Slow waves in circular muscle of porcine ileum: structural and electrophysiological studies

M. Jiménez, J. R. Borderies, P. Vergara, Y.-F. Wang, and E. E. Daniel. Slow waves in circular muscle of porcine ileum: structural and electrophysiological studies. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G393–G406, 1999.—The structural and functional bases of pacemaking (slow waves) in porcine ileal circular muscle were studied. The myenteric plexus contained two, structurally distinct types of interstitial cells of Cajal (ICC) interconnected by gap junctions and connected by close contacts to muscle layers. At the deep muscular plexus, ICC were present, not regularly close to nerve axons or in gap junction contact with one another or outer circular muscle, which had many gap junctions. Slow waves (5.2 ± 2 mV amplitude and 4.6 ± 0.7 s duration) occurred at 9.9 ± 1.1 counts/min. Tissue length and time constants were 2.00 ± 0.3 mm and 11.1 ± 37 ms, respectively. Large electrical field-induced hyperpolarizations or depolarizations reduced amplitudes but not frequencies or durations of slow waves; hyperpolarizations progressively reduced inhibitory junction potentials as if the K⁺ channel opening mediated them. In conclusion, the myenteric plexus ICC of pig ileum, which appears to pace the muscle, appears insensitive to voltages applied to the syncytium of circular muscle cells. Limited coupling between ICC and circular muscle or voltage-insensitive pacemaking activity may explain these findings.

interstitial cells of Cajal; pacemaking; electrical coupling; cable properties

SLOW WAVES ARE cyclic depolarizations that occur in smooth muscle cells of the gastrointestinal tract and pace cell contraction by determining excitability of smooth muscle (14). The origin of slow waves is still controversial, but interstitial cells, first described by Cajal, are putative pacemaker cells. Recently, much evidence has accumulated that they are necessary for origination of slow waves (14, 23, 35, 37, 38, 42, 43, 46). The role of interstitial cells of Cajal (ICC) as pacemaker cells has been demonstrated using dissection methods (4, 24, 36–38), “selective” chemical lesions such as methylene blue or rhodamine (27, 35, 43), and more recently c-kit mutant mice, which lack ICC of the myenteric plexus and slow waves (23, 46). Moreover, as Cajal initially suggested, it has been postulated that ICC participate in neurotransmission between nerve endings and smooth muscle (15), and recent evidence in fundus of c-kit mice supports this possibility (7, 35). A close relationship exists between nerve varicosities and ICC (15, 14, 35). ICC express nitric oxide (NO) synthase (35, 48) and may synthesize NO, an inhibitory mediator of the gastrointestinal tract (9, 11, 12, 40).

Small slow waves can be recorded in the pig small intestine, and inhibitory junction potentials (IJPs) can be elicited by electrical field stimulation (6, 20). Although NO synthase is present in nerve endings in the pig intestine, ATP may be the nerve mediator responsible for the initiation of fast IJPs in this species (20).

One aim of this study was to study the structural relationships between ICC, nerve endings, and smooth muscle in the circular muscle of the pig ileum. An electrophysiological approach based on the Abe and Tomita (1) technique revealed passive properties of circular smooth muscle of the pig intestine.

METHODS

Tissue Preparation

We used 6- to 10-wk-old pigs fed a standard diet (Purina). The day before the experiment, animals were fasted overnight but were allowed ad libitum access to water. Pigs were killed by an intravenous overdose of Pentothal Sodium according to the requirements approved by the Universitat Autònoma de Barcelona Ethical Committee. After laparotomy, a segment of ileum was removed 10–15 cm oral to the ileocecal junction.

Morphological Studies

Tissues strips from four pigs were dissected (see Electrophysiological Experiments) and were fixed after 30–60 min of recovery with 2% glutaraldehyde with 4% paraformaldehyde in phosphate buffer for 2 h at room temperature. After fixation, all tissues were washed overnight in 0.1 M sodium phosphate buffer containing 6% sucrose and 1.24 mM CaCl₂ (pH 7.4) at 4°C. They were postfixed with 2% OsO₄ in 0.05 M phosphate buffer for 2 h at room temperature. After fixation, tissues from the third animal were transported to Canada in buffer before embedding, and tissues from one animal were embedded first. No morphological difference between these samples was observed. To locate suitable areas, 0.5-μm-thick sections were cut and stained with 2% toluidine blue. After the examination of the toluidine blue-stained sections, ultrathin sections were cut, mounted on either 200-mesh grids or 400-mesh Ultrathin sections were cut, mounted on either 200-mesh grids or 400-mesh UltraLight transmission grids (Marivac, Halifax, NS), and double stained with uranyl acetate and lead citrate. The grids were examined in a JEOL-1200 EX Biosystem electron microscope at 80 kV or in a Phillips 301 electron microscope at 60 kV.

