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Interstitial cells of Cajal: is their role in gastrointestinal function in view of therapeutic perspectives underestimated or exaggerated?

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This manuscript reviews the current views on morphology and function of the distinct subpopulations of interstitial cells of Cajal (ICC) in the digestive tract and their interrelationships with surrounding cells. Three different functions have been postulated so far, i.e. a pacemaker role, a mediator in enteric excitatory and inhibitory neurotransmission and a mechanosensor. Attention will also be paid to the interstitial cells of Cajal and their possible involvement in pathophysiological conditions. Finally, perspectives for interstitial cells of Cajal as targets for therapeutic intervention will be discussed.

key words: interstitial cells of Cajal, pacemaker, neurotransmission, slow wave mechanoreceptor, gut

INTRODUCTION

Discussing the regulation of motility within the gastrointestinal tract implies a good insight into the typical features of the intrinsic nervous system within the gut wall. Several recent reviews [18,20,22] have efficiently dealt with the morphological and functional properties of the distinct subpopulations of enteric neurones within the different gastrointestinal regions. Comprehensive schemes showing the largely independent involvement of this nervous system in secretomotoric and propulsive reflexes have been published. The fact that the regulation of gastrointestinal motility is intimately associated with the modulation of kinetic properties of smooth muscle cells and non-muscle cells has been somewhat neglected in most of these schematic representations. One particular cell group which plays an important role in this co-ordination is that of the interstitial cells of Cajal (ICC). Since their first defined description at the end of the last century [6], the ICC have been the subject of much controversy; especially their origin and role in the digestive tract have been much debated upon. The increasing interest in these cells and the very dynamic progress in this field during the past years is well reflected in the number of publications dealing with this topic (from the more than 500 publications on this subject, 200 of them were published during the last decade) [58]. Several morphological and physiological studies (for review see 28,31,48,56) have led us to assume that the gut wall harbours different classes of ICC, which either play a pacemaker role or are involved in inhibitory neurotransmission. Moreover, there is every indication (for review see 65,68) that ICC may play a role in the pathophysiology of certain gastrointestinal disorders such as hypertrophic infantile stenosis, Hirschsprung's disease, inflammatory bowel disease, severe constipation and intestinal pseudo-obstruction. ICC have even recently been suggested to be associated with gastrointestinal mesenchymal tumours.

This review aims at briefly summarising the current status of our knowledge of the morphological, pharmacological and physiological features of the distinct

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ICC subpopulations in both normal and pathological conditions. Furthermore, attention will be paid to the extent in which these cells might be the ideal targets for pharmacological intervention in gastrointestinal motor disorders and whether ICC should deserve the first priority in view of clinical relevance.

Embryological origin of ICC

The conflicting views regarding their origin and role mainly resulted from the lack of a specific marker for these cells. The discovery that c-kit, a proto-oncogene encoding for the tyrosine kinase receptor, is expressed in ICC, enabled identification of these cells at the light microscopic level, although no conclusive evidence has been provided yet that all electronmicroscopically identified ICC express c-kit or that c-kit expression in the digestive tract is specific for ICC and mast cells. Three recently performed studies in chicken/quail chimeras [38] and mouse [36,79] have refuted the long-time hypothesis that these cells are derived from the neural crest and provided clear evidence for their mesenchymal origin. In addition, in line with earlier reports of Imazumi and Hama [32] and Yamamoto [77] other developmental studies [36,62] suggest that at least some ICC and smooth muscle cells might have common precursor cells and that some ICC may also express smooth muscle markers [60,61].

Morphological features and topographical organisation of ICC

Apart from the first subdivision into two separate networks of ICC described by Li [39], pioneering work was performed by L. Thuneberg [57], who was the first to propose a classification of the different populations of ICC into four cell types, based on morphological and topographical criteria obtained from his supravital methylene blue stainings and ultrastructural studies of mouse small intestine. He distinguished ICC-type I, which are associated with the myenteric plexus; ICC-type II, which are located within the subserous layer and longitudinal smooth muscle layer; ICC-type III, which are situated at the level of the deep muscular plexus and ICC-type IV, which can be observed within the bulk of the circular muscle layer. As we are now also beginning to understand the physiology of at least part of these cell types, new classification schemes have emerged integrating some of the established functional properties and regional and species differences [16,47,48]. We now discern IC-MY_{ST}, IC-MY_{SI} and IC-MY_{C}, which include the ICC located in the myenteric region of stomach, small intestine and colon, respectively; IC-SM, which represent the ICC along the submucosal surface of the circular muscle bundles of the colon; IC-DMP located within the deep muscular plexus region of the small intestine and finally IC-IM_{ES}, IC-IM_{ST} and IC-IM_{C} representing ICC observed between the smooth muscle fibres of the bulk of the circular muscle layer in the esophagus, stomach and colon as well as at the level of the lower esophageal, the ileo-colonic and internal anal sphincters.

