap junctions to operate as larger functional mechanical units. Electrical stimuli can spread between the cells through the gap junctions, causing parts of the muscle to act as one single unit [2–4]. The level of muscular activity de-

pends on intrinsic, myogenic activity as well as on the neural apparatus. Electrical slow waves are cyclic changes in membrane potential that are responsible for rhythmic contractions of the muscles. The factors that trigger these slow waves are a network of pacemaker cells called interstitial cells of Cajal (ICC) [2–4].

7.2.3 Interstitial Cells of Cajal

The ICC are mesenchymal cells, spindle shaped or with several processes that form networks that are widely distributed within the submucosal, intramuscular and intermuscular layers of the gastrointestinal tract from the esophagus to the internal anal sphincter [5–7]. Immunohistochemically, they can be localized by the expression of c-Kit, a trans-cell membrane tyrosine-kinase receptor. ICC act as pacemakers in the gut wall, by developing spontaneous slow waves, which spread to the smooth muscle cells. Recent studies have demonstrated that ICC also mediate enteric motor neurotransmission via synaptic-like contacts that exist between varicose nerve terminals and intramuscular ICC [6]. However, the integrative role of the ICC and the enteric nervous system (ENS) in the control of gastrointestinal function is still unknown [8].

7.2.4 Extrinsic Innervation

In addition to intrinsic myogenic activity and the involvement of ICC discussed above, the autonomic nervous system controls gut motility [9]. The autonomic nervous system controls several visceral functions that are not under conscious control. It can be divided into three main divisions: the cranial (parasympathetic) and the spinal (sympathetic and parasympathetic) systems, which relay extrinsic control, and the ENS, which is the intrinsic nervous system of the gut and not only regulates the intestinal motility but also secretions, blood flow, immune and endocrine functions [2, 10]. The extrinsic innervation of the gut involves the vagus nerve and splanchnic nerves to the stomach and upper intestine and the pelvic nerves supplying the distal intestinal segments. Parasympathetic fibers running in the vagus nerve innervate the stomach; however, the majority of the fibers in the vagus are sensory fibers with their nerve cell bodies in the nodose ganglion. These fibers convey information from the stomach and other peripheral organs to the central nervous system [11]. The splanchnic nerves are sympathetic, while the pelvic nerve contains both parasympathetic and sympathetic fibers. Sensory nerve fibers within the spinal nerves, running from the gut to the central nervous system, have their cell bodies located in the dorsal root ganglia [11].

7.2.5 Intrinsic Innervation: the Enteric Nervous System

The ENS is the system of neurons and their supporting cells that is present within the wall of the gastrointestinal tract. It may act independently of extrinsic input but both sympathetic and parasympathetic nerves can influence gut motility via enteric nerves. The ENS is the largest division of the autonomic nervous system, it contains about 100 million neurons, only comparable to the ones of the spinal cord [12, 13]. The neuron cell bodies are clustered together in ganglia. The ENS has two ganglionated plexuses, the myenteric and submucosal plexuses [14]. The myenteric plexus (Auerbach plexus) is positioned between the outer longitudinal and circular muscle layers throughout the digestive tract, from the esophagus to the anus. The submucous plexus is subdivided into separate plexuses: the inner submucous plexus (Meissner plexus) directly below the muscularis mucosae and the outer submucous plexus (Schabadasch or Henle plexus) directly adjacent to the circular muscle layer [13]. The submucosal plexus is absent from the esophagus and stomach, being only prominent in the intestines [3]. This topography has functional relevance is that the myenteric plexus mainly regulates motor function whereas the submucous plexus is mainly involved in control of blood flow, secretion and absorption [13]. The density of neurons varies between myenteric and submucosal ganglia and between gut regions. Typically, myenteric ganglia are considerably larger than submucosal ganglia. The ENS neurons, although clustered into ganglia, do not form nuclei of morphologically similar neuron types as occur, for example, in the brain. Instead, each enteric ganglion contains many different neuron types and neighboring ganglia will contain similar types of neurons although not always in the same proportions [12].

7.2.5.1 Classification of the Neurons of the ENS

Neurons of the ENS can be classified according to their morphological, neurochemical or functional properties. These properties have been disclosed by different methods including light and electron microscopy, immunohistochemistry, electrophysiological analysis, intracellular dyes and retrograde tracing of neuronal projections [3, 15]. In the small intestine 17 different neuronal types, only 14 of which are functionally important, have been identified [14].

