Pediatr Surg Int (2009) 25:1043–1052
DOI 10.1007/s00383-009-2484-x

REVIEW ARTICLE

New insights into the pathogenesis of infantile pyloric stenosis

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Accepted: 25 August 2009 / Published online: 16 September 2009

Abstract Infantile hypertrophic pyloric stenosis (IHPS) is the most common surgical cause of vomiting in infants. Despite numerous hypotheses, the aetiopathogenesis of IHPS is not fully understood. Genetic, extrinsic and hormonal factors have been implicated in the pathogenesis of the disease. Furthermore, abnormalities of various components of the pyloric muscle such as smooth muscle cells, growth factors, extracellular matrix elements, nerve and ganglion cells, synapses, nerve supporting cells, neurotransmitters and interstitial cells of Cajal have been reported. Recently, genetic studies have identified susceptibility loci for IHPS and molecular studies have concluded that smooth muscle cells are not properly innervated in IHPS.

Introduction

Infantile hypertrophic pyloric stenosis (IHPS) is the most common surgical cause of vomiting in infants. The pyloric muscle is hypertrophied and the pyloric channel becomes narrow and elongated, causing gastric outlet obstruction. The onset of symptoms typically occurs at 2–8 weeks of age with a peak occurrence at 3–5 weeks [70]. The incidence of IHPS has been reported to be approximately 3 per 1,000 live births although wide variations have been observed with geographic location, season and ethnic origin [73]. Recently, a decline in IHPS incidence has been reported in a number of countries [4, 68, 79, 98].

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The first complete description of IHPS as a clinical entity was by Hirschsprung in 1888, whereas the first successful pyloromyotomy was performed by Ramstedt in 1912. At that time IHPS was a lethal condition in the majority of cases. Since, medical knowledge along with advances in fluid resuscitation and paediatric anaesthesia has led to zero mortality and minimal morbidity. However, despite extensive research, the aetiology of IHPS remains unclear.

The occurrence of IHPS has been associated with several variables such as genetic [9, 10, 15, 25, 26, 55, 91], environmental [16, 61, 79, 99, 100] and mechanical factors [36, 48, 58]. The pyloric sphincter, a zone of intermittently increased pressure, is able to contract tonically and phasically and produce an effect on gastric emptying [19]. Pyloric sphincter function and motility is under a complex control system which involves the enteric nervous system, gastrointestinal hormones and interstitial cells of Cajal (ICC); these pathways have been investigated in IHPS and abnormalities in hormonal control [7, 21, 35, 56, 102], extracellular matrix [12, 30, 65, 76], smooth muscle fibres [22, 30, 33, 57, 89], growth factors [45, 71–73, 94, 95] and ICC [57, 77, 80, 107, 112] have been implicated in the pathogenesis of the disease. Lastly, a significant amount of research has focused on abnormalities in pyloric innervation [1–3, 6, 29, 30, 32, 34, 40, 47, 49–53, 55, 57, 62, 73–75, 84, 91, 92, 104, 107, 108, 110].

Genetic factors

Genetic factors have been implicated in the pathogenesis of IHPS on the basis of male prevalence and familial distribution. Boys are affected four times more frequently than girls, whereas 5.5% of the sons and 2.5% of the daughters of an affected father develop IHPS in comparison to 20% of the sons and 7% of the daughters of an affected mother

[11]. Siblings of affected children carry a risk up to 30 times greater than that of IHPS in the general population [60]; a similar risk of both infants being affected has been reported in twins, although increased in monozygotic twins [64].

IHPS has been associated with genetic syndromes such as Smith-Lemli-Opitz [20] and Cornelia de Lange [46] as well as chromosomal abnormalities including partial trisomy of chromosome 9 [37], partial trisomy of chromosome 13 and partial monosomy of chromosome 18 [13], and a translocation of chromosome 8 and 17 [38]. Autosomal dominant inheritance has also been reported [9, 28].

