Basic research

Study of expression of two transient receptor potential (TRP) channels, TRPC5 and TRPC6, in children with infantile hypertrophic pyloric stenosis

Janusz Jabłoński[1], Maciej Borowiec[2], Wojciech Fendler[2], Wojciech Młynarski[2], Małgorzata Lewandowska[3], Ewa Andrzejewska[1], Józef Kobos[3]

[1]Department of Pediatric Surgery and Oncology, Institute of Pediatrics, Medical University of Lodz, Lodz, Poland
[2]Department of Pediatrics, Oncology, Hematology and Diabetology, Medical University of Lodz, Lodz, Poland
[3]Laboratory of Pathomorphology, Institute of Pediatrics, Medical University of Lodz, Lodz, Poland

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Corresponding author:
Janusz Jabłoński
Department
of Pediatric Surgery
and Oncology
Institute of Pediatrics
Medical University of Lodz
36/50 Sporna St
91-738 Lodz, Poland
Phone: +48 42 617 77 77
E-mail: gosia.lek@wp.pl

Abstract

Introduction: The transient receptor potential (TRP) channel, so named after the role they play in *Drosophila* phototransduction, takes a prominent position among these sensors and transducers in the digestive system. However, the roles of TRP channels in digestive function are incompletely understood. Changes in TRP channel expression or function are associated with a variety of diseases. The aim of this study was to analyze the expression of TRPC5 and TRPC6 by immunohistochemistry in the pyloric muscle in children with infantile hypertrophic pyloric stenosis (IHPS).
Material and methods: Fifty-three muscle biopsies were obtained at the time of pyloromyotomy. The control group consisted of 10 children. Specimens were evaluated by routine histopathological methods and by immunohistochemistry using monoclonal mouse anti-TRPC5 or -TRPC6 antibodies. Cells showing positive reaction were counted in five random 200× high-power fields.
Results: Immunohistochemical studies showed higher expression of both receptors in the muscularis propria and in the ganglia in children with infantile hypertrophic pyloric stenosis compared with the control group. The result for TRPC5 was statistically significant.
Conclusions: These results show a local increase in the expression of TRPC5 and TRPC6 in the hypertrophic pyloric muscle in children with infantile hypertrophic pyloric stenosis, which may have implications for the pathogenesis of the disease.

Key words: children, infantile hypertrophic pyloric stenosis, transient receptor potential channel.

Introduction

Approximately 20 of the 30 mammalian transient receptor potential (TRP) channel subunits are expressed by specific neurons and cells within the alimentary canal. They serve important roles in taste, chemesthesis, mechanosensation, pain and hyperalgesia and contribute to the regulation of gastrointestinal motility, absorptive and secretory processes,

blood flow, and mucosal homeostasis. In a cellular perspective, TRP channels operate either as primary detectors of chemical and physical stimuli, as secondary transducers of ionotropic or metabotropic receptors, or as ion transport channels [1]. However, the roles of TRP channels in digestive function are incompletely understood. Changes in TRP channel expression or function are associated with a variety of diseases/disorders of the digestive system [1, 2]. These implications have raised enormous interest in the role of TRP channels in the pathogenesis of infantile hypertrophic pyloric stenosis (IHPS).

The IHPS is one of the most common congenital defects requiring surgical intervention in the neonatal period. Infantile hypertrophic pyloric stenosis is characterized by progressive hypertrophy of pyloric muscle. The prevalence of IHPS is 5 in 1000 live births [3]. It typically presents in infants 2–6 weeks after birth with projectile vomiting, weight loss, and dehydration. Surgical treatment by pyloromyotomy was introduced a century ago [4].

The etiology of IHPS is not fully understood. There are many theories concerning the pathogenesis of IHPS. The IHPS appears to arise from a defect in gastrointestinal motility which shows both development and location-specific expression, rather than resulting from a defect in the developmental anatomy of the gastrointestinal tract [5].

Based on the available information, we have chosen to focus the investigation on two receptors: TRPC5 and TRPC6. The aim of the study was thus to assess TRPC5 and TRPC6 expression in the muscularis propria of the hypertrophic pyloric muscle in children with IHPS.

Material and methods

A study was carried out on a group of 53 children (70% boys) with IHPS who underwent pyloromyotomy, aged 4 to 8 week. Vomiting and lack of weight gain were observed between 2 and 6 weeks of age. Electrolyte imbalance and, in rare cases (n = 3), metabolic alkalosis were noted. All patients were discharged home after 3–5 days of hospitalization in good general condition without complications.

Intraoperatively, at the time of pyloromyotomy, 2–3-mm full-thickness biopsy specimens of the pyloric portion of the stomach were obtained. The specimens were fixed in 10% formalin, routinely processed, and embedded in paraffin blocks. The control group consisted of 7 children undergoing surgery because of other alimentary tract defects. Full-thickness biopsy specimens of the pyloric portion of the stomach were obtained intraoperatively.