Morphology

According to their morphology, neurons are classified into Dogiel type I to type VII and giant neurons. Most neurons are Dogiel types I–III [14]. Dogiel type I neu-

rons have flat cell bodies with many short, lamellar dendrites and a single long axon, and they are considered as enteric motor neurons. Dogiel type II neurons have relatively smooth cell bodies with short and long processes arising in a variety of configurations. The long processes may extend through interganglionic fiber tracts across several rows of ganglia. Shorter processes may project only within the home ganglion. Dogiel type III neurons are similar to type II neurons except that they have more processes and more of the processes are shorter in length [16].

Neurochemistry

Neurons usually express a combination of different neurotransmitters, a phenomenon known as *chemical coding* [17]. The chemical code depends on the type of neuron and the intestinal segment. The general mechanism of chemically mediated synaptic transmission is the same in the ENS as elsewhere in the body, and seemingly as complex as in the central nervous system. More than 30 neurotransmitters have been identified in the ENS, which are usually colocalized according to their function, as shown in Table 7.1 [3, 14]. Enteric neurotransmitters are either small molecules (norepinephrine, 5-HT), larger molecules (peptides) or gases including nitric oxide (NO) and carbon monoxide.

Functional Classification

Neurons are classified according to their function into sensory neurons, interneurons and motor neurons.

Sensory neurons: The sensory neurons are a dense network of extrinsic (vagal and spinal afferents with their cell bodies outside the gut wall) and intrinsic primary afferent neurons (IPAN, with their cell bodies within the gut wall) [18]. They communicate with each other and function together with enteroendocrine and immune cells. Whereas IPAN are essential for the control of the digestion by the ENS, extrinsic afferents notify the brain about processes that are relevant to energy and fluid homeostasis and the sensations of discomfort and pain [19]. Sensory neurons include mechano-, chemo- and thermoreceptors. Mechanoreceptors are activated by distension and generate tonic muscle contractions, but if distension is maintained, they respond by generating peristaltic activity (Fig. 7.1) [10].

Besides direct activation of the IPANs, there are other specialized transducers, the enteroendocrine cells [20]. These cells are strategically positioned in the mucosa to "taste" and sense luminal contents and release their mediators at the basolateral side to activate sensory nerve endings within the lamina propria, which synapse on excitatory or inhibitory motor neurons. While enteroendocrine cells are specialized for luminal nutrient sensing, subepithelial IPANs may also respond to luminal chemicals that freely diffuse across the epithelium [21]. There are regional and topographic differences in the distribution of enteroendocrine cells, with the highest frequency in the duodenum. The major transmitters are cholecystokinin (CCK), secretin, somatostatin, serotonin (5-hydroxytryptamine, 5-HT), and corticotropin releasing factor. Cells containing 5-HT are present in all regions of the intestine and comprise the single largest endocrine cell population [3].

Interneurons: Interneurons are usually Dogiel type II. At least one type of ascending and three types of descending interneurons have been described, most of them being the descending type. The ascending interneurons are mainly cholinergic, whereas the descending ones have a complex chemical coding including acetylcholine, NO, vasoactive intestinal polypeptide, 5-HT and somatostatin (Table 7.1 and Fig. 7.1) [3].

Motor neurons: Motor neurons are Dogiel type I. There are three types: muscle motor neurons, secretomotor neurons that are or are not vasodilators and neurons in-

Table 7.1 Chemical coding of the enteric neurons (5-HT 5-hydroxytryptamine, *Ach* acetylcholine, *Calb* calbindin, *Calret* calretinin, *CCK* cholecystokinin, *CGRP* calcitonin generated peptide, *DYN* dynorphin, *ENK* enkephalins, *GRP* gastrin releasing peptide, *NO* nitric oxide, *NPY* neuropeptide Y, *SP* substance P, *VIP* vasoactive intestinal peptide)