Non-syndromic IHPS is considered to be an example of the multifactorial, sex-modified threshold model of inheritance as proposed by Carter in 1961. According to this model various genetic and environmental factors contribute to the "liability" of an individual towards development of a disorder. Each single factor has a small effect, but the effects add up and when a critical liability "threshold" is crossed, disease results. In IHPS the threshold is higher for females who require a stronger liability to express the trait [10].

Although a specific gene responsible for IHPS has not yet been discovered, several susceptible loci have been identified, such as two distinct regions on chromosome 16p12-p13 [9] and 16q24 [25] and loci on chromosome 11q14-q22 and Xq23 [26]. In view of the implication of the enzyme neuronal nitric oxide synthase (nNOS) in the pathogenesis of IHPS, *NOS1*, the gene encoding nNOS on chromosome 12q has been investigated by linkage analysis [15] and evaluation of nNOS mRNA expression [55, 91] and suggested as a susceptibility locus.

Extrinsic factors

Various environmental and mechanical factors have been proposed as potential causes of IHPS. Data on the role of infant feeding have been inconsistent as IHPS has been reported to be more common in breast-fed babies [24] as well as in formula-fed babies [81]. Recently, a possible involvement of the sleeping position has been suggested. The incidence of IHPS in Sweden between 1970 and 1997 has been reported to parallel the incidence of Sudden Infant Death Syndrome (SIDS) for the same period, raising the question of a common causative factor for IHPS and SIDS. The prone sleeping position has been suggested as a possible risk factor given the fact that it has been associated with increased risk of SIDS and the launch of the "back to sleep" campaign to prevent SIDS has coincided with the decline in the incidence of both IHPS and SIDS in Denmark and Sweden [68, 79]. Maternal smoking, another recognised risk factor for SIDS, has been reported to double the risk for IHPS [99].

Erythromycin has been associated with an increased risk of IHPS as it acts as a motilin agonist and induces strong gastric and pyloric contractions that may lead to pyloric hypertrophy. Infants of mothers exposed to erythromycin during lactation have been reported to be at a higher risk of IHPS [100] while prenatal exposure has not been found to be associated with an increased risk [17, 59]. Although neonates treated with erythromycin for different types of infections in the first 2 weeks of life have been found to carry an up to tenfold risk of IHPS [16, 61], cases of IHPS have not been reported in premature infants treated with erythromycin for feeding intolerance [67].

Recently, Paulozzi, based on clinical features and epidemiological data, hypothesised that IHPS is caused by *Helicobacter pylori* (HP) and postulated that the infection causes antral inflammation which leads to spasm and work hypertrophy resulting in gastric outlet obstruction [78]. Two studies tested this hypothesis using immunohistochemical staining of gastric biopsies and rapid urease test [18] or HP stool antigen immunoassay [93], but failed to confirm HP infection in infants with IHPS.

In a small number of cases IHPS has been believed to develop as secondary effect to a primary gastric outlet obstruction by mechanical factors. Transpyloric feeding tubes [58], an antral polyp [48] and a pyloric cyst [36] have been associated with IHPS. In the same manner, the mucosal and submucosal hypertrophy in eosinophilic gastroenteritis [43, 97] and focal foveolar hyperplasia [39] have been reported to act as obstructive factors resulting in IHPS.

Hormonal control

The pyloric sphincter is under hormonal control by gastrin, secretin, cholecystokinin and somatostatin. Gastrin stimulates gastric acid secretion via histamine release while secretin and cholecystokinin are released in response to the acidity and consistency of the chyme entering the duodenum and contract the pyloric sphincter. Somatostatin is the main physiological antagonist of gastrin.