At the light microscopic level, ICC have been mainly described as stellate cells, each of their processes giving off secondary branches. Exceptions include ICC described in several species and several regions of the gut whose cell shape ranges from simple bipolar to bipolar with few secondary branches [4,5,7]. At an ultrastructural level, regional and species differences can be found with regard to the more or less prominent presence of smooth and rough endoplasmic reticulum, mitochondria, thin and intermediate filaments, caveolae and the presence or absence of a (dis)continuous basal lamina (see 16). A direct correlation between ultrastructural data and c-kit immunoreactive cells has till now been greatly hampered by the incompatibility of the available c-kit antibodies with glutaraldehyde-based fixatives. The recent availability of a transgenic mouse model in which the E.Coli lacZ gene has been inserted in the W/kit locus [2] has opened new possibilities in this respect [62,63]. Indeed, the permanent presence in heterozygotes of a functional kit allele, next to the zero allele W^{lacZ}, indicated that these cells are still capable of expressing c-kit, while the inserted lacZ reporter gene also allows visualisation of the ultrastructure of these cells either by histochemistry or by immunocytochemistry using specific antibodies raised against the gene product of lacZ, i.e. β-galactosidase.

Functional role(s) of ICC-subtypes

The gradual availability of a neutralising kit antibody (ACK2) and a range of c-kit or stem cell factor (i.e. the natural ligand for c-kit) mutants like Sl/Sl^{d} mice, W/W^{v} mice and Ws/Ws rats, which provided us with viable models lacking specific subpopulations of ICC, have greatly contributed to the hypotheses regarding the assignment of different functions to the above-mentioned subsets. The concept that IC-MY and IC-SM are primarily involved in the generation and propagation of slow waves and that IC-DMP and IC-IM might be rather playing a role in mediating neuronal input (see 28,48) has nowadays found ac-

ceptance in the majority of the scientific community. Prior to the introduction of these mutant models, the association of IC-MY with pacemaking was questioned since the morphological substrate was not directly in support of this hypothesis: both conventional EM and immunocytochemical studies visualising isoforms of gap junction proteins (see 1,10, 11,16,51) indicated, in contrast to the IC-DMP, a poor coupling of IC-MY and IC-SM (except for the IC-SM of the dog colon) with the circular smooth muscle layer. Therefore, it was put forward that coupling of ICC to circular muscle may utilise but does not require gap junctions [12]. Furthermore, it should be kept in mind that a high density of gap junctions does not necessarily have to be present to play a pacemaker role and that the presence of gap junctions is a dynamic and not a static event. New immunological markers and molecular biology tools have provided further evidence that IC-DMP and IC--IM in stomach and small intestine play a role in modulating inhibitory and/or excitatory neurotransmission. An intimate relationship between intrinsic nitrergic nerve fibres and IC-DMP has been shown [41] and using the W/W^{v} mutant model it has been demonstrated that ICC-IM can mediate nitrergic neurotransmission [5,74]. The latter study also produced evidence for the assumption of a parallel nitrergic innervation of IC-IM and of smooth muscle at least at the level of the lower esophageal and pyloric sphincters. In laboratory animals, immunoreactivity for the constitutive isoform of NOS [74,76] and of the CO-forming enzyme heme oxygenase 2 [15,42] has been described in different types of ICC as well, which might be suggestive of an amplifying effect of ICC on inhibitory neural signalling [45], but this is still a matter of debate. In foetal human small intestine, NOS-immunoreactive and NADPH-diaphorase stained non-neuronal cells were observed in the myenteric and circular muscle region [59]. Although controversial and species-related results were obtained in vivo [34] and in vitro [19] regarding the effect of somatostatin on intestinal smooth muscle, the presence of the sst2A receptor, a particular subtype of somatostatin receptor [54], on IC-DMP which were shown to be surrounded by somatostatin--immunoreactive nerve fibres, gives further support for a mediating role of ICC on smooth muscle activity. Similarly, immunohistochemical studies demonstrated the presence of one type of tachykinin receptors (NK1) on the surface of IC-DMP [21,44,53,70] and a few were also detected on IC-MY [37,69], suggesting that some ICC may be involved in mediation of excitatory neurotransmission as well. In addition, recent evidence has been provided that IC-IM$_{ST}$ play a major role in receiving cholinergic excitatory inputs from enteric motor neurones [73].

