Physiol. Res. 52: 275-284, 2003

MINIREVIEW

The Pacemaker Activity of Interstitial Cells of Cajal and Gastric Electrical Activity

P. ČAMBOROVÁ, P. HUBKA[1], I. ŠULKOVÁ, I. HULÍN

Department of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic, [1]Third Department of Physiology, JW Goethe University, Frankfurt am Main, Germany

Received January 16, 2002
Accepted July 15, 2002

Summary

Interstitial cells of Cajal (ICC) are the pacemaker cells in the gut. They have special properties that make them unique in their ability to generate and propagate slow waves in gastrointestinal muscles. The electrical slow wave activity determines the characteristic frequency of phasic contractions of the stomach, intestine and colon. Slow waves also determine the direction and velocity of propagation of peristaltic activity, in concert with the enteric nervous system. Characterization of receptors and ion channels in the ICC membrane is under way, and manipulation of slow wave activity markedly alters the movement of contents through the gut. Gastric myoelectrical slow wave activity produced by pacemaker cells (ICC) can be reflected by electrogastrography (EGG). Electrogastrography is a perspective non-invasive method that can detect gastric dysrhythmias associated with symptoms of nausea or delayed gastric emptying.

Key words

Interstitial cells of Cajal • Gastric electrical activity • Pacemaker of the stomach • Kit-receptor • Electrogastrography

Introduction

In 1911, Santiago Ramon y Cajal described a network of cells in gastrointestinal tissues and called them the "primitive neurons". Cajal thought that these stellate cells with long branched processes are a special type of neurons – the interstitial neurons. He characterized them as accessory components that modify the smooth muscle contraction and are themselves the subject of nervous system regulation (Cajal 1893, 1911). Thuneberg (1982) stated that only a subset of cells identified by Cajal were associated with smooth muscle. These cells were qualified as "interstitial cells of Cajal" (ICC). With the development of transmission electron microscopy an increased number of publications dealing with morphology and physiology of Cajal's cells appeared. There are some structural variations between species and differences due to their different location in the gut, but the ultrastructure of ICC can be characterized by the following features: 1) numerous large mitochondria present in processes, 2) large bundles of intermediate filaments (vimentin), 3) absence of thick myosin filaments, 4) presence of surface caveolae, 5) differently developed basement membrane, 6) synapse-like contacts between the ICC and tertiary nerve bundles, 7) well developed smooth and rough endoplasmic reticulum, and 8) close apposition of gap junctions with smooth muscle cells (Huizinga *et al.* 1997).

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

Although the interstitial cells of Cajal were identified at the end of the 19th century, their developmental origin and functions remain uncertain. The discovery that ICCs express the protooncogene c-kit, and that its gene product is the tyrosine kinase receptor Kit, opened new possibilities for studying ICCs (Maeda *et al*. 1992, Ward *et al*. 1994). The interstitial cells of Cajal form a three-dimensional network of cells placed between and in the smooth muscle layers. Isolated ICCs are electrically active and create ion currents for pacemaker function. They also have electrical slow-wave activity, which determines the phasic contraction frequency in the stomach, small intestine and colon. Sanders (1996, 1999) uses in his reviews following classification of ICCs: *IC-MY* are cells in the myenteric region of the stomach, small intestine and colon; *IC-SM* (submucosal ICCs) are cells located along the submucosal surface of the circular smooth muscle layer in the colon; *IC-DMP* are Cajal's cells along the deep muscular plexus in the small intestine; *IC-IM* are the intramuscular Cajal's cells in esophagus, stomach and colon (Fig. 1).

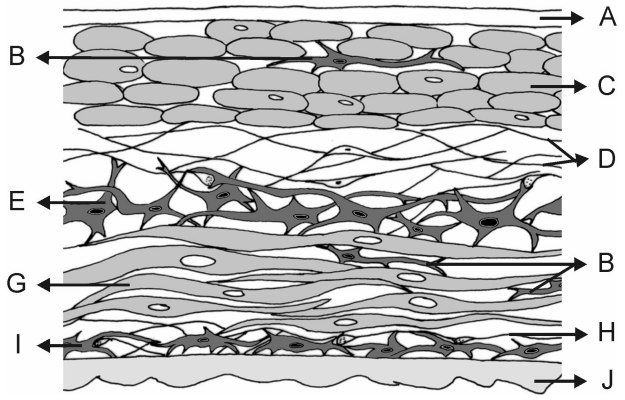

Fig. 1. *Scheme of the gut wall anatomical configuration. Detailed organization and the presence of ICC subclasses varies along the gut. A) serosa; B) intramuscular ICC (IC-IM) present in the esophagus, stomach and colon; C) longitudinal muscle layer; D) plexus myentericus Auerbachi; E) myenteric ICC (IC-MY) mainly in the stomach, small intestine and colon; G) circular muscle layer; H) plexus submucosus Meissneri; I) submucosal ICC (IC-SM) in the colon; J) submucosa.*

The results of many studies and detailed observations in the GIT have served as the basis for this classification. The individual subpopulations play important, but varying roles in the motility of GIT. Assuming that the ICCs are unique for the gut and have properties which are not present in neurons and smooth muscle cells suggests that they could be an ideal target for pharmacological intervention in motility disorders (Huizinga *et al*. 1997).