Function	Neurochemical coding
Sensory	Ach, Calb, CGRP, SP
Ascending interneurons	Ach, Calret, ENK, SP
Descending interneurons	5-HT, DYN, GRP, NO, somatostatin, VIP
Short excitatory muscle motor neurons	Ach, SP
Long excitatory muscle motor neurons	Ach, Calret, SP
Inhibitory muscle motor neurons	DYN, ENK, GRP, NO, VIP
Secretomotor neurons	Ach, CCK, CGRP, DYN, NPY, somatostatin, VIP

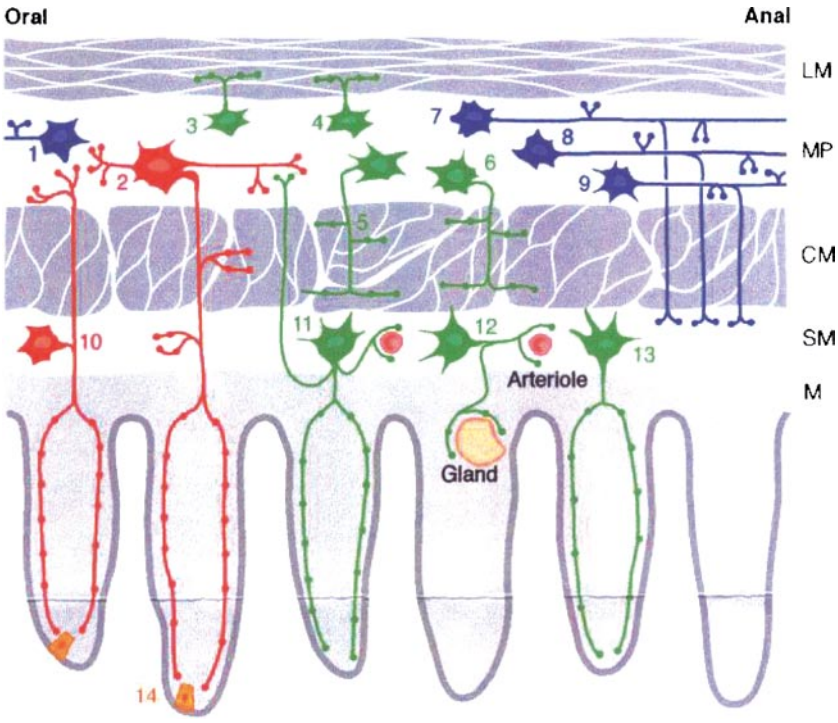

Fig. 7.1 Types of neurons in the small intestine: *1* ascending interneuron, *2* myenteric intrinsic primary afferent neuron, *3* excitatory longitudinal muscle motor neuron, *4* inhibitory longitudinal muscle motor neuron, *5* excitatory circular muscle motor neuron, *6* inhibitory circular muscle motor neuron, *7* descending interneuron (local reflex), *8* descending interneuron (secretomotor reflex), *9* descending interneuron (migrating myoelectric complex), *10* submucosal intrinsic primary afferent neuron, *11* non-cholinergic secretomotor/vasodilator neuron, *12* cholinergic secretomotor/vasodilator neuron, *13* cholinergic secretomotor (non-vasodilator) neuron, *14* enteroendocrine cell; *CM* circular muscle, *LM* longitudinal muscle, *M* mucosa, *MP* myenteric plexus, *SM* submucosal plexus

nervating enteroendocrine cells. Muscle motor neurons innervate the longitudinal and circular muscles and the muscularis mucosae throughout the digestive tract. The muscle motor neurons are either excitatory or inhibitory and release transmitters that provoke muscle contraction or relaxation. For the excitatory neurons, transmission is predominantly muscarinic cholinergic and tachynergic (substance P and neurokinin A). For the inhibitory neurons, the primary transmitter is NO [22, 23], but also vasoactive intestine polypeptide, ATP, pituitary adenylate cyclase-activating polypeptide and carbon monoxide (Table 7.1 and Fig. 7.1) [14].

7.3 Motility of the Gut

Two patterns of activity are recognized in the mammalian intestine, the activity of the interdigestive state and the fed pattern of activity [24].