Dodge in 1970 induced hypertrophic pyloric stenosis in newborn puppies after prolonged maternal stimulation with pentagastrin, prompting the hypothesis that gastrin is a causative factor for IHPS [23]. At that time it was suggested that elevated gastrin levels in infants cause pyloric contractions and eventually work hypertrophy [88]. However, several attempts to demonstrate the role of gastrin in IHPS have produced inconsistent results. Spitz et al. found elevated preoperative fasting serum gastrin levels in IHPS infants compared to controls whereas further postoperative increase was noted [102]. Bleicher et al. found significantly higher preoperative and postoperative fasting gastrin levels in IHPS

infants in comparison to vomiting and non-vomiting infants without IHPS [7]. Hambourg et al. reported similar fasting serum gastrin levels in controls and IHPS infants preoperatively, although the postoperative fasting levels in IHPS infants were significantly higher. Postprandial gastrin levels were similar for the two groups [35]. Grochowski et al. did not observe significant differences in fasting gastrin levels between controls and IHPS preoperatively, but reported elevated gastrin levels in IHPS patients postoperatively and attributed this finding to the trophic role of gastrin on the gastrointestinal mucosa [31]. Rogers et al. found no significant differences between fasting gastrin levels in controls and preoperative or postoperative IHPS patients [87], while Moazam et al. investigated fasting and postprandial serum gastrin levels in IHPS and controls and noted no differences [66]. Dick et al. reported no differences in gastrin levels between IHPS infants and controls, but found increased levels of somatostatin in IHPS infants compared to controls and this difference was statistically significant, suggesting that somatostatin may have a role in the pathogenesis of IHPS [21].

Conflicting findings on the levels of gastrin in IHPS led to the formulation of the hyperacidity theory according to which IHPS is caused by inherited high acid secretion possibly due to a greater parietal cell mass, rather than by gastrin itself [85, 87]. Gastric hyperacidity results in acid contents entering the duodenum and, in response, secretin and cholecystokinin are released causing pyloric contractions and secondary hypertrophy [66, 87]. Subsequent measurements of cholecystokinin levels in infants with IHPS have failed to confirm this hypothesis [86].

Prostaglandins are produced in response to acid secretion and have a role in gastrointestinal motility as well as cytoprotective and trophic effects. Prostaglandins PGE2 and PGF2a in the gastric juice have been found to be elevated in IHPS as compared with controls and, based on the belief that they influence pyloric contraction, it has been suggested that these substances may be responsible for pylorospasm and pyloric hypertrophy [56]. Although the finding of elevated PGE2 in IHPS has been confirmed, evidence on prostaglandins causing relaxation of circular smooth muscle has challenged the hypothesis that prostaglandins cause pyloric hypertrophy [96]. Interestingly, prostaglandin treatment for cyanotic congenital heart disease has been shown to induce antral hyperplasia and gastric outlet obstruction that mimics IHPS [63], whereas one case of IHPS has been reported in an infant with prostaglandin induced foveolar hyperplasia [8].

The increased risk of IHPS in infants treated with erythromycin, a motilin agonist, has led to the hypothesis that MLN, the motilin gene, is involved in the pathogenesis of IHPS. However, MLN mutation screening and investigation for an association between V15A polymorphism and IHPS failed to support this hypothesis [105].

Many investigators have raised the question of hormonal abnormalities observed in IHPS being the consequence rather than the cause of the disease. Further studies on gastrointestinal hormones which have interrelated effects are necessary to elucidate their role in the pathophysiology of IHPS.

Smooth muscle cells

The tone of the smooth muscle sphincters of the gastrointestinal tract is an intrinsic property of myogenic origin, independent of the nervous system [90]. Ultrastructural and molecular studies of smooth muscle cells in IHPS have produced varied results. Dieler et al. studied pyloric muscle biopsies from IHPS infants with light and electron microscopy and having identified two groups of patients with distinct types of changes, suggested that two types of IHPS exist, a primarily myogenic and a primarily neurogenic type, although transitional forms were also identified. In the myogenic type, dilated rough endoplasmic reticulum, glycogen accumulation, swollen and necrotic smooth muscle cells, enlarged mitochondria as well as nuclear vacuoles, inclusions and cytoplasmic bodies were observed [22]. Langer et al. found smooth muscle cells in IHPS to be morphologically normal, although frequently in a proliferative phase with dilated rough endoplasmic reticulum and a lower proportion of contractile filaments. Numerous gap junctions and intermediate contacts were observed in control circular muscle, whereas in IHPS cell to cell junctions were rare [57].