ICC and the peristalsis in GIT

In the 1970s, information about the pacemaker regions in the GIT began to appear gradually. Electrophysiological studies with isolated portions of intestinal wall confirmed that the circular muscle layer, if separated from the longitudinal muscle layer, ceases to generate slow waves, and that the longitudinal layer remains electrically active only at some sites. Suzuki *et al.* (1986) confirmed that the slow waves can be observed only at the sites where the presence of Cajal's cells has been demonstrated by *ex post* staining; thus, ICCs can be considered as the initiators of slow waves. The slow waves represent the pacemaker activity of GIT contractility and are ready to transform the excitatory neural stimulus into coordinated peristaltic movements for 24 h daily. Measurements performed on the cat, dog, and rabbit small intestine, even on human jejunum (Hara *et al*. 1986) have demonstrated that the myenteric pacemaker region is the dominant source of slow waves in the small intestine. The dominant pacemaker in the stomach is localized in the proximal portion of its body, in the myenteric region. Each part of the stomach, with the exception of fundus, includes the pacemaker mechanism, a dense network of IC-MY, with an increasing number of these cells towards the antrum (Faussone-Pelegrini *et al*. 1989). The pacemaker in the colon resides at the submucosal surface of the circular muscle layer (Smith *et al*. 1987a,b). There is, however, an additional pacemaker in the colon, which is located along the septa separating the individual circular muscle bundles. IC-SM cells are present in proximal colon only whereas they are completely absent in its distal portion.

The myenteric pacemaker generates small membrane potential oscillations, spreading into both circular and longitudinal muscle layers. Current investigations have demonstrated, that only the specific networks of ICCs exhibit pacemaker activity (i.e. those coupled with plexus submucosus Meissneri in the colon or plexus myentericus Auerbachi in the stomach and small intestine). Remaining ICCs (those connected with the plexus muscularis profundus in the small intestine or those coupled with colonic plexus myentericus

Auerbachi) do not perform this function. Nevertheless, some organs without slow wave activity (e.g. the esophagus) are equipped with an ICC network in the circular muscle layer. Specific function of these ICCs is not yet known, but they could be a link between the enteral nerves and muscle cells.

It is difficult to determine what is the role of ICCs in the spreading of the slow waves. As the ICC network becomes impaired, slow waves are abolished and their spreading is not measurable. To compare the propagation of slow waves with that of fast action potentials is not convenient, because these processes have different threshold potentials. The time needed for spreading of slow and fast activation differs in its dependence on muscle syncytium resistance (Publicover and Sanders 1989). Intracellular measurements have revealed that the slow waves spread along the long axis and around the circumference of the colon so that the ICCs represent a basal pathway of activation in the colon (Sanders *et al*. 1990). Electric activity of the circular and longitudinal muscle layers of gastrointestinal tract organs is synchronized. Slow waves of circular and longitudinal muscle cells are in phase, indicating that a link must exist between these two layers. This connection is mediated by IC-MY, forming gap junctions with muscular cells in the small intestine and colon.

Ion channels in ICCs

Isolated IC-SM are spontaneously active at resting potential values (measured by means of the patch clamp technique), while muscle cells remain at the same potential values. In experimental conditions the influx ion channels in interstitial Cajal's cells are activated with lower potential values (more negative) than the channels in muscle cells in the same region. Such a relation between the current and potential is typical of low-threshold Ca^{2+} channels (T-type of Ca^{2+} channels). This is the ideal type of channels for pacemaker activity, and it can also be found in other pacemaker cells in the organism. The low-threshold Ca^{2+} channels are not inactivated by low depolarization. Close to the resting potential of pacemaker ICCs these channels are able to create small influx currents, which very probably are responsible for IC-SM depolarization towards the threshold potential. Activation of pacemaker currents depends upon the periodic release of Ca^{2+} from IP_3 receptor-operated stores. Mitochondrial Ca^{2+} uptake is linked in a hitherto unknown way to the activation of pacemaker currents. The uptake and periodic release of Ca^{2+} from IP_3 receptor-operated stores appears to be the main oscillatory process responsible for GI autorhythmicity (Ward *et al*. 2000). Because of the high total resistance of IC-SM (at least 1 G Ω) a very feeble current is needed to induce significant polarization. With progressing depolarization, the L-type calcium channels (the second source of Ca^{2+} influx) are progressively activated. These L-type calcium channels are important for electrical activity transmission and a threshold potential increase in smooth muscle cells (Sanders 1996). Some authors suggested the occurrence of some ionic flow direction rectifier in ICCs, important for pacemaker activity, as in the heart. Non-specific Na^+ or K^+ channels activated by hyperpolarization perform the function of a rectifier in myocytes. Ca^{2+} channels in IC-SM, initiate the pacemaker activity and the voltage-gated K^+ channel terminates the cycle by depolarization. It is therefore also necessary to investigate the voltage-gated channels in other types of ICCs (Langton *et al*. 1989, Lee and Sanders 1993).