7.3.1 Migrating Myoelectric Complex

In the interdigestive state, complexes of contractions traveling in an anal direction have been recorded. This is known as migrating myoelectric complex (MMC), which passes along the intestine every 80–110 minutes in humans. The complex takes about 6–10 minutes to pass any point in the intestine, and as it passes, that region undergoes intense rhythmic contractions of the circular muscle [24]. These MMC probably act as housekeepers, to transport waste products in the interdigestive stage and they also control the bacterial flora, preventing overgrowth and returning bacteria to the large intestine [25, 26]. The MMCs disappear soon after a meal is taken, to be replaced by the fed pattern of activity, the peristaltic movements. Both the interdigestive pattern and the fed pattern are generated through the ENS but are modified by the extrinsic nerves. The continuity of the ENS is necessary for the orderly progress of the MMC; if the intestine is interrupted surgically and then rejoined,

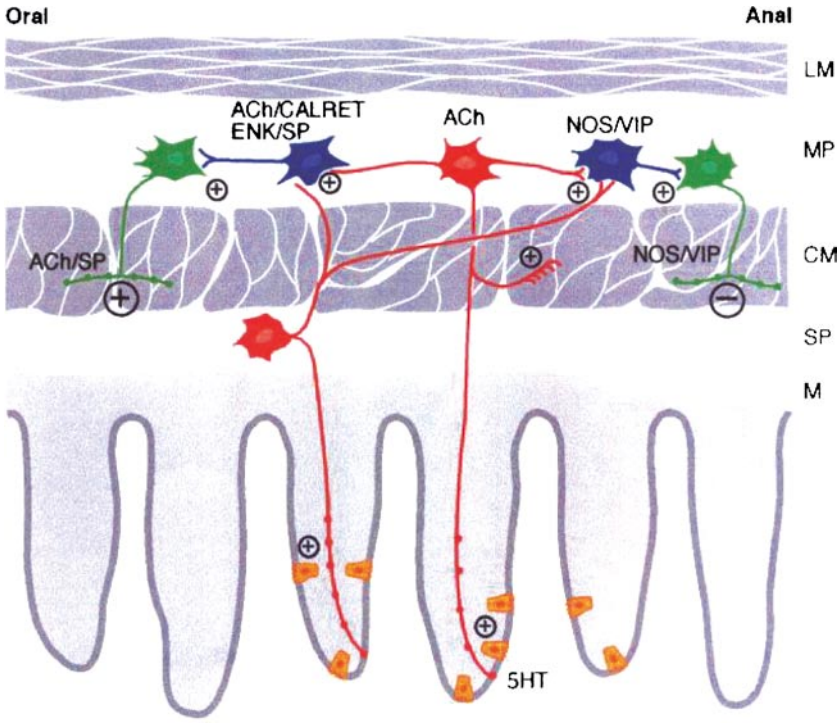

Fig. 7.2 Generalized picture of ascending and descending reflex pathways controlling intestinal peristalsis. The passage of food may cause release of 5-HT from enteroendocrine cells (*yellow*) in the mucosa stimulating sensory nerve endings from IPAN projecting from cell bodies in the myenteric or submucous plexus (*red*). In addition, IPAN may be directly stimulated by distension of the gut wall. The IPAN activate ascending (*oral*) and descending (*anal*) interneurons (*blue*). Orally projecting interneurons release acetylcholine, calretinin, enkephalins and substance P that stimulate excitatory motor neurons innervating the circular muscle, which in turn release acetylcholine and substance P (*green*). Anally projecting interneurons contain NO, and vasoactive intestinal peptide. These interneurons stimulate inhibitory motor neurons that release NO and vasoactive intestinal peptide among other neurotransmitters (*green*) (*5HT* 5-hydroxytryptamine, *ACh* acetylcholine, *CALRET* calretinin, *ENK* enkephalins, *NOS* nitric oxide synthase, *SP* substance P, *VIP* vasoactive intestinal peptide; *CM* circular muscle, *LM* longitudinal muscle, *M* mucosa, *MP* myenteric plexus, *SP* submucous plexus)

the MMC does not always pass the lesion and ectopic MMCs occur on the anal side [24].

7.3.2 Peristalsis

The fed pattern of activity both mixes and propels the contents. In one human study, about 45% of individual contractions did not propagate and about 35% propagated for less than 9 cm [27]. These nonpropagating contractions correspond to the mixing activity. The propagated contractions are peristaltic waves, which consist of contraction of the circular muscle oral to a bolus in the lumen, the ascending excitatory reflex; and relaxation on the anal side, the descending inhibitory reflex. In addition, longitudinal muscle on the anal side may contract while the oral longitudinal muscle relaxes. Total extrinsic denervation of the bowel does not affect peristalsis [24].

