New Insights into the Understanding of Gastrointestinal Dysmotility

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Abstract: Our understanding of the physiology of digestion, absorption, secretion, and motility in the gastrointestinal tract has improved immensely. Today it is well established that the gross functions of the gastrointestinal tract depend on the coordination between the muscles, nerves and hormones. The enteric nervous system (ENS) is involved in most of the physiological and pathophysiological processes in the gastrointestinal tract. Therefore, clinical and experimental studies on the ENS provide the basis for a better understanding of the mechanisms involved in gastrointestinal disorders and promote the development of therapeutic options. This review outlines some of the current views on the role of the ENS and its related hormones in gastrointestinal motility.

Keywords: gonadotropin-releasing hormone (GnRH), oxytocin, chronic intestinal pseudo-obstruction (CIPO), apoptosis

Introduction

Gastrointestinal motility is a term used to describe the contraction of the muscles in the gastrointestinal tract. The normal motility of the gastrointestinal tract is dependent on the function of the enteric nervous system (ENS), the smooth muscle layers, and the interstitial cells of Cajal (ICCs) (Fig. 1) (Goyal and Hirano, 1996; Wood, 2000). Diseases characterised by gastrointestinal dysmotility are highly prevalent conditions. For instance, the mild form of dysmotility, also called irritable bowel syndrome (IBS), is assumed to affect almost 15%–20% of the population (Gwee, 2005). Chronic intestinal pseudo-obstruction (CIPO) is the most difficult of these clinical challenges, characterised by the presence of chronic dysmotility and intestinal dilatation in the absence of mechanical obstruction (De Giorgio et al. 2004a). The pathogenesis of IBS and CIPO remains unclear, although putative mechanisms, including inflammation, altered calcium signalling, mitochondrial dysfunction, free radical production, and others, may contribute to the degeneration and loss of enteric neurons (Hall and Wiley, 1998; Spiller, 2003).

Over recent years much effort has been made to try to improve the motility and decrease the abdominal pain occasioned by these conditions by targeting enteric peptides and their receptors. One example is motilin, an important peptide in digestive motility whose receptor is the site of action for erythromycin in the treatment of gastro paresis (Galligan and Vanner, 2005). The peptide ghrelin, related to motilin, which originates primarily in the stomach (Möller et al. 2003), has recently also been shown to enhance gastric emptying in idiopathic gastro paresis (Tack et al. 2005). Serotonin and its receptor have been the goal for an intensive effort to develop different agonists and antagonists (Johanson, 2004; Cash and Chey, 2005; Wessinger et al. 2005). So far, these attempts have not been very successful. Recent findings support a new concept that alterations in intracellular mechanisms of neuronal survival might play a crucial role in the degeneration of the enteric nervous system (De Giorgio et al. 2000; Bassotti et al. 2006). Furthermore, two newly discovered peptides localized in the human gastrointestinal tract, oxytocin and gonadotropin releasing hormone (GnRH) seem to play crucial roles in the regulation of gastrointestinal dysmotility (Monstein et al. 2004; Ohlsson et al. 2006a; Ohlsson et al. 2007).

Oxytocin is known to enhance gastric emptying (Hashmonai et al. 1979; Petring, 1989). Our own studies have shown that oxytocin is expressed in the myenteric and submucous ganglia and nerve fibres along the entire human gastrointestinal tract (Monstein et al. 2004; Ohlsson et al. 2006b), and that it increases colonic peristalsis while the receptor antagonist delays the gastric emptying rate (Ohlsson et al. 2004; Ohlsson et al. 2006a). Similarly, GnRH has also been shown to stimulate intestinal motor