Smooth muscle cells and rhythmicity

From a general point of view, isolated smooth muscle depolarization is mediated by L-type Ca^{2+} channel activation and this depolarization activates voltage-gated K^+ channels. However, the spontaneous activity is stopped at a potential value of approximately –60 mV, soon after the isolation from the ICC network (Sanders 1996). The phasic electrical activity in GIT muscles is composed of pacemaker ICC activity and the response of the muscles. Ionic channels in smooth muscle cells are very important for the rhythmicity in GIT, because it is sufficient if pacemaker cells depolarize the adjacent cells by only 10-30 mV, in order to attain the threshold potential value. Thus, the excitability of muscle cells facilitates the entry and spreading of pacemaker activity. The activation of voltage-gated ion channels in muscle cells is, in fact, the main pathway of calcium entry into the cell, and is important for excitation-contraction coupling (Ozaki *et al*. 1991).

The current intensity produced by Cajal's cells is not high enough to depolarize the muscle syncytium. The fact that ICCs are electrically coupled facilitates the current potentiation in the ICC network. The current intensity needed for pacemaker function is reduced when the spreading is divided into steps, probably due to differences in the threshold potential of pacemaker and muscle cells (Publicover 1995). Coupling of ICCs with muscle cells is a further critical factor for impulse

propagation. Publicover (1995) suggests the possibility that the small connexin clumps forming gap junctions could be responsible for the coupling. In contrast to this suggestion, theoretical calculations based on connexin properties in gap junctions indicate that about 20 open channels between ICC and muscle cell would be needed to create a sufficiently large current influx for muscle cell depolarization. The apparatus necessary for rhythmic activity need not occur exclusively in ICCs, but partially also in smooth muscle cells. In fact, the combination of ICCs and the muscle complex can create rhythmicity *in situ*.

The gap junctions are composed of connexin protein complexes (hemi-channels), forming intercellular channels between the coupled cells. Ions and molecules with low molecular weight (less than 1 kDa) can pass through channels of gap junctions; this makes the transfer of electrical current or molecules possible. Sixteen proteins are coded by genes for gap junctions (Sanders *et al*. 1999). Connexin 43 (Cx43) is the most frequently present connexin in GIT muscles, but other isoforms of proteins can also be of importance. There are significant differences in connexin expression. For example, Cx43 was found in muscles of the stomach and small intestine, but not in the colon. The data about different Cx43 expression in individual ICC classes can be explained by the fact that the individual classes of ICCs are coupled with surrounding cells at various levels or that other isoforms of connexins can participate in the coupling (Li *et al*. 1993, Seki *et al*. 1998). Different expression and changes in individual connexin isoforms can play an important role in the development, plasticity and functions of ICCs. The presence of connexin 43 in ICC couplings was demonstrated in the intestinal wall by immunolocalization. A comparison of findings in the healthy intestine with those in the intestine affected by Hirschprung's disease revealed that Cx43 is not present in the aganglionic portion of affected intestine and is considerably reduced in its intermediate part. Motility disturbances in Hirschprung's disease can therefore be explained by the lack of Cx43 (Nemeth *et al*. 1999).

Our knowledge of pacemaker activity regulation is very limited. The slow wave frequency is often considered to be an indicator of interventions into the mechanism responsible for pacemaker activity in intact muscles. At present we do not know, whether the low-threshold Ca^{2+} channels in ICCs are regulated by cAMP-dependent protein kinase. Nevertheless, similar channels in the myocardium do not seem to be governed by this factor (Tytgat *et al*. 1988, Huizinga *et al.* 1991). It would be necessary to investigate the influence of the second messenger pathway upon the frequency changes. Only then can the target site of these regulation factors be determined.

ICCs and neurotransmission

Pharmacological studies should take into consideration the functional location of ICCs between the non-adrenergic as well as non-cholinergic nerves and smooth muscle cells, and the mechanical and electrical activities of muscle layer, which reflect the properties of ICCs. It is supposed that ICCs play an important role in enteral inhibitory neurotransmission because of the close gap junctions between nerve varicosities and ICCs. There is another possibility that the pathway of neurotransmission runs in parallel between nerve-muscle cells and the ICC-muscle cells (Daniel and Posey-Daniel 1984).

Experiments were performed with isolated cultured ICCs, using optic measurements of Ca^{2+} transit in order to monitor the ICC activity (Publicover *et al*. 1992). During these measurements ICCs responded to many enteral transmitters, e.g. acetylcholine, NO, VIP, ATP, NK1, and substance P. The transmitters elicited measurable biochemical responses in subjunctional cells. Certain effects can be monitored by means of antibodies against products of responses to other messengers. For example nitric oxide (NO) enhances the guanylyl-cyclase activity, which increases cGMP concentration in GIT muscles (Ward *et al*. 1992). The use of antibodies against cGMP makes it possible to evaluate the NO effect (de Vente *et al*. 1989).