Guarino et al. analysed the expression of desmin in IHPS. Desmin is the main protein of intermediate filaments and is important for the organisation and function of muscle fibres. It is strongly expressed during myogenesis and has been found to be increased in some forms of myopathies. A strong expression of desmin was observed in pyloric muscle biospies from infants with IHPS, as opposed to absent or weak expression in matched controls. A similar pattern of strong desmin expression has been demonstrated in the fetal pylorus, suggesting than in IHPS the organisation of intermediate filaments is in a fetal state of development [33].

Gentile et al. studied cytoskeletal elements of pyloric smooth muscle cells in IHPS using immunohistochemical staining and confocal laser microscopy. The immunostaining pattern was similar in controls and IHPS for desmin, vinculin, a protein mainly involved in signal transduction and α-smooth muscle actin, responsible for contraction. Talin, a protein responsible for smooth muscle cell-extracellular matrix interaction, and dystrophin, a

protein with adhesion properties, were present in controls but absent in IHPS patients. The authors concluded that in IHPS the muscle cells are mature with contractile capability but the membrane-cytoskeleton interactions and the cell-matrix communications are altered [30]. Romeo et al. investigated dystroglycans and sarcoglycans, two proteins that, along with dystrophin, form the Dystrophin–Glycoprotein Complex which is important for maintaining the structural integrity and function of muscle fibres. They reported that although dystroglycans showed similar expressions in IHPS and controls, sarcoglycans were present in controls but absent in IHPS and suggested that lack of sarcoglycans can alter the physiology of smooth muscle cells and predispose to IHPS [89].

Growth factors

Growth factors are peptides that control cell proliferation and modulate cellular functions by binding to specific high-affinity cell membrane receptors. Although the exact mechanism that leads to muscle hypertrophy in IHPS is unknown, there is evidence that the growth of smooth muscle cells is regulated by growth factors [71, 72].

Insulin-like growth factor-I (IGF-I) stimulates proliferation and differentiation in many tissues and acts as the mediator of most anabolic effects of growth hormone. Using immunohistochemical staining, the expression of IGF-I and its receptors in controls was either absent or weak as opposed to increased expressions in the hypertrophied pylorus in IHPS infants [45, 72]. The circular muscle layer of the hypertrophied pylorus demonstrated stronger immunoreactivities in comparison to the longitudal muscle layer [72]. Similarly, using in situ hybridization histochemistry, IGF-I mRNA expression in IHPS was strong in the circular muscle layer and moderate in the longitudal muscle layer while it was weak or absent in controls [71].

Platelet-derived growth factor-BB (PDGF-BB), a potent smooth muscle mitogen, requires other growth factors such as IGF-I to exert its mitogenic effects. Both PDGF-BB and its receptor were found to be markedly increased in the circular muscle layer of the pylorus and to a lesser degree in the longitudal muscle layer in IHPS compared to controls [72]. Platelet-derived endothelial cell growth factor (PDEGF) is an endothelial cell mitogen that is not expressed in normal smooth muscle but is believed to have a role in pathological angiogenesis. Using immunohistochemical staining, the expression of PDEGF in the hypertrophied pyloric muscle in IHPS was found to be significantly increased in comparison to control specimens [45].

Transforming growth factor-α (TGF-α) has growth promoting effects in vascular and visceral smooth muscle cells. In IHPS specimens both TGF-α immunoreactivity and mRNA expression have been found to be increased in the circular and longitudal muscle layers compared to controls [95]. Transforming growth factor-β (TGF-β) induces growth inhibition associated with a marked increase in the cell-cycle transit time so that the hypertrophic component of cell-cycle progression is prolonged and cells attain a large mass. In IHPS, immunoreactivities of TGF-β1 and its receptors as well as mRNA expression of TGF-β1 have been reported to be significantly increased compared to normal pyloric muscle [73].