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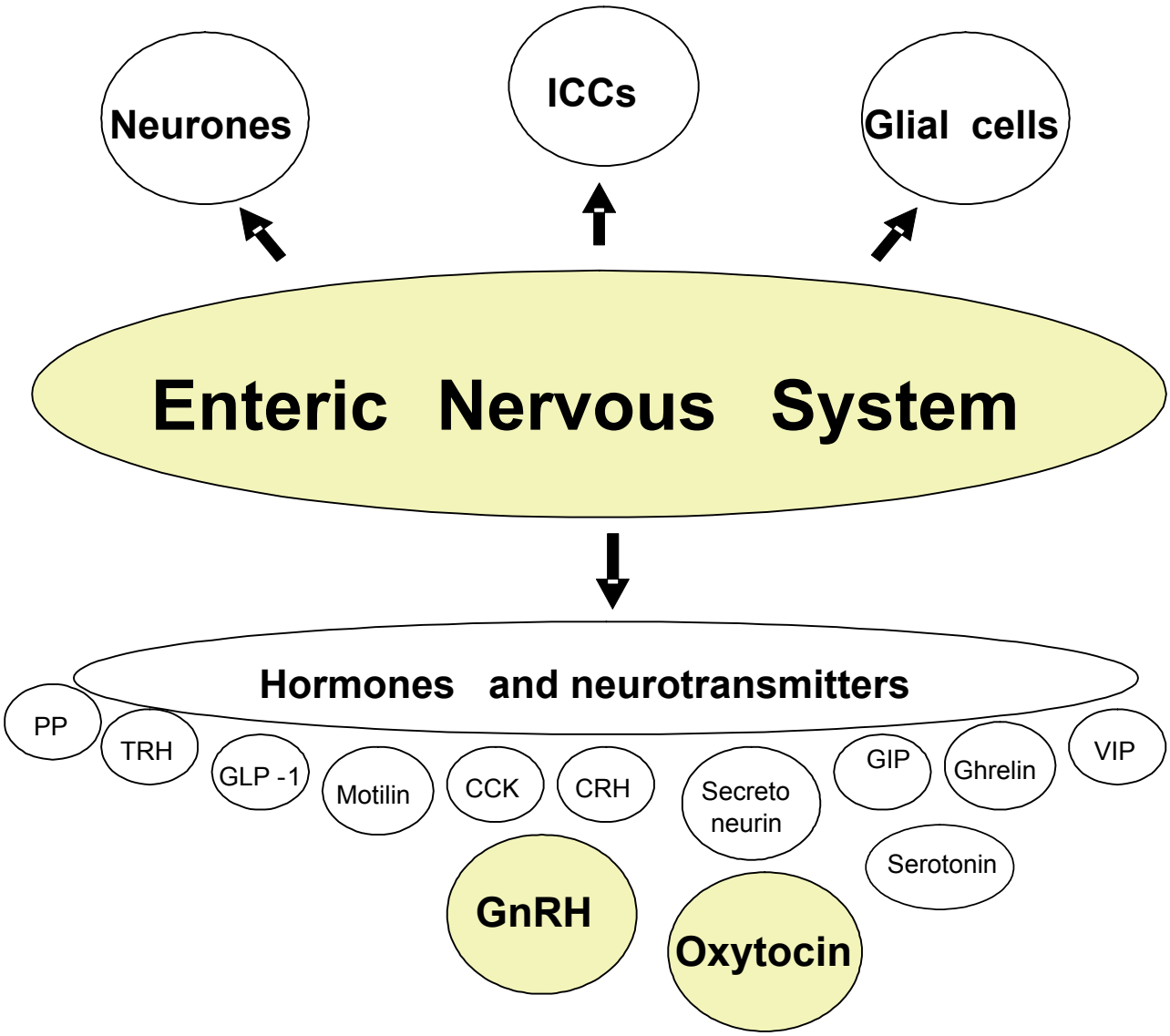

Figure 1. A schematic overview over the enteric nervous system and its most important peptides according to motility. CCK = cholecystokinin; CRH = corticotrophin-releasing hormone; ICCs = Interstitiasl cells of Cajal; GIP = gastric inhibitory polypeptide; GLP-1 = glucagon-like peptide 1; GnRH = gonadotropin-releasing hormone; PP = pancreatic polypeptide; TRH = thyrotropin-releasing hormone; VIP = vasoactive intestinal peptide.

activity in rat (Khanna et al. 1992; Ducker et al. 1996), and a loss of GnRH-containing neurons in the ENS has been related to CIPO development (Ohlsson et al. 2007).