Already 25 years ago, the strategic positioning of ICC between neurones and smooth muscle cells of the circular layer led to the assumption that ICC might also fulfil a role as mechanoreceptors [9], i.e. sensing distension or muscle contraction. This hypothesis has been revived by a recently introduced concept based on the presence of so-called "peg-and-socket" junctions between individual smooth muscle cells of the inner circular muscle layer and between those cells and IC-DMP, between IC-DMP and the bulk of the circular layer, between individual cells of the circular layer and between the latter cells and IC-MY, and between IC-MY and the longitudinal muscle layer which is devoid of gap junctions [58] (Fig. 1). This morphological substrate, which had already been demonstrated at the beginning of the seventies [25], might also provide an answer to the somewhat puzzling results on species and region-dependent gap junction distribution and the lack of a direct coupling between circular and longitudinal muscle layer, which is after all essential for a co-ordinated peristaltic movement. Thuneberg [58] postulated in this concept that the pegs act as stretch sensors of the smooth muscle. In a similar way, a unidirectional mechanical coupling between the muscle and the pacemaker network can be effectuated. Interestingly, comparable structures have been described in the pacemaker region of the heart [55,64]. Since the number of peg-and-socket junctions, particularly at the level of IC-MY and IC-DMP, is strongly affected by distension, a plausible assumption would also be to assign a general role for these structures in the autoregulation of smooth muscle tonus whereby ICC can function as a mechanoreceptor and, in response to the degree of stretch, modulate neural transmission to the muscle. Similarly, a role of tension receptor can be postulated for ICC in the stomach, which have been shown to bear a close relationship to vagal afferents [3]. Moreover, a reduced vagal afferent response to gastric distension has been reported in c-kit mutant mice [72]. Additional arguments for a possible sensory role of ICC have recently been put forward by Huizinga et al. [30]. They found a degeneration of ICC in the feline distal esophagus both after chemical ablation of the nodosal ganglion and following vagotomy. These findings led them to hypothesise that ICC in the distal cat esophagus may act as the sensory receptor cells for vagal