Other authors demonstrated the expression of heme oxygenase (HO) in ICCs of the small intestine (Ny *et al*. 1997, Miller *et al*. 1998) and the expression of NO synthase (NOS) in IC-SM of the colon and pyloric sphincter (Ward *et al*. 1998, Xue *et al*. 1994). NOS and HO serve as a source for NO and CO production.

Nitric oxide as an inhibitory neurotransmitter exerts a different influence upon the Ca^{2+} concentration. In addition, the ICCs produce NO in response to increased Ca^{2+} intracellular concentration. Thereafter, NO released from enteral inhibitory neurons sends signals through Ca^{2+} to Cajal's cells about the increase in NO concentration; thus, NOS participates in the potentiation of inhibitory signals (Xue *et al*. 1993). Carbon monoxide (CO) is considered to be the second gaseous transmitter. CO is produced as byproduct of hemoglobin metabolism mediated by heme oxygenase together with cytochrome-

P450-reductase and biliverdin-reductase (Maines 1997). The isoform of HO-2 mediates the production of CO in neurons of the enteral nerve plexuses, smooth muscle cells and Cajal's cells (Miller *et al*. 1998). The exogenous CO leads to an increase in cGMP concentration, activation of K^+ channels, membrane hyperpolarization, and thus to GIT smooth muscle relaxation (Faruggia *et al*. 1998). Experimental studies with mice deficient in HO-2 and nNOS demonstrated that HO-2 and NOS determine the resting membrane potential in smooth muscle cells of the small intestine (Xue *et al*. 2000).

Receptors for a subunit of cholera toxin were found in small intestine ICCs of mice and rats, indicating that a glycophospholipidic or gangliosidic GM1 surface cellular receptor is present. The potential function of this receptor is not yet known (Anderson and Edwards 1993).

It was observed during investigation of stromal gastrointestinal tumors that CD34, originally an endothelial cellular marker, is localized together with the Kit receptor at ICCs. This marker is a surface antigen useful in ICCs isolation from other cells in tunica muscularis and also involves CD34. All the above mentioned markers occur in ICCs, but only Kit is specific for ICCs (Vanderwinden *et al*. 1998).

The gene product of protooncogene Kit, a tyrosine-kinase receptor, is structurally and functionally similar to the receptor for platelet-derived growth factor (PDGF). The c-KIT is a protein with a molecular weight of 160 kDa and has its transmembrane and cytoplasmatic domains. The extracellular part of the c-KIT receptor includes receptor for the Steel factor (otherwise the stem cell factor) which is a natural ligand for c-Kit receptors. The cytoplasmatic portion exerts the tyrosine-kinase activity and includes binding sites for cytoplasmic signal proteins. Binding of the Steel factor activates the tyrosine-kinase activity and leads to autophosphorylation of c-Kit receptor tyrosine residues, binding of IP3-kinase and phospholipase C, and to activation of the phosphorylation cascade which has influence upon the growth, differentiation and migration of cells. Triggering of cascades through the c-Kit receptor in ICCs seems to be essential for the ICC phenotype, development and for electrical rhythmicity (Rottapel *et al*. 1991).

Embryonic origin of ICCs

It has been demonstrated in animal experiments that Kit-positive ICC precursors are of mesodermal origin (Torihashi *et al*. 1997). Investigation of the fetal small intestine revealed that ICCs coupled with the plexus myentericus Auerbachi contain the tyrosine-kinase receptor from the beginning of the 13th week of gestation. From about the 17th week the Kit-immunoreactive ICCs form a layer surrounding the myenteric ganglia and nerve fascicles (Wester *et al*. 1999). Kit is important for normal postnatal development, for maintenance of the ICC phenotype and for division of the original line of mesenchymal precursors during the development towards the ICCs or smooth muscle cells (Sanders *et al*. 1999).

The IC-MY network in the small intestine seems to be completely developed immediately after birth, but its ultrastructure is not yet mature as shown by the electron microscope. These immature cells ($IC\text{-}MY_{blasts}$) contain numerous ribosomes, well-developed Golgi's complexes and numerous mitochondria, but do not contain the membranous caveollae and their contact with muscle cells occurs only rarely. Nevertheless, in 10 days after birth they exhibit all signs of maturity (Sanders 1996). In studies concerning the Kit-receptor function, neutralizing Kit antibodies-ACK2 are used. Mastocytes exhibit the Kit positivity as well, but are absent in the intestinal muscle layer (Maeda *et al*. 1992). When neutralizing antibodies against the Kit-receptor are applied soon after birth, extinction of Kit-immunoreactivity and abnormal contractility appear. To $IC\text{-}MY_{blasts}$ similar cells can be found microscopically, however, the population of the new cells is dominant. ICC differentiated towards the phenotype of smooth muscle cells are probably involved. Thus, the presence and functional capacity of the Kit-receptor are inevitable for the formation and maintenance of the ICC phenotype (Kluppel *et al*. 1998). We do not know at present, whether this plasticity in phenotype also progresses in adulthood, or what happens with the ICCs during aging. The Kit-receptor function is therefore important for the development of ICC network. In white mice, the locus for the white color (W) is allelic with protooncogene c-kit. Mutations in locus W induce loss or decline in tyrosine-kinase activity Kit. Mutations which eliminate completely the c-Kit protein are incompatible with life, and the homozygotes die *in utero*. In heterozygotes, W/W^v point mutations occur, not disturbing the tyrosine-kinase activity completely. Nevertheless, the number of ICCs drops and the integrity of cellular networks is impaired. In W/W^v mutants, it was impossible to measure the slow wave activity intracelullarly (Nocka *et al*. 1990, Zsebo *et al*. 1990).