All the neural elements for the peristaltic reflex are in the intestine; these are the IPAN, interneurons and motor neurons. Passing the food may cause release of 5-HT from enteroendocrine cells in the mucosa stimulating sensory nerve endings from IPAN projecting from cell bodies in the myenteric or submucous plexus (Table 7.1 and Fig. 7.2). In addition, IPAN may be directly stimulated by distension of the gut wall. The IPAN activate ascending (oral) and descending (anal) interneurons. Orally projecting interneurons release acetylcholine, calretinin, enkephalins and substance P that stimulate excitatory motor neurons innervating the circular muscle, which in turn release acetylcholine and substance P. Anally projecting interneurons contain NO, and vasoactive intestinal peptide. These interneurons stimulate inhibitory motor neurons that release NO and vasoactive intestinal peptide among other neurotransmitters (Table 7.1 and Fig. 7.2)

7.4 The Gut in Hirschsprung's Disease

The characteristic gross pathological feature of HD is a narrowed distal colon with a funnel shaped transition zone to a dilated and hypertrophied proximal colon. However, these features may vary with the duration of untreated disease. In the neonatal period, the intestine may appear normal, but as the child ages, the proximal intestine hypertrophies and becomes thicker and longer than normal. The taeniae disappear and the longitudinal muscle layer seems to completely surround the colon [28]. It has long been recognized that the obstructive symptoms in HD are secondary to the abnormal motility of the distal narrow segment, but there is still no clear explanation for the occurrence of contracted intestinal wall in the distal bowel in HD [29].

7.4.1 Aganglionosis

The most striking finding in the distal intestine in HD is the absence of ganglion cells in the myenteric and submucous plexuses [30]. Aganglionosis typically extends to the rectosigmoid region in approximately 80% of patients. The aganglionosis is continuous and uninterrupted until the proximal transitional zone is reached. The length of this zone may vary and extend for several centimeters and is characterized by hypoganglionosis. Several other abnormalities have been described associated with HD that may contribute to its pathophysiology and may explain the clear discrepancy between the length of the nonfunctional bowel and the degree of obstruction.

7.4.2 Cholinergic Hyperinnervation

In association with aganglionosis, there is a marked increase in cholinergic nerve fibers in the intermuscular zone and submucosa of the aganglionic segment. These fibers appear as thick nerve trunks and correspond to extrinsic preganglionic parasympathetic nerves [31–35]. The continuous acetylcholine release from the axons of these parasympathetic nerves result in an excessive accumulation of the enzyme acetylcholinesterase that is typically found using histochemical staining techniques in the lamina propria mucosae, muscularis mucosae and circular muscle [30]. Both the thick nerve trunks and the increased acetylcholinesterase activity are most pronounced in the most distal aganglionic rectum and progressively diminish proximally as normal bowel is approached [36]. The proximal extent of increased cholinergic activity does not necessarily correspond to the extent of the aganglionosis, which usually extends more proximally to a variable degree. Pharmacological investigations of the colon in HD have demonstrated higher acetylcholine release in the aganglionic segment at rest and after stimulation compared with the proximal ganglionic bowel [37, 38]. Acetylcholinesterase concentrations have also been found to be higher in the serum and erythrocytes from children suffering from HD [39]. Cholinergic nerve hyperplasia has been proposed as the cause of spasticity of the aganglionic segment since acetylcholine is the main excitatory neurotransmitter. However, in the chemical animal model of aganglionosis, after application of benzalkonium chloride or corrosive sublimate, the aganglionic bowel does not show hypertrophic nerve bundles and the bowel still appears narrow, and animals exhibit typical obstructive symptoms [40, 41]. Therefore, the cholinergic hyperinnervation does not seem to be a prerequisite for the appearance of a narrow spastic segment.