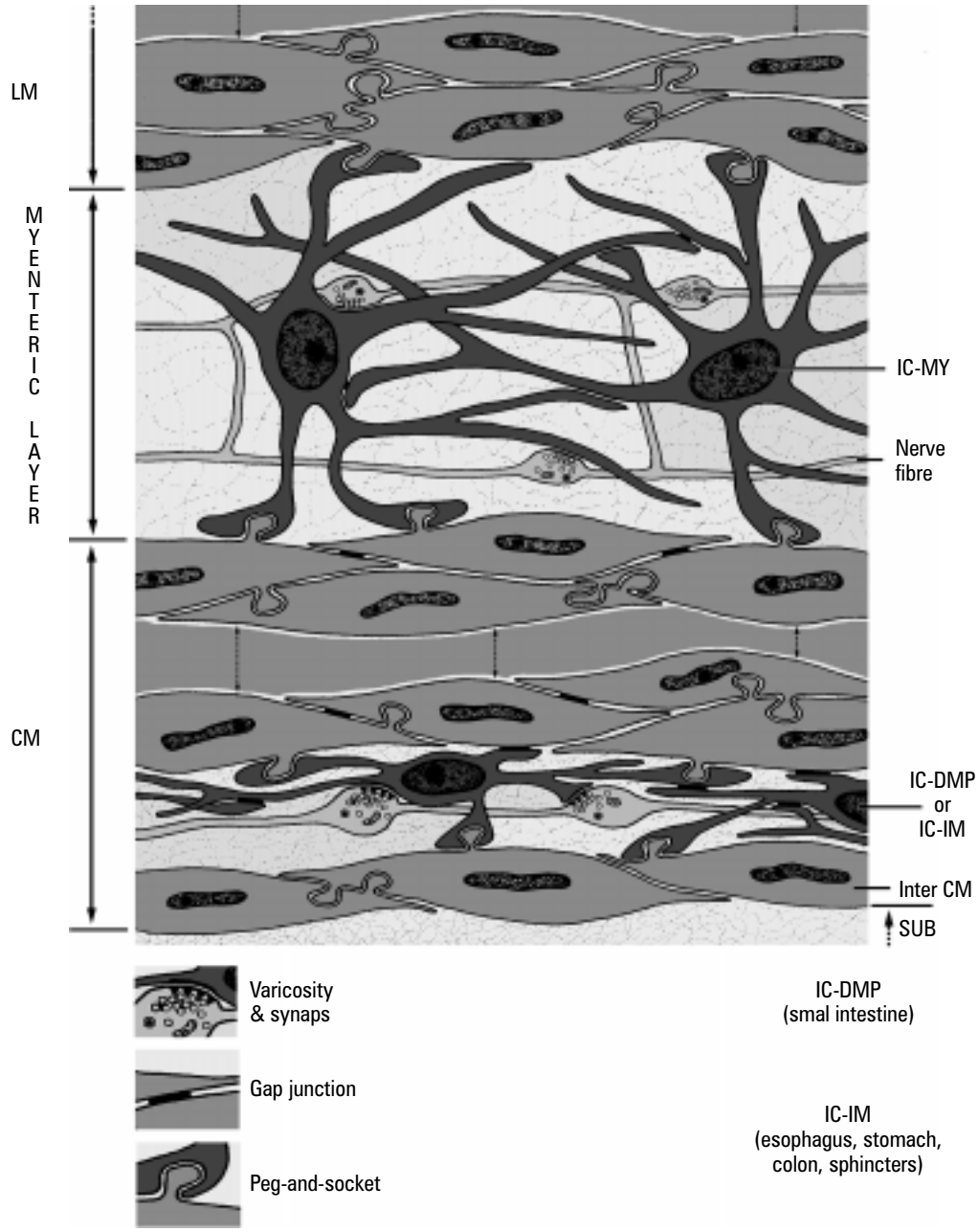

Figure 1. Schematic drawing showing the distinct types of connections between ICC, nerves and smooth muscle cells. Please note that peg-and-socket junctions occur between ICC, between all smooth muscle cells and between ICC and muscle cells. Gap junctions appear to be absent from the longitudinal muscle layer, the inner circular muscular layer and between IC-DMP and the inner circular muscle layer, and are rare between IC-MY. For reasons of clarity, neither IC-IM nor IC-SM in the colon have been depicted. Functionally, they can be regarded as equivalents to the IC-DMP and IC-MY, respectively. CM — circular muscle layer; LM — longitudinal muscle layer; SUB — submucous layer (data adapted from 5, 8).

afferent innervation. What is interesting in this respect is also the observation that c-kit immunoreactive cells, resembling ICC, are present in the striated muscle part of the esophagus of control and transgenic mice [66] and of rat and pig (unpublished observations) (Fig. 2). Although the striated esophageal portion is supposed to be controlled entirely by the swallowing centre in the brainstem via vagal pathways, it is also known that the contraction strength of the esophageal striated musculature is modulated by a variety of sensory inputs such as bolus volume, bolus composition and bolus temperature, which might be fine-tuned via myenteric co-innervation of the motor endplates. On the other hand, given the common precursor of smooth muscle cells and ICC, and considering the current concepts of