The Steel factor stimulates the tyrosine-kinase activity of Kit. At present the source of the Steel factor is

not clear. In mice, the Steel factor is produced by enteral neurons; ICCs develop independently of neurons. In the human pylorus and colon, Steel factor immunoreactivity was found in unidentified submucosal cells, but not in neurons. It is not precisely known whether Kit receptor stimulation by the Steel factor passes *via* "cell-to-cell" interactions or if a soluble form of this factor is involved. The studies on mutants indicate that only the IC-MY class is affected in mutants in which a considerably reduced body weight is caused by abnormal GIT motility. The fact that these animals reach adulthood suggests that GIT motility and nutrient resorption must persist to a certain extent. The slow wave activity loss can result in disorders of segmentation and speed of chymus shifting. Because the muscles can generate Ca^{2+}-dependent action potentials and contract, and the enteral nerves are functioning, there is a possibility, that peristalsis and propulsion remain intact. It means that the neuromuscular apparatus probably has the ability to compensate the ICCs loss and provide the normal electrical activity (Sanders 1996). Genetic models with selective impairment of various ICCs populations can be useful in understanding the contribution of ICCs to resting potential of smooth muscle syncytium/ICCs.

The relation between the ICCs and GIT in clinical pathophysiology is a new domain of investigation, because the etiology is not known in many motility disorders. It is necessary to strictly diagnose the patient with a pacemaker activity disturbance. The abnormal slow wave frequency (bradygastria or tachygastria) as well as the absence of slow wave activity can be associated with abnormal transit and gastroparesis (Chen *et al*. 1996). The progress in treatment is slow due to inadequate understanding of the mechanism of the abnormal frequencies of slow waves and due to a lack of knowledge about how the drugs affect ICCs. It has been demonstrated that erythromycin restores the normal gastric rhythm and improves gastric function in patients with postoperative gastroparesis. Experiments with pacemaker implantation were partially successful and this can be a promising field of investigation because the identification of slow wave origin and regulation can result in rational changes in the treatment of gastroparesis (Huizinga *et al*. 1997).

Electrogastrography

Electrogastrography (EGG) is a non-invasive method for recording the gastric myoelectric activity, which regulates the contractility, by means of surface electrodes. The first electrogastric recordings were performed by Alvarez *et al*. (1922). Further progress in this domain was very slow, but it is still ongoing. The validity of the surface EGG is dependent on the quality of the technical equipment. General conditions of EGG are not yet standardized. Electrical activity of the stomach can be recorded from the serous gastric surface, from mucosa (invasive methods) or by non-invasive methods using the cutaneous electrodes. Many studies were performed, comparing cutaneous measurements with those utilizing implanted serous electrodes. The quantitative comparison of EGG signal variability showed a 94 % sensitivity and 79 % specificity (Mintchev and Bowes 1998). Diseases manifesting themselves by disorders of electric activity, frequency and rhythm can be detected by means of EGG with acceptable precision, provided that the whole organism electrical connection is preserved. Using current methods the EGG is of sinusoidal shape with a certain periodicity determined by slow-wave frequency (gastric electrical control activity: ECA). Previous studies (Chen *et al*. 1993) showed that the dominant EGG frequency determines the maximal frequency of stomach contractions. The rhythmic activity 2.4-3.7 cpm is considered to be the norm. Bradygastria (0.2-2.4 cpm), and tachygastria (3.7-9.0 cpm) together with arrhythmias (tachyarrhythmia and bradyarrhythmia) reflect the abnormality of slow waves. These gastric dysrhythmias were observed in many clinical conditions, using intraluminal serous measurements or the EGG. Gastric dysrhythmias are components of many syndromes associated with motility disorders and nausea. Eradication of dysrhythmia and restoration of normal electric activity is accompanied by the improvement of symptoms, e.g. tachygastria and gastroparesis caused by mesenteric ischemia are completely reversible following mesenteric revascularization (Liberski *et al*. 1990). Gastroparesis in gastric outlet obstruction (due to ulcus) or in duodenal obstruction contrasts with above mentioned findings associated with normal (3 cpm) electric activity (Koch and Stern 1994). It is therefore probable that the gastroparesis in these cases is not a consequence of neural or muscular dysfunction, but is of mechanical origin. Exact patterns by which it would be possible to diagnose accurately various diseases are not yet clearly defined. Comparison of gastric electrical activity and motility in patients with dyspepsia during gastroinstestinal or extraintestinal diseases have led to the conclusion that tachygastria is always present in patients with dyspepsia (Pfaffenbach *et al*. 1998). The electrogastrogram is abnormal in 93 % of patients with functional dyspepsia and in various diseases accompanied with dyspepsia (Leahy *et al*. 1999).