7.4.3 Adrenergic Innervation

Fluorescent-histochemical studies for localization of adrenergic nerves have demonstrated that they are increased in number in the aganglionic colon of HD and have a chaotic distribution. They are also present in the circular and longitudinal muscle layers as well as in the mucosa, whereas they are almost absent from normal ganglionic colon [42–44]. However, the sensitivity of the aganglionic bowel to epinephrine is apparently not increased, despite the elevated number of adrenergic fibers [45, 46]. The tissue concentration of norepinephrine is two to three times higher in the aganglionic bowel than in the normal colon; and also there is a corresponding increase in tyrosine hydroxylase, an enzyme that regulates norepinephrine biosynthesis [43]. Because adrenergic nerves normally act to relax the bowel, it is unlikely that adrenergic hyperactivity is responsible for increased tone in the aganglionic colon [47].

7.4.4 Nitrergic Innervation

NO is considered to be one of the most important neurotransmitters involved in relaxation of the smooth muscle of the gut [48]. It is synthesized in a reaction catalyzed by nitric oxide synthase (NOS) and depends on l-arginine and molecular oxygen as cosubstrates to form l-citrulline and NO. NO binds to cytosolic guanylate cyclase and increases the production of 3′5′-cyclic guanosine monophosphate (cGMP) with subsequent relaxation of smooth muscle [49]. NOS has been shown to be colocalized with reduced nicotine adenine dinucleotide phosphate (NADPH) diaphorase, which has been demonstrated to have identical functions [50, 51]. Several investigators have studied NOS distribution in the ganglionic and aganglionic bowel in patients with HD using NOS

immunohistochemistry or NADPH diaphorase histochemistry [52–57]. In normal and ganglionic colon from patients with HD, there is a strong NADPH diaphorase staining of the submucous and myenteric plexuses and a large number of positive nerve fibers in the circular and longitudinal muscle as well as in the muscularis mucosae [49]. In the aganglionic segment of HD patients, there are no ganglia and there is an absence or marked reduction of nerve fibers positive for NADPH diaphorase in both muscle layers and in the muscularis mucosae. The typical hypertrophied nerve trunks appear weakly stained [49]. Kusafuka and Puri [58] examined the expression of neural NOS mRNA in the aganglionic segment from seven patients who had HD and demonstrated that NOS mRNA expression was at least 1/50 to 1/100 of the level expressed in ganglionic bowel. These findings indicate that there is impaired NO synthesis in the aganglionic bowel in HD and this deficiency could prevent smooth muscle relaxation, thereby causing the lack of peristalsis in HD. In an interesting experiment, Bealer et al. [59] compared the effect of an exogenous source of NO, *S*-nitroso-*N*-acetylpenicillamine (SNAP) on the isometric tension of smooth muscle strips from aganglionic bowel and demonstrated a 70% reduction of resting tension. These results suggest that the defective distribution of nerves containing NOS may be involved in the pathogenesis of HD.

7.4.5 Interstitial Cells of Cajal

Abnormalities of ICC have been described in several disorders of human intestinal motility including HD. Vanderwinden et al. [52] using c-kit immunohistochemistry were the first to report that ICC were scarce and the network appeared to be disrupted in aganglionic segments of HD whereas the distribution of ICC in the ganglionic bowel of HD was similar to that observed in controls. Yamataka et al. [60, 61] found few c-kit-positive cells in the muscle layers in HD and a moderate number around the thick nerve bundles in the space between the two muscle layers in the aganglionic bowel. Horisawa et al. [62] found no differences in c-kit immunopositive cells in aganglionic segments compared with the corresponding area of ganglionic bowel. Rolle et al. [63] using whole-mount and frozen sections stained with c-kit immunohistochemistry preparations found an altered distribution of ICC in the entire resected bowel of HD patients and not only in the aganglionic segment. Moreover, gap junctions connecting ICC were immunolocalized by anti-connexin 43 antibody and found to be absent from the aganglionic part of HD bowel and highly reduced from the transitional zone [64]. Rolle et al. proposed that persistent dysmotility problems after a pull-through operation in HD may be due to altered distribution and impaired function of ICC.

7.4.6 Enteroendocrine Cells

Using the generic enteroendocrine cell immunohistochemical markers chromogranin A and synaptophysin, Soeda et al. [65] demonstrated that the number of enteroendocrine cells in the aganglionic colon in patients with HD is significantly increased compared with the number in the normal ganglionic segment. The increase of enteroendocrine cells in the mucosa of aganglionic colon may well influence sustained contraction of the bowel wall mainly mediated by the release of 5-hydroxytryptamine.