The Enteric Nervous System and Gastrointestinal Dysmotility

Enteric neuron apoptosis

The ENS is a highly integrated neural system which consists of distinct subclasses of enteric neurons localized within the wall of the alimentary tract throughout its entire length. The ENS closely resembles the central nervous system (Gershon et al. 1994), and has a unique ability to control virtually all gut functions, including motility, independently of the central nervous system (CNS) (Furness et al. 2005).

Remarkably, in response to different types of stimuli/conditions, enteric neurons are able to change their structural, functional and chemical phenotype (Lomax et al. 2005). These changes in the functional and/or structural integrity of the ENS may occur as a consequence of normal aging or due to pathologies ranging from enteric neuropathies

(i.e. Hirschsprung's disease) to intestinal or extra intestinal diseases (i.e. ulcerative colitis and Crohn`s disease, amyloidosis, scleroderma and etc) (Di Lorenzo, 1999; De Giorgio and Camilleri, 2004b; De Giorgio et al. 2004c).

It has been suggested that some motility disorders originate from developmental defects, i.e. Hirschsprung's disease (Kim et al. 2006), whereas others are due to neurodegeneration. In fact, pathological changes of the ENS are often accompanied by nerve process degeneration and necrosis (Dvorak et al. 1993). Ultra structural evaluation of tissue specimens from patients with Crohn's disease and ulcerative colitis have shown swollen, empty axons, filled with large vacuoles, swollen mitochondria and concentrated neurofibrils (Vasina et al. 2006).

The B-cell leukaemia/lymphoma-2 (bcl-2) protein has the functional role of blocking apoptosis, i.e. programmed cell death. This protein is widely expressed in the developing central and peripheral nervous systems. The expression of bcl-2 is also displayed in enteric neurons (Wester et al. 1999). The reduced expression of bcl-2 has been demonstrated in degenerative disorders of both the central nervous system and ENS (Merry and Korsmeyer, 1997). Thus, the current state of knowledge allows speculations that alterations in the intracellular mechanisms involved in neuronal survival may play a critical role in various gastrointestinal motility disorders. To support of this postulation, the decreased expression of the Bcl-2 protein in enteric neurons has been demonstrated in patients with severe forms of CIPO (De Giorgio et al. 2000; De Giorgio et al. 2004a; De Giorgio and Camilleri, 2004b).

We found further support when, examining full thickness biopsies, we noticed significantly lower bcl-2 expression in a patient with CIPO than in controls. In parallel, histological examination revealed the presence of swollen or shrunken neurons of the myenteric plexus with or without vacuolisation of the cytoplasm (Ohlsson et al. 2007). The increased number of apoptotic enteric neurons and decreased expression of bcl-2 have also been found in patients with slow-transit constipation (Bassotti et al. 2006) and in patients with Hirschsprung's disease (Song et al. 2002).

Thus, the improved knowledge on the changes and regulation in proteins associated with apoptosis in the cells of ENS may be crucial for regulating the apoptosis program. A number of animal models such as a rat hemispheric ischemia/reperfusion model (Gabryel et al. 2006) and an apoptosis-dependent emphysema mouse model (Petrache et al. 2006), as well as age-related macular degeneration (Glotin et al. 2006) have demonstrated that substances controlling intracellular pathways of cell apoptosis can reduce disease processes characterised by excessive cell apoptosis. Therefore, we believe that clinical and experimental studies on the role of enteric neuronal apoptosis are of fundamental importance because they may improve our understanding regarding the pathophysiology of gastrointestinal dysmotility and may also provide the basis for new therapeutic approaches.