The unique properties of EGG make it an attractive tool in the diagnosis of gastrointestinal diseases in pediatric patients. Relatively few studies have been performed in children, in whom the range of functional disorders consists of unique conditions. Recent technological and clinical advances have contributed to the improved survival of premature and neurologically impaired infants. The fact that they have severe disorders in the motility of the gastrointestinal tract necessitates a systemic consideration. Further trials in the pediatric population are mandatory and will probably improve our understanding of additional diseases and processes (Levanon and Chen 1998). Moreover, electrogastrography may provide a therapeutic approach. Experiments with gastric pacing (electrical stimulation) have been conducted. Promising results have been shown in the improvement of gastric emptying and symptoms in patients with gastroparesis refractory to pharmacological treatment (McCallum *et al.* 1998). Further studies are required to determine the contribution of EGG.

Conclusion

The present review incorporates the interstitial cells of Cajal (ICCs) into control mechanisms of the gut electrical and motor activity. These cells generate slow wave activity spreading to involve the whole musculature. In addition, ICCs may act as intermediaries between enteric nerves and smooth muscle cells. Because ICCs have unique features that are specific for the gut musculature, they are an ideal target for pharmacological action. Possible sites of action on ICCs, receptors and ion channels, are being characterized. One of the most exciting aspects of ICC biology is the growing possibility that loss or defects of ICC might contribute to human motility disorders. Motor disorders of the gastrointestinal tract are common and costly, and current pharmacological approaches are unsatisfactory. Considerably more studies are needed to evaluate the susceptibility of ICCs to a variety of potentially noxious environmental and/or disease factors. New alternatives for treatment might be possible by changing abnormal motor patterns using the control system of the musculature, which includes pacemaker activity and neuromodulation by interstitial cells of Cajal.

Acknowledgements

This work was supported by Grant Agency of Slovak Republic, grant Vega No. 1/7537/20.

References

ALVAREZ WC: The electrogastrogram and what it shows. *JAMA* **78**: 1116-1119, 1922.

ANDERSON CR, EDWARDS SL: Subunit b of cholera toxin labels interstitial cells of Cajal in the gut of rat and mouse. *Histochemistry* **100**: 457-64, 1993.

CAJAL SR: Sur les ganglions et plexus nerveux de l´intestin. *C R Soc Biol* **45**: 217-223, 1893.

CAJAL SR: *Histologie du Systéme Nerveux de l´Homme et des Vertébrés*. Maloine, Paris, 1911, pp 891-945.

CHEN JD, RICHARDS RD, MCCALLUM RW: Frequency components of the electrogastrogram and their correlations with gastrointestinal motility. *Med Biol Eng Comput* **31**: 60-67, 1993.

CHEN JD, LIN Z, PAN J, MCCALLUM RW: Abnormal gastric myoelectrical activity and delayed gastric emptying in patients with symptoms suggestive of gastroparesis. *Dig Dis Sci* **41**: 1538-1545, 1996.

CONNOR JA, KREULEN D, PROSSER CL, WEIGEL R: Interaction between longitudinal and circular muscle in intestine of cat. *J Physiol Lond* **273**: 665-689, 1977.

DANIEL EE, POSEY-DANIEL V: Neuromuscular structures in opossum esophagus: role of interstitial cells of Cajal. *Am J Physiol* **246**: G305-G315, 1984.

DE VENTE J, SCHIPPER J, STEINBUSCH HWM: Formaldehyde fixation of cGMP in distinct cellular pools and their recognition by different cGMP-antisera: an immunocytochemical study into the problem of serum specificity. *Histochem J* **91**: 401-412, 1989.

FARRUGIA G, MILLER SM, RICH A, LIU X, MAINES MD, RAE JL, SZURSZEWSKI JH: Distribution of heme oxygenase and effects of exogenous carbon monoxide in canine jejunum. *Am J Physiol* **274**: G350-G358, 1998.

FAUSSONE-PELLEGRINI MS, PANTALONE D, CORTESINI C: An ultrastructural study of interstitial cells of Cajal of the human stomach. *J Submicrosc Cytol Pathol* **21**: 439-460, 1989.

HARA Y, KUBOTA M, SZURSZEWSKI JH: Electrophysiology of smooth muscle of the small intestine of some mammals. *J Physiol Lond* **372**: 501-520, 1986.

HUIZINGA JD, THUNEBERG L, VANDERWINDEN J-M, RUMESSEN JJ: Interstitial cells of Cajal as targets of pharmacological intervention in gastrointestinal motor disorders. *Trends Pharmacol Sci* **18**: 393-403, 1997.