7.4.7 Smooth Muscle

Since smooth muscle is the final effector for bowel motility, it is likely that it could also be abnormal in HD. The smooth muscle cell cytoskeleton consists of proteins whose primary function is as a structural framework that surrounds and supports the contractile apparatus of actin and myosin filaments in the body of the smooth muscle cell. Nemeth et al. [66] studied the distribution of cytoskeleton in the smooth muscle of HD bowel by means of immunohistochemistry and found that dystrophin, vinculin and desmin immunoreactivities are either absent or weak in the smooth muscle of aganglionic bowel, whereas they are moderate to strong in the smooth muscle of normal bowel and ganglionic bowel from patients with HD. Neural cell adhesion molecule (NCAM) is a cell surface glycoprotein involved in cell–cell adhesion during development that has been suggested to play an important role in development and maintenance of the neuromuscular system [67–69]. NCAM is present in the innervation of normal infant bowel and, less densely, in some components of the enteric smooth muscle. Contradictory results have been published regarding NCAM expression in the smooth muscle of aganglionic bowel. Kobayashi et al. [53] have described a lack of expression of NCAM in the muscularis propria of the aganglionic bowel compared with the ganglionic segment, whereas Romanska et al. [70] have found an increase in NCAM expression in muscle, particularly in the muscularis mucosae. Anyhow, both authors agree that there is a strong expression of NCAM in the hypertrophied nerve trunks from the aganglionic segment.

7.4.8 Extracellular Matrix

Although extracellular matrix (EM) abnormalities have been described mainly related to the pathogenesis of HD, they could also have an influence on its pathophysiology. The lethal spotted mouse, an animal model which develops aganglionosis in its distal bowel, displays an

abnormal distribution of EM components including laminin, collagen type IV, glycosaminoglycans and proteoglycans in the smooth muscle layer [71, 72]. Parikh et al. [73] have demonstrated that the laminin concentration in aganglionic bowel is twice as high as in the normoganglionic bowel of HD and three times higher than in an age-matched control. Moreover, by means of immunohistochemistry, they found an uneven distribution of laminin and collagen type IV in the muscularis propria of aganglionic bowel, being more intensely expressed in the circular layer than in the longitudinal layer [74]. The same authors have reported that the EM components tenascin and fibronectin are more intensely expressed in aganglionic bowel from HD [75].

7.4.9 Alterations in the Proximal Ganglionic Segment

Several recent studies [76–79] have shown that gastrointestinal motor dysfunction persists in a subset of HD patients long after surgical correction, indicating that morphological and functional abnormalities of the gut are not necessarily restricted to the aganglionic segment. Intestinal neuronal dysplasia (IND) is a malformation of the ENS characterized by the presence of giant ganglia in the submucous plexus, ectopic ganglion cells in the lamina propria of the mucosa and an increased acetylcholinesterase activity in the lamina propria and around submucosal blood vessels [80]. In 1977, Puri et al. reported the first case of IND immediately proximal to a segment of aganglionic colon [81]. Since then, there have been several reports of the combined occurrence of these disorders. Some investigators have reported that 25–35% of patients with HD have associated IND [82, 83] and stress that this could be the cause of persistent bowel symptoms after a pull-through operation for HD [84–86]. Recently, Sandgren et al. [87] have studied in depth the proximal ganglionic bowel in the lethal spotted mouse, a natural mutant model of rectosigmoid HD. They showed that the number of neurons is increased in the submucous plexus from the ileum and colon proximal to the aganglionosis, resembling human IND. They suggested that these findings might explain the persistence of dysmotility after operation for Hirschsprung's disease. Sandgren et al. also demonstrated that the expression of NO and vasoactive intestinal peptide are upregulated in the proximal ganglionic segment, whereas the expression of substance P is downregulated [87].