Enteric glial cells

The ENS is composed of both neurons and enteric glial cells, which play a central role in sustaining the structural and functional integrity of enteric neurons. Enteric glial cells were first described by Dogiel in 1899 as nucleated satellite cells accompanying enteric neuronal cells. Dogiel assumed that enteric glia represented a kind of connective tissue, and consequently very little research was conducted to reveal their functions (Dogiel, 1889). Today, there is evidence from transgenic animal models that enteric glial cells are essential for gastrointestinal integrity and function, but still little is known about the underlying mechanisms. For example, in genetically modified animals, loss of enteric glia results in neuronal degeneration and changes in the neurochemical coding of enteric neurons (Bush et al. 1998; Aube et al. 2003). New data suggest that enteric glial cells have an important role in maintaining the integrity of the mucosal barrier of the gut, and may also serve as a link between the nervous and immune systems of the gut as indicated by their potential to synthesize cytokines, present antigens and respond to inflammatory insults. The role of enteric glia in human disease has not yet been systematically studied, but, based on the evidence available it can be predicted that enteric glia are involved in the aetiopathogenesis of various pathological processes in the gut, particularly those with neuroinflammatory or neurodegenerative components (Ruhl, 2005). The number of glia cells has been shown to increase in response to pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumour necrose factor alpha (TNFα), or lipopolysaccharide (von Boyen et al. 2004). Notably, several studies have described

an association between increased glial cell proliferation and neuronal disintegration in patients with inflammatory bowel diseases (Cabarrocas et al. 2003; Lomax et al. 2005). In another study, examination of non-involved intestinal tissue from patients with Crohn's disease, ulcerative colitis, or histological normal controls demonstrated that the enteric glia cell network was significantly disrupted in Crohn's disease, but not in ulcerative colitis (Cornet et al. 2001).

In patients with slow transit constipation a remarkable decrease both in the number of enteric neurons and interstitial cells of Cajal (ICCs), and also in the number of glial cells has been found. These patients had significantly more apoptotic enteric neurons than controls (Bassotti et al. 2006). It is likely that a dynamic equilibrium between enteric neurons and glia plays an important role *in vivo*. Therefore, the insufficient support of enteric neurons by glial cells may lead to enhanced neuronal apoptosis and neurodegeneration, characteristic features of gastrointestinal dysmotility disorders such as idiopathic chronic constipation, IBS and CIPO (Törnblom et al. 2002; De Giorgio et al. 2004a; De Giorgio and Camilleri, 2004b; De Giorgio et al. 2004c; Bassotti et al. 2006).

Interstitial Cells of Cajal (ICCs)

Interstitial cells of Cajal (ICCs) were originally described in the gut more than a century ago by Ramóny Cajal (He et al. 2001). ICCs are a unique class of mesenchymal cells found in the gastrointestinal tract of mammals. In the region of the gastric corpus and antrum, multipolar ICCs form two-dimensional networks, and have been mistaken for neurons, glial cells, smooth muscle cells, macrophages and fibroblasts. ICCs are the pacemaker cells responsible both for initiating slow wave activity in gastrointestinal muscles and for the active propagation of the electrical slow waves (Thomsen et al. 1998). ICCs can be recognised either by their characteristic ultra structure by electron microscopy or by the immunohistochemical demonstration of their surface receptor tyrosine kinase Kit. Recent studies demonstrated that the c-Kit receptor is essential for the development of ICCs. Mesenchymal ICC precursors that carry the c-Kit receptor require the *kit* ligand, which can be provided by neuronal cells or smooth muscle cells. Accordingly the ICCs develop as either myenteric or muscular ICCs (Wu et al. 2000).

The evidence from experimental models and human diseases increasingly point to a central role of ICCs in the aetiology of human gastrointestinal dysmotility. Many gastrointestinal motor disorders like gastro paresis, abnormal small bowel motility, inflammatory bowel disease, CIPO, gastrointestinal stromal and multiple autonomic tumours, achalasia and Hirschsprung's disease show a changed number and/or structure of ICCs (He et al. 2000; Sanders et al. 1999; Hagger et al. 2000). Gastro paresis is associated with electrical abnormalities, and deviations from normal slow-wave rhythm (dysrhythmias) have been reported to result in delayed gastric emptying. In a diabetic rat model it has been demonstrated that degeneration of ICCs is responsible for these gastro-electrical dysrhythmias (Ordög et al. 2000). Therefore, the identification of abnormalities in ICCs which are linked to specific gastrointestinal motor disorders should be taken more into focus in the future.