Epidermal growth factors (EGF) are polypeptides with a variety of biological effects that act as powerful smooth muscle cell mitogens. Increased immunoreactivity for EGF and their receptors and strong expression of EGF mRNA have been reported in both circular and longitudal muscle in IHPS. Weak immunostaining was observed in control specimens [94].

Strong expression of growth factors within the pyloric muscle along with increased expression at the mRNA level shows that in IHPS the local synthesis of growth factors is increased. These studies suggest that upregulated growth factors in IHPS may induce altered growth regulation in pyloric smooth muscle cells contributing to pyloric muscle hypertrophy.

Extracellular matrix proteins

Smooth muscle cells have been reported to form a close relationship and interact with extracellular matrix elements [103]. Early investigators have reported an increase in connective tissue in IHPS [3, 6], whereas more recent studies have explored extracellular matrix proteins.

Chondroitin sulphate was significantly increased in specimens of pyloric muscle in IHPS compared to controls. Increased staining was observed both among the circular muscle fibres and in the connective tissue septa between circular muscle bundles. Fibronectin and laminin were also found to be increased in IHPS although to a lesser degree [12, 30].

On electron microscopy, collagen fibres have been reported to be increased and grouped in a bundle fashion in the extracellular matrix and the connective tissue septa in IHPS [12, 30]. Furthermore, immunohistochemical staining for newly synthesised type I procollagen has demonstrated abnormal amounts in the circular muscle in IHPS, indicating that the hypertrophic muscle in IHPS is actively synthesising collagen [65]. Immunoreactivity for elastin fibres in IHPS has been found to be increased in connective

tissue septa and present among the muscle fibres, whereas in controls it is moderate and absent, respectively [76].

Studies on extracellular matrix proteins demonstrated that in IHPS the distribution and organisation of extracellular matrix components is altered and this may account for the consistency of the pyloric tumour.

Pyloric innervation

Although the smooth-muscle sphincter tone is myogenic, contraction and relaxation are under neural control via activation of excitatory and inhibitory pathways, respectively. Sympathetic stimulation is believed to exert an excitatory effect on the pyloric sphincter, while parasympathetic stimulation has either an excitatory effect via cholinergic neurons or an inhibitory effect via non adrenergic non cholinergic neurons [90].

Many investigators have sought evidence for an abnormality in pyloric innervation in IHPS. Defective innervation would explain the failure of the pylorus to relax resulting in functional gastric outlet obstruction and subsequently muscle hypertrophy. In view of similarities between Hirschsprung's disease and IHPS, early investigators concentrated on the myenteric plexus searching for abnormalities in ganglion cells [2, 3, 6, 29, 47, 57, 74, 84, 106] and nerve fibres [2, 47, 49, 57]. Since, accumulating knowledge on the role of the enteric nervous system along with advances in laboratory techniques and equipment have facilitated more sophisticated studies on glial cells [32, 34, 51], synaptic function [52, 53, 75] and neurotransmitters [1, 30, 40, 50, 52, 55, 62, 92, 104, 108, 110] in the hypertrophied pyloric muscle.

Ganglion cells

Studies on ganglion cells in IHPS have produced conflicting results. Light microscopy studies showed that myenteric ganglia were fewer and contained many undifferentiated cells and suggested that maturation of ganglia is either arrested or delayed [29]. Various degrees and types of degenerative changes as well as decreased number and size of ganglion cells have also been reported [3, 6, 69, 101]. Rintoul et al. found that one type of ganglion cells, Dogiel type I cells, were virtually absent in the hypertrophied pylorus but failed to attribute this finding to immaturity or degeneration [84]. Using electron microscopy or immunohistochemical staining, some investigators reported that ganglion cells in IHPS were of adequate number and size and of normal morphology [47, 74, 106] while others found fewer ganglia of smaller size in IHPS compared to controls [2, 57].