The specimens were fixed in 10% formalin, routinely processed, and embedded in paraffin.

The study was conducted after obtaining the approval of the Bioethics Committee of the Medical University of Lodz and the signing of a consent form by the parents.

The full-thickness specimens of the muscularis propria of the pyloric portion of the stomach were evaluated by routine histopathological methods. Immunohistochemical staining was performed on the paraffin tissue sections using the monoclonal antibodies anti-TRPC5 and anti-TRPC6. The immunoreactivity was quantified by morphometric methods using a MultiScanBase V.808 analyzer. Cells showing positive reaction were counted in 5 random 200× high-power fields.

TRPC5 and TRPC6 expression was assessed in the muscularis propria and in the ganglia of the pyloric muscle. To evaluate the expression of receptors the authors used a semiquantitative scale (1 point – reaction absent; 2 points – reaction exhibits a single cell; 3 points – reaction has quite a number of cells; 4 points – reaction seen in numerous cells).

Statistical analysis

The results were analyzed statistically. Statistical analysis was performed using a χ^2 test. The level of statistical significance was p less than 0.05.

Results

Standard histopathological evaluation with hematoxylin and eosin staining showed hypertrophy of the muscularis propria of the pyloric muscle and connective tissue remodeling in all the analyzed children with IHPS. Our studies showed statistically significantly greater expression of TRPC5 5 in the study group compared with the control group in both the muscle of the pylorus ($p < 0.001$) and in the ganglia ($p = 0.002$). Expression of TRPC5 6 was higher in the study group compared with the control group in both the muscle of the pylorus ($p = 0.310$) and in the ganglia ($p = 0.149$), but the differences were not statistically significant.

Examples of observed immunohistochemistry are shown in Figures 1–4.

Discussion

The mammalian TRP superfamily of ion channels consists of 28 cation selective ion channels encoded by 28 genes [5]. The TRP channels are widely expressed calcium-permeable channels with diverse functions and activated by a wide variety of stimuli [6]. They are grouped into six subfamilies according to their amino acid sequence homology (TRPC, TRPV, TRPM, TRPA, TRPP and TRPML) [5]. The TRPC cation channel family has seven members (*TRPC1–7*) with widespread

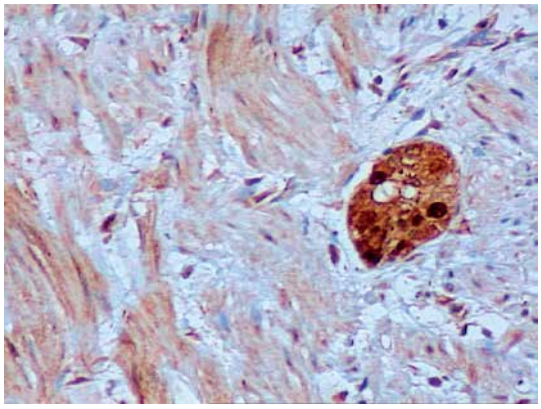

Figure 1. Presence of TRPC5 expression in both muscle and ganglia. The intensity of the reaction was estimated to be 2 points in muscle and 3 points in ganglia according to the adopted scale

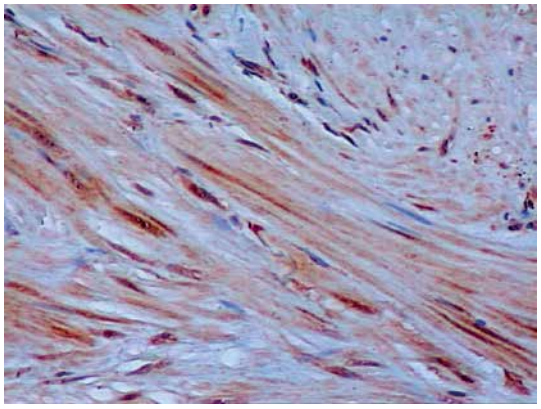

Figure 2. TRPC5 expression in muscle. The intensity of the reaction was estimated to be 3 points according to the adopted scale

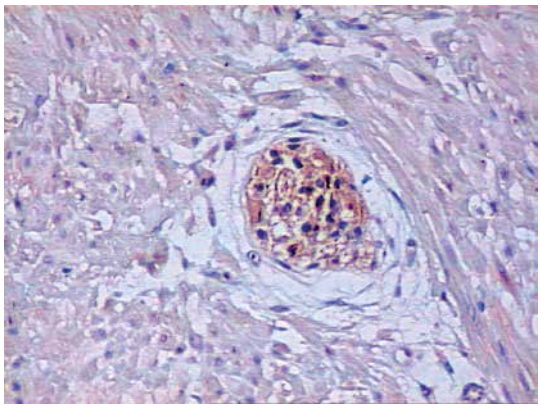

Figure 3. Presence of expression of TRPC6 in ganglia and its absence in muscle. The intensity of the reaction was estimated to be 2 points according to the adopted scale