Newly Discovered Peptides in the Enteric Nervous System (ENS)

A wide range of peptides are described as having a decreased expression in dysmotility. It is not known whether these down-regulated peptides are primary or secondary to development of the disease (Krischnamurthy and Schuffler, 1993; De Giorgio and Camilleri, 2004b). Gut peptides exert diverse effects, regulating gastrointestinal motility and acid secretion, epithelial integrity, and both nutrient absorption and disposal. These actions are initiated by the activation of specific G protein-coupled receptors and may be mediated by direct or indirect effects on target cells (Kutchai, 2004). More recent evidence demonstrates that gut peptides, such as glucagon-like peptides-1 and 2, also directly regulate signalling pathways coupled to cell proliferation and apoptosis (Drucker, 2003).

A number of signalling pathways between mesenchymal and neural crest cells are required for the development of the ENS (Natarajan et al. 2002). These signalling pathways involve peptides secreted by intestinal mesenchymal cells such as endothelin-3, glial cell line-derived neurotrophic factor (GDNF), neuroturin, neurotrophin-3 (NT-3), and netrin-1 (Chalazonitis et al. 1998; Young et al. 2004; Nagy and Goldstein, 2006). The presence of both motilin and ghrelin in guinea-pig myenteric neurons is suggested to play a role in the

activation of the ENS and hence in the regulation of gastrointestinal motility (Xu et al. 2006), which is further supported by a close relationship between Ghrelin and gastric motility in rats (Masuda et al. 2000). The findings that patients with functional dyspepsia (FD) have altered plasma profile of ghrelin suggest a possible role for this peptide in the pathophysiology of FD (Takamori, 2006). Obestatin, a newly discovered ghrelin-associated peptide, was initially suggested to decrease gastric emptying (Zhang, 2005). Unfortunately, recent studies have not been able to confirm these results, and existing reports do not support obestatin as a regulator of digestive motility (Gourcerol and Taché, 2007).

Serotonin is a biochemical neurotransmitter, found primarily in the CNS, gastrointestinal tract, and blood platelets (Vialli, 1966). The bowel exhibits reflexes in the absence of CNS input. To do so, epithelial sensory transducers, such as enterochromaffin (EC) cells, activate the mucosal processes of intrinsic and extrinsic primary afferent (sensory) neurons by secretion of serotonin (5-HT) in response to mucosal stimuli (Gershon, 2005). The enteric serotonin reuptake transporter has been proposed to play a critical role in serotonergic neurotransmision and in the initiation of peristaltic and secretory reflexes (Chen et al. 2001). The current knowledge suggests that serotonin initiates peristaltic and secretory reflexes because of its ability to stimulate secretion of acetylcholine (Ach) and calcitonin gene related peptide (CGRP) (Pan et al. 1994; Sidhu et al. 1995; Grider, 1994, 2003). These afferent reflex pathways also lead to perceptions of nausea, and discomfort and pain from the gastrointestinal tract (Grundy, 2002). Serotonin is thus implicated in the pathology of irritable bowel syndrome (IBS), which is characterised by visceral hypersensitivity and altered motility (Simrén et al. 2003; Costedio et al. 2006). Multiple receptor families explain the broad physiological actions and distribution of serotonin, therefore, many agonists and antagonists to the serotonin receptors have been developed and clinically used. So far, no one has given successful results in the treatment of IBS (McLean et al. 2006).

The neuropeptide vasoactive intestinal peptide (VIP) is the most important peptidergic transmitter in intestinal relaxation, which regulates smooth muscle- and epithelial function. For the first time, VIP/pituitary adenylate cyclase activating peptide (PACAP) receptors have been detected in the human gastrointestinal tract by the use of specific antibodies (Rettenbacher and Reubi, 2001). Observed correlation between delayed gastrointestinal transit and an increase of VIP neurons in a rat ischemia/reperfusion model suggests that changes in enteric transmitters might contribute to gastrointestinal dysmotility (Calcina et al. 2005).