HUIZINGA JD, FARRAWAY L, DEN HERTOG A: Effect of voltage and cyclic AMP on frequency of slow wave-type action potentials in canine colon smooth muscle. *J Physiol Lond* **442**: 31-45, 1991.

KLUPPEL M, HUIZINGA JD, MALYSZ J, BERNSTEIN A: Developmental origin and Kit-dependent development of the interstitial cells of Cajal in the mammalian small intestine. *Dev Dyn* **211**: 60-71, 1998.

KOCH KL, STERN RM: Nausea and vomiting and gastric dysrhytmias. In: *Electrogastrography: Principles and Applications*. JZ CHEN, RW MCCALLUM (eds), Raven Press, New York, 1994, pp 309-330.

LANGTON P, WARD SM, CARL A, NORELL MA, SANDERS KM: Spontaneous electrical activity of interstitial cells of Cajal isolated from canine proximal colon. *Proc Natl Acad Sci USA* **86**: 7280-7284, 1989.

LEAHY A, BESHERDAS K, CLAYMAN C, MASON I, EPSTEIN O: Abnormalities of the electrogastrogram in functional gastrointestinal disorders. *Am J Gastroenterol* **94**: 1023-1028, 1999.

LEE HK, SANDERS KM: Comparison of ionic currents from interstitial cells and smooth muscle cells of canine colon. *J Physiol Lond* **460**: 135-152, 1993.

LEVANON D, CHEN JZ Electrogastrography: its role in managing gastric disorders. *J Pediatr Gastroenterol Nutrition* **27**: 431-443, 1998.

LI Z, ZHOU Z, DANIEL EE: Expression of gap junction connexin 43 and connexin mRNA in different regional tissues of intestine in dog. *Am J Physiol* **265**: G911-G916, 1993.

LIBERSKI SM, KOCH KL, ATNIP RG, STERN RM: Ischemic gastroparesis: resolution after revascularization. *Gastroenterology* **99**: 252-257, 1990.

MAEDA H, YAMAGATA A, NISHIKAWA S, YOSHINAGA K, KOBAYASHI S, NISHI K, NISHIKAWA S: Requirement of c-kit for development of interstitial pacemaker system. *Development* **116**: 369-375, 1992.

MAINES MD: The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* **37**: 517-554, 1997.

MCCALLUM RW, CHEN JD, LIN Z, SCHRIMER BD, WILLIAMS RD, ROSS RA: Gastric pacing improves emptying and symptoms in patients with gastroparesis. *Gastroenterology* **114**: 456-461, 1998.

MILLER SM, FARRUGIA G, SCHMALZ PF, ERMILOV RG, MAINES MD, SZURSZEWSKI JH: Heme oxygenase 2 is present in interstitial cell networks of mouse small intestine. *Gastroenterology* **114**: 239-244, 1998.

MINTCHEV MP, BOWES KL: Comparative quantification of gastric electrical activity and electrogastrograms. *Med Biol Eng Comput* **36**: 96-100, 1998.

NEMETH L, MADDUR S, PURI P: Immunolocalization of gap junction protein, connexin-43, in the interstitial cells of Cajal of the normal and Hirschprung´s disease bowel. *Pediatrics* **104**: 766-767, 1999.

NOCKA K, TAN JC, CHIU E, CHU TY, RAY P, TRACTMAN P, BESMER P: Molecular bases of dominant negative and loss of function mutations at the murine c-kit/white spotting locus: W^{37} , W^{v} , W^{41} and W. *EMBO J* **9**: 1805-1813, 1990.

NY L, ALM P, LARSSON B, ANDERSSON KE: Morphological relations between haem oxygenases, NO-synthase and VIP in the canine and feline gastrointestinal tracts. *J Auton Nerv Syst* **65**: 49-56, 1997.

OZAKI H, BLONDFIELD DP, STEVENS RJ, PUBLICOVER NG, SANDERS KM: Simultaneous measurement of membrane potential, cytosolic calcium and muscle tension in smooth muscle tissue. *Am J Physiol* **260**: C917-C925, 1991.

PFAFFENBACH B, ADAMEK RJ, LUX G: The value of electrogastrography for the diagnosis of gastroenterologic function. *Dtsch Med Wochenschr* **123**: 855-860, 1998.

PUBLICOVER NG: Generation and propagation of rhytmicity in gastrointestinal smooth muscle. In: *Pacemaker Activity and Intercelular Communication.* JD HUIZINGA (ed), CRC Press, Boca Raton, FL, 1995, pp 175-192.

PUBLICOVER NG, SANDERS KM: Are relaxation oscillators an appropriate model of gastrointestinal electrical activity? *Am J Physiol* **256**: G265-G274, 1989.

PUBLICOVER NG, HOROWITZ NN, SANDERS KM: Calcium oscillations in freshly dispersed and cultured interstitial cells from canine colon. *Am J Physiol* **262**: C589-C597, 1992.