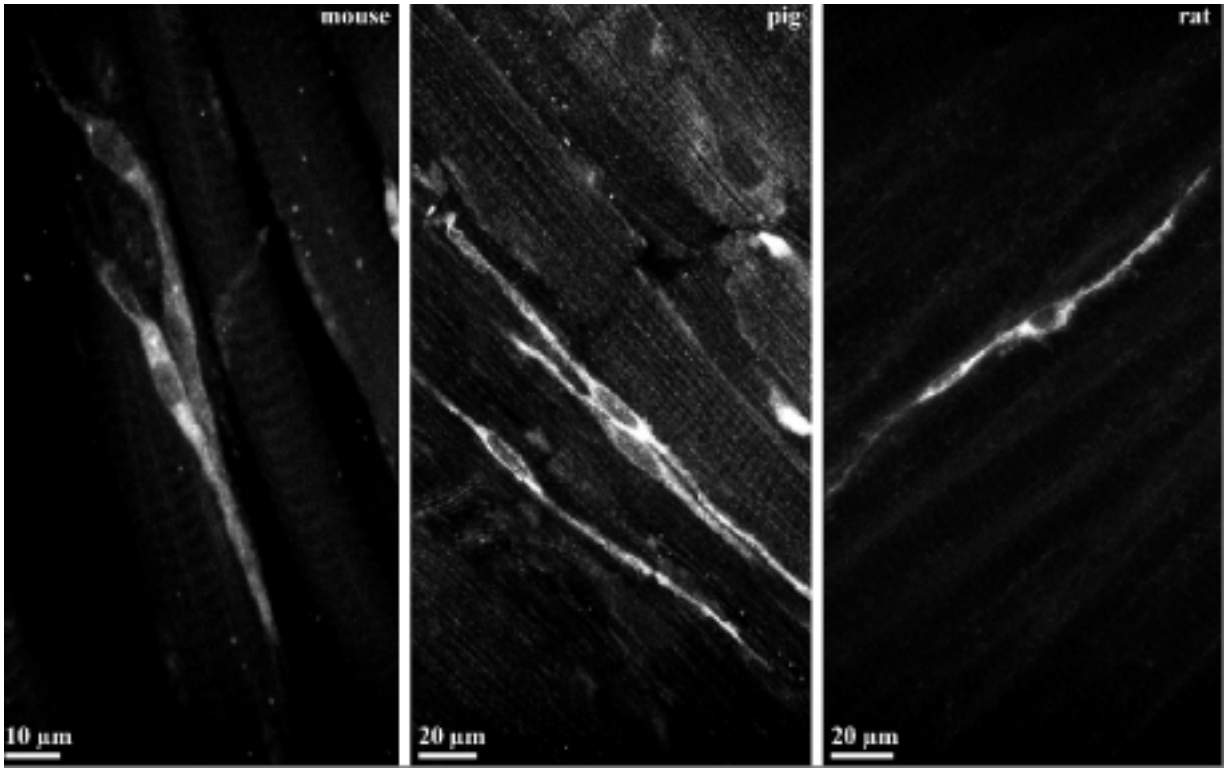

Figure 2. C-kit-immunoreactive cells resembling ICC in the striated portion of mouse, pig and rat esophagus.

transdifferentiation of smooth muscle cells in striated musculature [43,49,50], it cannot be ruled out that the c-kit-positive cells (excluding mast cells) at the level of the striated region in the esophagus might be a relict of incomplete transdifferentiation rather than a functional finding.

ICC in human gut disorders

The absence, reduction or structural alteration of subpopulations of ICC observed in several human gut disorders have comprehensively been reviewed in the recent paper of Vanderwinden and Rumessen [67] (Table 1). However, the reader should exercise caution when interpreting and generalising these data and should take into account the limitations and possible pitfalls of the methods employed. First, the number of studies of and the amount of data on ICC in human intestinal disorders are still limited and therefore can provide little information on individual variability as yet; moreover, the majority of these studies are based exclusively on either classical transmission electron microscopy or c-kit immunostaining. The absence of c-kit immunoreactivity does not necessarily implicate the absence of ICC. A "normal" distribution pattern of ICC (for which no objective, clear-cut criteria have been formulated yet) does not exclude the occurrence of significant changes in ultrastructure and expression of receptors. In view of density assessments, light or electron microscopical sections do not warrant representative sampling and adequate quantification, which might explain the contradictory findings regarding the distribution pattern of ICC in Hirschsprung's disease [27,78] and slow transit constipation [23,24,40]. One should also bear in mind that the great majority of the biopsies and resection material has been collected from patients who received medical treatment for a longer period, which alone might have affected the features of ICC. Moreover, a long-lasting dilatation/compression of an intestinal segment may equally influence the survival rate of ICC [8,13] and interconnections between ICC, smooth muscle and nerves, rendering cause and effect difficult to distinguish. This might, next to a possible delay in ICC maturation [67], also explain the normalisation of the ICC distribution pattern which can be observed following surgical recovery of intestinal transit in infantile hypertrophic pyloric stenosis and transient idiopathic neonatal pseudo-obstruction.