Secretoneurin is a functional neuropeptide derived from secretogranin II (chromogranin C). Both in the myenteric and submucous plexuses, nerve fibres and the majority of ganglion cells were found to be secretoneurin-immunoreactive. Thus, secretoneurin is a new major peptide within the human enteric neuroendocrine system. Its abundant presence in myenteric ganglion cells may imply a role in the modulation of gastrointestinal motility. The chemotactic properties of secretoneurin and its possible localization in sensory fibres suggest that this peptide may be involved in the genesis of intestinal inflammation (Schurmann et al. 1995).

Oxytocin

Oxytocin is a hormone with its most well-known effects on myoepithelial cells of the breast during lactation and the uterine contractions during parturition. Oxytocin is detected not only in plasma but also in almost all segments of the gastrointestinal tract (Monstein et al. 2004). The indirect immunofluorescence approach has shown that oxytocin is expressed in myenteric and submucous ganglia, suggesting that it is important for both gastrointestinal sensitivity and motility (Ohlsson et al. 2006b). Oxytocin is released into plasma in response to a meal (Ohlsson et al. 2002), and has been shown to stimulate gastric emptying (Hashmoni et al. 1979; Petring, 1989) and colonic peristalsis (Ohlsson et al. 2004). In addition, inhibition of the binding of endogenous oxytocin by the receptor antagonist atosiban delayed gastric emptying (Ohlsson et al. 2006a). The prokinetic effect of oxytocin on the gastrointestinal tract is speculated to be similar to the one in uterine myometrium and mammary myoepitheal cells; intracellular release of Ca2+ which leads to muscle contraction via myosin light kinase activity (Gimpl and Fahrenholz, 2001).

A woman with chronic gastro paresis demanding continuous treatment with prokinetic drugs, was completely out of symptoms during pregnancy and breast feeding, and could stop medicamentation

every time she was pregnant (Ohlsson, 2006c). Although the mechanism behind this phenomenon is not proven, these states are characterised by elevated oxytocin levels in plasma (Chiodera et al. 1991, Silber et al. 1991), and together with other observations mentioned above, one may speculate whether oxytocin deficiency may be the aetiology to the gastro paresis in this woman (Ohlsson, 2006c). Despite the stimulatory effect of oxytocin on peristalsis, treatment with nasally administered oxytocin did not improve the stool habits in women with refractory constipation (Ohlsson et al. 2005).

Oxytocin is also known to have analgesic effects (Petersson et al. 1996), and its plasma levels are found to be decreased in patients suffering from dyspepsia and IBS, conditions characterised by abdominal pain and discomfort (Uvnäs-Moberg et al. 1991). Furthermore, children suffering from recurrent abdominal pain exhibit lower plasma levels of oxytocin than healthy controls (Alfven, 2004). Interestingly, both depression and fibromylagia are associated with IBS (Lydiard et al. 1993; Sperber et al. 1999), and both of these conditions are also characterised by low plasma levels of oxytocin (Frash et al. 1995; Anderberg and Uvnäs-Moberg, 2000). Accordingly, treatment of IBS patients with intravenously (Louvel et al. 1996) or nasally (Ohlsson et al. 2005) administered oxytocin resulted in the reduction of abdominal pain and reduced depression. The questions remain as to whether oxytocin could be used clinically to improve the suffering of patients with IBS and CIPO by reducing their pain and their depressive mood rather than by attempting to improve motility. Further randomised clinical trials are needed to answer these questions.