ROTTAPEL R, REEDIJK M, WILLIAMS DE, LYMAN SD, ANDERSON DM, PAWSON T, BERNSTEIN A: The Steel/W transduction pathway: kit autophosphorylation and its association with a unique subset of cytoplasmic signaling proteins is induced by Steel factor. *Mol Cell Biol* **11**: 3043-3051, 1991.

SANDERS KM: A case of interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. *Gastroenterology* **111**: 492-515, 1996.

SANDERS KM, STEVENS R, BURKE EP, WARD SM: Slow waves actively propagate at submucosal surface of circular layer in canine colon. *Am J Physiol* **259**: G258-G263, 1990.

SANDERS KM, ORDOG T, KOH SD, TORIHASHI S, WARD SM: Development and plasticity of interstitial cells of Cajal. *Neurogastroent Motil* **11**: 311-338, 1999.

SEKI K, ZHOU DS, KOMURO T: Immunohistochemical study of the c-kit expressing cells and connexin 43 in the guinea-pig digestive tract. *J Auton Nerv Syst* **68**: 182-187, 1998.

SMITH TK, REED JB, SANDERS KM: Origin and propagation of electrical slow waves in circular muscle of the canine proximal colon. *Am J Physiol* **252**: C215-C224, 1987a.

SMITH TK, REED JB, SANDERS KM: Interaction of two electrical pacemakers in the muscularis of the canine proximal colon. *Am J Physiol* **252**: C290-299, 1987b.

SUZUKI N, PROSSER CL, DAHMS V: Boundary cells between longitudinal and circular layers: essential for electrical slow waves in cat intestine. *Am J Physiol* **250**: G287-G294, 1986.

THUNEBERG L: Interstitial cells of Cajal: intestinal pacemaker cells? *Adv Anat Embryol Cell Biol* **71**: 1-130, 1982.

TORIHASHI S, WARD SM, SANDERS KM: Development of c-kit positive cells and the onset of electrical rhytmicity in murine small intestine. *Gastroenterology* **112**: 144-155, 1997.

TYTGAT J, NILIUS B, VEREECKE J, CARMELIET E: The T-type Ca channel in guinea-pig ventricular myocytes is insensitive to isoproterenol. *Pfluegers Arch* **411**: 704-706, 1988.

VANDERWINDEN JM, VERSLIP M, GILLARD K, DELAET MH, VANDERHAEGHEN JJ: Distribution of CD34 and Kit immunoreactivity in normal human intestine and colon. *Neurogastroenterol Motil* **10**: 457, 1998.

WARD SM, DALZIEL HH, BRADLEY ME, BUXTON ILO, KEEF K, WESTFALL DP, SANDERS KM: Involvement of cyclic GMP in non-adrenergic, non-cholinergic inhibitory neurotransmission in dog proximal colon. *Br J Pharmacol* **107**: 1075-1082, 1992.

WARD SM, BURNS AJ, TORIHASHI S, SANDERS KM: Mutation of the proto-oncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine. *J Physiol Lond* **480**: 91-97, 1994.

WARD SM, MORRIS G, REESE L, WARG XY, SANDERS KM: Interstitial cells of Cajal mediate enteric inhibitory neurotransmission in the lower esophageal and pyloric sphincters. *Gastroenterology* **115**: 314-329, 1998.

WARD SM, ORDOG T, KOH SD, BAKER SA, JUN JY, ABERG G, MONAGHAN K, SANDERS KM: Pacemaking in interstitial cells of Cajal depends upon calcium handling by endoplasmic reticulum and mitochondria. *J Physiol Lond* **525**: 355-361, 2000.

WESTER T, ERIKSSON L, OLSSON Y, OLSEN L: Interstitial cells of Cajal in the human fetal small bowel as shown by c-kit immunohistochemistry. *Gut* **44**: 65-71, 1999.

XUE C, WARD SM, SHUTTLEWORTH K, SANDERS KM: Identification of interstitial cells in canine proximal colon using NADH diaphorase histochemistry. *Histochemistry* **99**: 373-384, 1993.

XUE C, POLLOCK J, SCHMIDT HH, WARD SM, SANDERS KM: Expression of nitric oxide synthase immunoreactivity by interstitial cells of the canine proximal colon. *J Auton Nerv Syst* **49**: 1-14, 1994.

XUE L, FARRUGIA G, MILLER SM, FERRIS CD, SNYDER SH, SZURSZEWSKI JH: Carbon monoxide and nitric oxide as coneurotransmitters in the enteric nervous system: evidence from genomic deletion of biosynthetic enzymes. *Proc Nat Acad Sci USA* **97**: 1851-1855, 2000.

ZSEBO KM, WILLIAMS DA, GEISSLER EN, BROUDY VC, MARTIN FH, ATKINS HL, HSU RY, BIRKETT NC, OKINO KH, MURDOCK DC: Stem cell factor encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell* **63**: 213-224, 1990.

Reprint requests

Petra Čamborová, M.D., Department of Pathophysiology, Faculty of Medicine, Commenius University, Sasinkova 4, 81108 Bratislava, Slovak Republic, Fax: 00421 2 59357 601, e-mail: pcambor@email.cz