7.5 Gut motility in Hirschsprung's Disease

In the 1940s Swenson et al. recorded the peristaltic tracings of HD specimens. They found that the progressive contractions of the dilated proximal colon do not enter the more distal narrow segment [88]. These findings provided the evidence for a physiological defect in the distal segment and led to the creation of a novel curative surgical procedure involving the resection of the rectosigmoid in these patients [89]. Kubota et al. [90–94] have studied for many years the electrophysiological and pharmacological characteristics of the different bowel segments in surgically resected specimens of HD. They have found that, while a single pulse stimulation is sufficient to induce a rapid membrane hyperpolarization followed by a spike generation in the majority of cells in the dilated ganglionic bowel, in the transitional region, the amplitude of the hyperpolarization response decreases and repeated stimulations are necessary to induce a response. Even more, in the narrow aganglionic segment, repeated stimulations evoke only a membrane depolarization in about 20% of the cells and spike potentials are generated only when the number of pulses is increased (Fig. 7.3). They have shown that atropine completely abolishes the depolarization response in all the segments and that a membrane hyperpolarization is insensitive to both cholinergic and adrenergic blockers and is completely abolished by tetrodotoxin, demonstrating electrophysiologically the presence of a nonadrenergic non-cholinergic inhibitory innervation. Then, by studying the regional changes in the ampli-

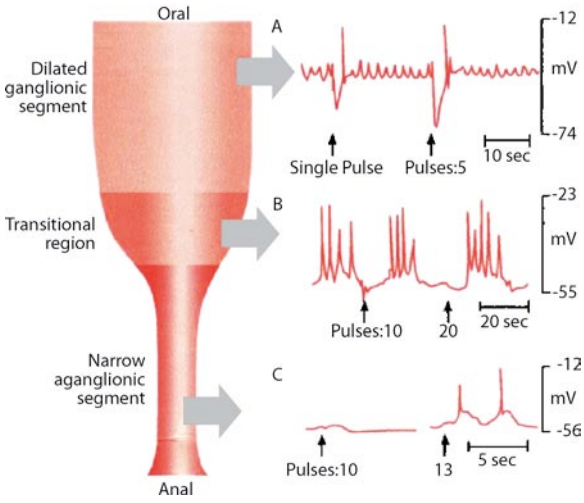

Fig. 7.3 Electrophysiological characteristics of the bowel in Hirschsprung's disease. In the dilated ganglionic bowel, a single pulse stimulation is sufficient to induce a rapid membrane hyperpolarization followed by a spike generation in the majority of cells. In the transitional region, the amplitude of the hyperpolarization response decreases and repetitive stimulations are necessary to induce a response. In the aganglionic segment, repetitive stimulations evoke only a membrane depolarization in about 20% of the cells and spike potential are generated only when the number of pulses is increased

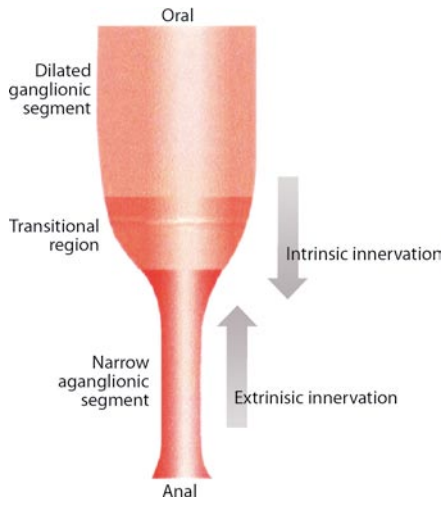

Fig. 7.4 Schematic view of the aganglionic bowel, which receives two nervous flows of different origins: the intrinsic inhibitory nervous flow from the ganglionic segment through the transitional region and the extrinsic excitatory nervous flow from the lower end of the aganglionic segment

tudes of the non-adrenergic non-cholinergic inhibitory junction potentials, they have concluded that the aganglionic segment receives two nervous flows of different origins: one is the intrinsic inhibitory nervous flow from the ganglionic segment through the transitional region, while the other is the extrinsic excitatory nervous flow from the lower end of the aganglionic segment (Fig. 7.4). Since the transitional zone is the place where the stagnation of intestinal contents takes place, they conclude that a decrease in the intrinsic inhibitory nervous flow might be the cause of the intestinal obstruction.

7.6 Final Remarks

Although the more striking histological feature in HD is the absence of ganglion cells, it is unlikely that this is the only cause of the increased intestinal wall tone provoking a functional intestinal obstruction. There are numbers of other histopathological findings both in the aganglionic segment and in the proximal ganglionic segment in HD which may account for the frequent discrepancy encountered between the length of the non-functional bowel and the degree of obstruction and also for the persistent obstructive symptoms after a pull-through operation for HD.

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