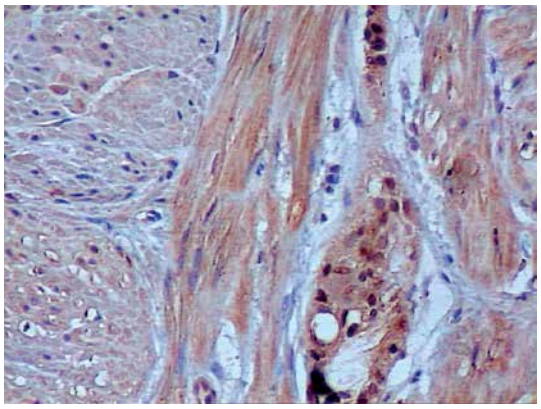

Figure 4. Presence of TRPC6 expression in both muscle and ganglia. The intensity of the reaction was estimated to be 2 points in muscle and ganglia according to the adopted scale

expression, including in smooth muscle cells [7]. They have a variety of functions, and their dysfunction has been implicated in hypertrophic phenotypes. Both *TRPC5* and *TRPC6* have a potential role in smooth muscle control and hypertrophy [5].

TRPC6 has developmentally specific expression in the gut of zebrafish. TRPC6 is thought to contribute to the stability, contractility and elasticity of the zebrafish gut [8]. Furthermore, *TRPC6* is purported to be mechanosensitive [9]. According to Everett *et al.* this is relevant to IHPS because the pylorus is responsible for the control of passage of food from the stomach into the gut and as such responds to mechanical stretch stimuli caused by the presence of food in the stomach. The smooth muscle hypertrophy which characterizes IHPS may itself be caused by overstimulation of the pylorus. It is quite plausible that a variant which increased the sensitivity of *TRPC6* to this stimulation could result in hypertrophy of the pyloric musculature [5]. Our study shows a local increase in the expression of TRPC6 in the muscularis propria of the hypertrophic pyloric muscle in children with IHPS. Although this was not a statistically significant difference, the role of this receptor in the pathogenesis of the disease cannot be ruled out. Everett *et al.* concluded that a single nucleotide polymorphism in the promoter region of the TRPC6 gene and a missense variant in exon 4 of the TRPC6 gene may contribute to infantile hypertrophic pyloric stenosis. They reported that TRPC6 is the only TRPC gene analyzed in which plausible functional variants were identified upon re-sequencing and found to be statistically associated [5].

There are numerous studies providing evidence for an important role of TRPC6 in vascular and pulmonary SMC. TRPC6 has been implicated in animal models of vascular hypertension [10]. In addition, mice overexpressing calcineurin in the heart, a model of cardiac hypertrophy, demonstrate enhanced TRPC6 expression that correlated with the extent of hypertrophy [11]. Furthermore, TRPC6 expression is up-regulated by hypoxia in pulmonary artery smooth muscle, and these channels have been implicated in hypoxia-induced hypertension [12, 13]. TRPC6 expression is enhanced

in pulmonary artery smooth muscle from patients with idiopathic pulmonary arterial hypertension [14]. Overexpression of TRPC6 is implicated in increased pulmonary smooth-muscle proliferation, and it is possible that there might be a similar effect on the smooth muscle cells of the pylorus [15]. Interestingly, a case of co-existing pulmonary hypertension and hypertrophic pyloric stenosis has recently been reported [16]. The authors speculate that this phenotype could be due to deficient NO synthesis, but *TRPC6* has also been reported to play a role in pulmonary hypertension, indicating that it could also be due to *TRPC6* dysfunction [16].

TRPC5 is predominantly expressed in the brain, but it is also detected in smooth muscle cells and in the stomach, where a role in gastrointestinal tract muscle contraction has been proposed [7, 17]. There is evidence that TRPC5 might be involved in cardiac muscle hypertrophy [18], and it has been shown to be activated by nitric oxide [19], which is important in smooth muscle relaxation in the gastrointestinal tract. Our studies have shown statistically significantly greater expression of TRPC5 in the study group compared with the control group in both the muscle of the pylorus and in the ganglia. In contrast, Everett *et al.* did not provide compelling evidence for *TRPC5* being the disease gene for IHPS; but its relevance on the other hand cannot be definitively excluded, because they did not re-sequence the entire genomic region [5].

In conclusion, our study has provided some suggestive evidence for the role of TRPC in physiological and pathophysiological processes of smooth muscle cell. The analysis of these two candidates for pathogenesis of IHPS produces the best evidence of a role for TRPC5. The pathogenesis of IHPS is a complicated process that is not fully understood and requires further multi-center studies. These works will help explain the etiology of IHPS and will also clarify the biological functions of TRPC, a fascinating family of proteins.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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