Gonadotropin releasing hormone (GnRH)

The central core of the hypothalamic-pituitary-gonadal axis, in all vertebrate species, is the group of neurons that produce and secrete gonadotropin-releasing hormone (GnRH). Over the past 20 years, techniques have become available to identify the GnRH-producing neurons and measure both GnRH content and levels of GnRH mRNA in brain tissue. Indeed, several types of differentiated lymphocytes, including spleenocytes, thymocytes, peripheral T- and B-lymphocytes, and mast cells have been demonstrated to produce GnRH or a GnRH-like peptide (Marchetti et al. 1996). Although it is not known whether different forms of GnRH might have different receptor types, GnRH receptors have been found throughout the human body (Fekete et al. 1989; Kakar and Jennes, 1995), but have not been studied in the gastrointestinal tract. In rats, GnRH mRNA has been found in parietal cells of gastric glands, the epithelium of the small and large intestine, and in parasympathetic ganglion cells of the myenteric plexus. In addition, the GnRH receptor has been found in the epithelium of gastric pits (Huang et al. 2001) and GnRH receptor mRNA in the myenteric neurons in the rat (Ho et al. 1996). GnRH has also been detected in rat pancreas (Wang et al. 2001). In the dog, GnRH has been shown to inhibit the release of gastric secretions and gastrin release (Soldani et al. 1982), possibly due to diminished vagal activity. Apart from its effects on reproduction, these findings suggest a role for GnRH also in the regulation of the gastrointestinal tract. Accordingly, the GnRH analogue leuprolide (pGlu-His-Trp-Ser-Tyr-DLeu-Arg-Pro-EtNH_2) has been shown to stimulate intestinal motor activity in rats (Khanna et al. 1992; Ducker et al. 1996). Furthermore, symptom resolution and alleviation of intestinal motility abnormality after treatment with leuprolide have been reported in a patient with CIPO (Mathias et al. 1992). In a study of the effect of leuprolide in the treatment of IBS, the overall symptom score was improved, but the greatest therapeutic effect was seen on abdominal pain and nausea (Mathias et al. 1994a; Mathias et al. 1998). This effect persisted when the treatment was continued for up to 6–12 months (Mathias et al. 1994b; Palomba et al. 2005).

Other GnRH analogues, such as buserelin, are used in the treatment of *in vitro* fertilization (IVF), endometriosis, polycystic ovary syndrome, prostate cancer, uterine leiomyoma and precocious puberty. Gastrointestinal side effects are considered infrequent (WHO) but nausea and abdominal pain have been reported in 7%–17% of women treated with buserelin for endometriosis and uterine leiomyoma (FASS, Micromedex). The aetiology to these side effects is not known.

Recently we demonstrated for the first time GnRH positive neurons in the human gastrointestinal tract and have shown a decreased number of GnRH positive neurons in a CIPO patient. The patient, who had been treated with buserelin,

developed CIPO with pronounced abdominal pain and nausea/vomiting. Remarkably, immunohistochemical analysis of intestinal resects revealed that in the patient only 3% of myenteric neurons are GnRH positive as compared to 53% in controls (Ohlsson et al. 2007). The patient had high plasma titres of anti-GnRH antibodies that correlated with the occasions of the treatment with buserelin. The latter led us to the hypothesis that the patient developed CIPO due to buserelin-induced formation of anti-GnRH antibodies which destroyed GnRH-producing neurons of the myenteric plexus. We believe that GnRH plays a pivotal role not only in the regulation of different hormones involved in reproduction, but also in the regulation of the motor activity of the gastrointestinal tract. Degeneration of GnRH neurons might be of central importance for the pathophysiology of different forms of IBS and CIPO.

Concluding Remarks

Over recent decades, a number of peptides have been characterised, which led to an explosion in our understanding of their biological action and function in the central and enteric nervous system. Gut hormones, including cholecystokinin, corticotrophin-releasing hormone, gastrin, gastric inhibitory polypeptide, ghrelin, glucagon-like peptide-1, motilin, neurotensin, pancreatic polypeptide, secretoneurin, serotonin, thyrotrophic-releasing hormone and VIP have been shown to play a role in modulating gastrointestinal motility. New experimental and clinical data point to GnRH and oxytocin, two other peptide candidates, as being involved in controlling gastrointestinal motility. These findings open new, fascinating perspectives for research and the therapeutic potential of the peptidal role in gastrointestinal diseases.

Furthermore, the role of neuronal apoptosis and agents which improve neuronal survival deserves further attention concerning their function in preventing neuronal degeneration which results in dysmotility.

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