The fact that the available data should be interpreted with caution is also clearly exemplified by two recent papers reporting on CD34-positive gastrointestinal stromal tumours colocalising c-kit and

Table 1. Morphological and topographical features of ICC in human gut disorders

		Human motility disorders and ICC*
1.	Achalasia (adults)	Altered ICC ultrastructure (electron-lucent cytoplasma, fewer mitochondria, scarce smooth endoplasmic reticulum) in lower esophageal sphincter (LES) and proximal stomach Reduced number of contacts between nerves and ICC in LES and proximal stomach Normal ultrastructure of ICC in LES in patients with hypertensive sphincter
	Achalasia (childhood)	ICC in cardia markedly diminished or completely absent Normal presence of ICC in pylorus
2.	Infantile hypertrophic pyloric stenosis	Lack of ICC in hypertrophic pyloric circular smooth muscle layer
3.	Chronic intestinal pseudo-obstruction	Reduced density of ICC; lack of IC-MY; normal distribution of IC-IM
4.	Transient idiopathic neonatal pseudo-obstruction	Lack of ICC in small intestine (due to delayed maturation of ICC?)
5.	Hirschsprung's disease	Controversial findings reporting either a normal or a reduced density of ICC in aganglionic segment Normal ultrastructure of ICC
6.	Colonic hypoganglionosis or dysganglionosis	Lack of c-kit-immunoreactive cells
7.	Idiopathic slow transit constipation	Controversial findings reporting either a normal ultrastructural and normal distribution pattern or a reduced volume of ICC
8.	Chagas' megacolon	Colonic ICC absent or very scarce
9.	Ulcerative colitis	Altered ultrastructure of IC-SM (signs of apoptosis?) Abundance of macrophages in close association with IC-SM

*Data adapted from Vanderwinden and Rumessen (1999)

therefore suggesting origins from the ICC lineage [26,35]. In line with the editorial comment made by Huizinga et al. [29], that more research is needed to allow a positive identification of ICC in tumours, recent data [68] demonstrating that, in normal human gut, CD34- and c-kit-positive cells constitute closely adjacent but separate populations, do not fully support the view (but do not exclude it either) that gastrointestinal tumours may derive from ICC. On the other hand, another paper reporting c-kit gene abnormalities in gastrointestinal stromal tumours [46] appears to adhere to the initial view and recently a point mutation in the tyrosine kinase domain of the c-kit gene was found in two patients (mother and son) presenting multiple gastrointestinal stromal tumours with hyperplasia of c-kit-positive cells reminiscent of ICC, whereas other c-kit-expressing cell types like melanocytes and mast cells appeared unaffected [33].

ICC: ideal targets for pharmacological intervention?

The tremendous efforts which have been made by relatively few but excellent research groups to elucidate the basic mechanisms by which ICC are involved in gastrointestinal motility have already substantially furthered our understanding of the pathophysiology and possible causes of gastrointestinal disorders. The strict confinement of ICC to the gut only has made them ideal targets for pharmacological interventions [31]. Possible specific sites of action on ICC, i.e. receptors (see above) and channel properties (e.g. L-type and T-type-like Ca^{+} + channel blockers) (see 14, 48) are now being intensively explored, as is the mode of interaction between different cell types. The latter aspect is highly relevant in view of the search for efficient treatment of IBD, in which the primary targets should be the potential sources of cytokines and cytotoxic substances (for example, macrophages) rather than the ICC. A crucial question with regard to the validity of the attempts of pharmacologically influencing the pacemaker activity and/or action potential generation will be to what extent ICC are indeed unique to the gut. While there are indications that for example the slow wave is less sensitive to voltage changes than the cardiac action potentials, little is known about the organ-specific properties of ion conductances of cell types outside the gut sharing features with ICC. Given the presence of interstitial cells in several regions of the urinary tract [52,71,75], the interstitial cells of the atrioventricular and sigmoid heart valves [17] or even the myofibroblasts forming the network within the mucosal villus (see 58), the question arises whether these cells are sufficiently different so as to exclude undesired side effects in pharmacological intervention that is specifically directed to ICC.

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