Nerve cells

In comparison to ganglion cells little attention has been paid to the morphological features of nerve cells. On electron microscopy, Langer et al. found that in IHPS the numbers of nerve cell bodies identified within the circular muscle layer were decreased [57], while Jona observed that although most of the neurons appeared normal, a small proportion had very large axons with degenerative changes [47]. Immunohistochemical studies have demonstrated that in IHPS the nerves of the myenteric plexus were longer and thicker, forming numerous contorted bundles [2, 49].

Synapses

Using immunohistochemical staining techniques to label SVP-38, a synaptic vesicle protein, Okazaki et al. observed few neuromuscular junctions in the muscle layers of the pyloric tumour in IHPS despite the presence of dense clusters of synapses in the myenteric plexus. A similar staining pattern was observed in the myenteric plexus of controls, while many neuromuscular junctions were identified in the muscular layers [75].

Kobayashi et al. studied neural cell adhesion molecule (NCAM), a protein involved in cell adhesion, contact formation and interaction between nerve and muscle cells during development. They found strong immunoreactivity for NCAM in the myenteric plexuses of both controls and IHPS patients. In the muscle layers of the pyloric muscle NCAM immunoreactivity was moderate in controls, whereas it was either absent or markedly decreased in IHPS [52]. However, a study of older IHPS patients showed that NCAM immunoreactivity was "normalising" with age; therefore, it has been postulated that it may be age dependent [53]. These reports suggest that in IHPS the interaction between nerve and muscle cells is impaired.

Nerve supporting cells

Nerve supporting cells (NSC) are essential for the basic physiological functions of neurons and important for the spatial arrangement of their cell bodies and processes; they also stimulate neuronal growth and survival through the actions of neurotrophic factors. Neurotrophic factors, in turn, exert their biological functions by binding to high-affinity, signal-transducing receptors.

NSC have been reported to express various markers for astrocytes and Schwann cells such as S-100, a marker for astrocytes and Schwann cells, glial fibrillary acidic protein (GFAP), a marker for astrocytes and D_7, a marker for Schwann cells. In IHPS, S-100, GFAP and D_7 immunostaining was either absent or weak within the hypertrophied muscle layers as opposed to strong immunostaining in

controls. In both IHPS and control groups there was strong staining in the myenteric plexus by all three antibodies [51].

Glial-derived neurotrophic factor (GDNF) is important for the survival of a wide range of neuronal populations. The main sources of GDNF in the gut during fetal life are mesenchyme and smooth muscle cells, whereas after birth GDNF is produced by the enteric NSC. In IHPS, intense GDNF immunoreactivity was observed in the smooth muscle cells of both the circular and the longitudal muscle layers, whereas no GDNF immunoreactivity was detected in the smooth muscle cells of controls. The intensity of GDNF staining in the myenteric plexus was lower in IHPS compared to controls. These results suggest that in IHPS smooth muscle cells may retain the capacity to produce GDNF, possibly compensating for the immaturity of NSC [32].

Neurotrophins such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) and their receptors TrkA for NGF, TrkB for BDNF and NT-4/5 and TrkC for NT-3 were studied in IHPS patients using Enzyme-Linked Immunosorbent Assay Analysis and immunohistochemical staining. The levels of neurotrophins were found to be low in IHPS compared to controls. No significant differences were observed in the pattern of Trk staining between IHPS and controls, except for the finding of absent TrkA immunoreactive nerve fibres in IHPS as opposed to controls [34].

These observations suggest that abnormalities in NSC due to either quantitative impairment or functional immaturity may have a role in the defective innervation in IHPS.

Cholinergic and adrenergic innervation

Cholinergic nerve distribution has been studied using acetylcholinesterase (AChE) histochemical staining. Strong AChE staining was observed in the myenteric plexus and the muscle layers in controls, whereas in IHPS specimens AChE staining was markedly decreased in the muscular layers but strong in the myenteric plexus [50]. Adrenergic immunoreactivity has been reported to be absent in the muscular layers and markedly decreased in the myenteric plexus in IHPS in comparison to controls [73].

Peptidergic innervation

Enkephalin modifies transmitter release from other nerve fibres in smooth muscle and possibly acts as an excitatory neurotransmitter. In IHPS, nerves containing enkephalin were sparse or absent in the smooth muscle, whereas large numbers were observed in controls [62, 92, 110]. Substance P fibres, also believed to be excitatory, have been found to be absent or markedly decreased either in the smooth muscle [92, 110] or both in the smooth muscle and the myenteric plexus [1]. Neuropeptide Y inhibitory fibres have been reported to be decreased in the smooth muscle in IHPS [110]. Immunoreactive fibres for vasoactive intestinal polypeptide (VIP), an inhibitory neuropeptide, were found to be sparse in the circular muscle of IHPS specimens [1, 62, 92, 110]; however, some investigators observed increased VIP immunoreactivity in the myenteric plexus in IHPS compared to controls [1, 62].

These reports suggest that consumption of excitatory neuropeptides in IHPS may account for their depletion in the smooth muscle, whereas reduced inhibitory peptides may be responsible for the failure of pyloric relaxation. Increased VIP expression may represent a compensatory mechanism by which IHPS naturally resolves.

Nitrergic innervation

Nitric oxide (NO) is a gaseous free radical synthesised from L-arginine in a reaction catalysed by nNOS. NO has a well-described role as a major non-adrenergic non-cholinergic inhibitory neurotransmitter that mediates pyloric relaxation in the enteric nervous system. Furthermore, recent evidence suggests that it regulates several other functions in physiological and pathological conditions [111].

Vanderwinden et al. and Kobayashi et al. investigated nitrergic innervation in IHPS using NADPH diaphorase, an enzyme which was found to be identical to nitric oxide synthase. NADPH diaphorase activity in the myenteric plexus was similar in IHPS and controls, whereas it was selectively absent in the muscle layers of the hypertrophied pylorus in IHPS compared to controls [52, 108]. Gentile et al. and Huang et al. confirmed these observations using nNOS immunohistochemical staining, while Abel reported diminished nNOS expression in the muscle layers as well as the myenteric plexus in IHPS [1, 30, 40]. In addition to nNOS immunoreactivity, Huang et al. investigated the levels of plasma nitrates and nitrites in IHPS and controls and observed that plasma nitrite levels were significantly lower in IHPS patients compared to controls, but would rise to normal following pyloromyotomy. However, Subramaniam et al. [104] examined specimens of 20 IHPS patients and having observed lack of nNOS immunoreactivity in 13 but control-like staining in 7, suggested that NO is absent only in a subset of cases of IHPS.

At the mRNA level, Kusafuka et al. using Reverse Transcription Polymerase Chain Reaction, found the expression of nNOS gene to be significantly reduced in IHPS specimens when compared to controls [55]. Saur et al. confirmed this finding and added that the observed reduction

reflected a severe decrease of 88% in the expression of nNOS exon 1c, which is one of the predominant nNOS transcripts. They also found that the expression of exon 1f, an alternative variant, was increased and suggested that up-regulation of exon 1f may be compensatory in an attempt to increase nNOS expression, normalise pyloric function and eventually overcome IHPS [91].

Experimental animal studies have produced interesting results. Huang et al. generated mice that lack nNOS gene, using homologous recombination. The only abnormality noted in knockout mice was marked enlargement of the stomach with hypertrophy of the pyloric sphincter and the circular muscle layer [41]. Barbosa et al. administered nitro-L-arginine methyl ester hydrochloride (L-NAME), a known nitric oxide synthase inhibitor, to pregnant rats and their newborns and noted that the L-NAME rats had larger stomachs and pyloric hypertrophy [5]. These findings suggest that the stomach and the pylorus may be particularly dependent on NO and especially prone to dysfunction in its absence.

Studies on NO in IHPS suggest that, given the important role of NO in pyloric relaxation, diminished nNOS activity, possibly due to reduced expression of the nNOS gene, may be responsible for the contracted, hypertrophied pyloric muscle.

Interstitial cells of Cajal

Interstitial cells of Cajal are nonneuronal cells that form networks alongside the enteric nervous system and serve as electrical pacemakers and mediators of motor neurotransmission in the gastrointestinal tract. They express c-kit, a transmembrane protein kinase receptor, essential for their development and maintenance. ICC have been classified into several subtypes according to their anatomical locations; each subtype shows different morphological features and distribution pattern and performs different functions [54]. Myenteric ICC trigger the generation of spontaneous pacemaker currents, known as slow waves, which are essential for effective peristalsis, while intramuscular ICC mediate excitatory and inhibitory neurotransmission [42, 44]. Although ICC are a minor component of the tunica muscularis of the gastrointestinal tract, they are considered to be the most important cells coordinating gastrointestinal motility.

On electron microscopy, ICC have been reported to be either absent or rare both in the myenteric plexus and the circular muscle layer in IHPS specimens compared to controls [57].

These findings have been confirmed by studies using c-kit immunohistochemistry [107, 112]. Furthermore, a sharp transition between zones with and without c-kit immunopositivity was observed in IHPS specimens [107]. The lack of ICC in IHPS suggests a disruption in their network and therefore interruption of the generation of slow waves, which may be responsible for the motility disturbances of the pyloric sphincter.

Carbon monoxide acts as a neurotransmitter in the gastrointestinal tract and has been shown to cause smooth muscle relaxation. The main source of endogenous CO is through degradation of heme, catalysed by heme oxygenase (HO) [27]. Heme oxygenase -2 (HO-2), an isoform of HO, is present in the enteric neurons and in intramuscular ICC, suggesting that carbon monoxide may serve as an intercellular messenger between enteric neurons, ICC and smooth muscle cells [82]. Piotrowska et al. investigated immunocolocalization of HO-2 and ICC in IHPS and reported that although intramuscular ICC were HO-2 positive in controls, HO-2 and ICC colocalization was not detected in IHPS. They suggested that impaired intercellular communication between ICC and smooth muscle cells may contribute to motility dysfunction in infants with IHPS [80].

It has been suggested that ICC may produce NO and thus amplify nitrergic signalling [83]. Experimental studies on mutant animal models lacking intramuscular ICC have shown that intramuscular ICC are essential for nitrergic neurotransmission, as loss of this specific population of ICC results in loss of NO-dependent neurotransmission [109]. Conversely, studies on ICC in animals with targeted disruption of the nNOS gene have reported that ICC volumes were significantly reduced but would increase after incubation with a NO donor and suggested that NO is a survival factor for ICC [14]. In IHPS, the association between ICC and NO has recently been explored using immunohistochemical staining and semiquantitative analysis. Both ICC and nNOS expressions were found to be either absent or reduced in IHPS. Furthermore, a statistically significant correlation was observed between the degrees of c-kit and nNOS expression, suggesting the possibility of a causal relationship between ICC and NO. If that is the case, two factors that have been implicated in the pathogenesis of IHPS independently may be interrelated [77].

Conclusion

Although several potentially causative factors have been explored, the pathogenesis of IHPS is not yet fully understood. Two important issues have arisen from structural or molecular studies. First, it is possible that the abnormalities observed in IHPS patients in comparison to controls may be the result rather than the cause of IHPS. Second, factors that have been investigated separately and reported to be abnormal in IHPS patients may actually be interrelated or interdependent. Despite remaining questions, considerable progress has been made in the recent years, especially in the areas of genetics and pyloric innervation. Genetic

studies have identified a number of susceptibility loci for IHPS, and this may be the first step towards identification of the disease genes, although further studies are required. On the other hand, increasing evidence suggests that in IHPS there are quantitative or qualitative abnormalities in the innervation of smooth muscle cells. Of all the components of pyloric innervation, there is strong evidence to support the role of NO in IHPS. *NOS1* has been identified as a susceptibility locus for IHPS and the nNOS gene knockout model is the first genetic model resembling IHPS. The majority of pyloric muscle specimens from IHPS patients demonstrate lack or significant reduction of nNOS expression as opposed to controls. Future experimental studies testing the administration of either NO or a NO donor may elucidate further the role of NO in IHPS.

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