Electrophysiological Experiments

Strips of full-thickness muscularis externa had the mucosa removed and were then pinned flat in a chamber as described.
by Abe and Tomita (1) so that ~10 mm were inside the stimulating compartment. The stimulating chamber was delimited by two Ag-AgCl plates. The remainder of the tissue (~10 mm) protruded into the recording compartment. Two platinum wires were placed a few millimeters apart in the stimulating compartment along the tissue. The tissue was perfused constantly with prewarmed, preaerated (95% O₂-5% CO₂) Krebs solution (in mM: 115.5 NaCl, 1.6 NaH₂PO₄, 21.9 NaHCO₃, 4.2 KCl, 2.5 CaCl₂, 1.2 MgSO₄, and 11.1 glucose) at a rate of 3 ml/min (temperature 38 ± 0.5°C, pH at 7.4). Tissues were allowed to equilibrate for 2 h before starting the experiments. Circular muscle cells were impaled in the recording compartment, from the deep muscular plexus side, with glass microelectrodes (40–60 MΩ) filled with 3 M KCl. Membrane potential was measured using a standard electrometer (Duoo773; WPI). Electrical activities were displayed on a digital storage oscilloscope (4026; Racal-Dana) and simultaneously digitized (100 Hz) and collected using EGG software installed in a personal computer. To evaluate passive membrane properties of the tissue, the approach of Abe and Tomita (1) was used. Cells were impaled at several distances from the partition (from 0.5 to 3 mm). Electrotonic potentials were evoked by square electrical pulses applied across the Ag-AgCl plates at the time of the nadir of slow waves. The logarithm of the amplitude of the electrotonic potential recorded in response to electrical pulses was plotted against the distance from the partition. The amplitude, duration, and frequency of slow waves were measured before and after the pulse application. To normalize data, the amplitude, duration, and frequency of slow waves were measured before and after the pulse application. To normalize data, the amplitude, duration, and frequency of slow waves were measured before and after the pulse application. To normalize data, the amplitude, duration, and frequency of slow waves were measured before and after the pulse application. To normalize data, the amplitude, duration, and frequency of slow waves were measured before and after the pulse application.

RESULTS

Morphological Characterization of Electrical Control Systems of Pig Intestine

Interstitial cells of the myenteric plexus. As illustrated in Fig. 1A, the pig intestine has distinct longitudinal and circular muscle layers with no prominent inner circular muscle layer. In most regions as shown in Fig. 1A, the longitudinal and circular muscle layers are distinctly separated by the myenteric plexus with nerve ganglia and other cell types. Figure 1B shows, at higher magnification, the relationship of an ICC-like cell and associated profiles from similar cells to longitudinal muscle and to nerve bundles. Cells were labeled “ICC?” when they had many but not all ultrastructural characteristics found in other ICC, i.e., a large lobate nucleus surrounded by a thin perimeter of cytoplasm with most of the heterochromatin clumped at the periphery and what appears to be a nucleolus. This ICC and the associated processes have lysosomes, scattered intermediate filaments, and very little rough endoplasmic reticulum compared with fibroblasts. It also had very scant, scattered basal lamina. The associated ICC-like processes have intermediate filaments. In intestine of other species, we usually require the presence of caveolae or coupling by the gap junction to a structure with these organelles before accepting a cell process as belonging to an ICC.

One of the ICC-like processes in Fig. 1B makes an intermediate contact with a longitudinal muscle cell; gap junction contact between ICC-like and longitudinal muscle cells was never observed. Caveolae were rarely observed in these ICC-like cells or in the associated profiles. Note that one such process near the bottom of the micrograph had more electron-lucent cytoplasm along with mitochondria and rough endoplasmic reticulum. Neither cell process had myoid features like those in ICC of the deep muscular plexus in other species (18); dense bodies or bands and thin filaments were not obvious. Hereafter, for reasons discussed later, these and similar cells are considered likely to be ICC.

Figure 1C shows portions of two ICC cells with nuclei, similar to that in Fig. 1B and ICC processes. These cells were typically close to but not in contact with longitudinal muscle and a bundle of nerve axons. In the larger process at the top ICC, there was much rough endoplasmic reticulum, and mitochondria were scattered near the plasmalemma throughout the cell. An associated profile contained more electron-lucent cytoplasm than the others, and this profile, like ICC in other species, had numerous electron-lucent organelles, some of which were caveolae. As demonstrated in Fig. 2, A and B, the presence of ICC cells of different electron densities was a common feature. Lysosomes and large membrane-bounded structures unstained or poorly stained, which may have contained lipid material, often were present. Gap junctions were rarely present between two ICC processes. An shown in the
two processes in Fig. 1C, one has more electron-dense cytoplasm than the other.

Figure 2A shows ICC cells and numerous ICC processes and their relationship to circular smooth muscle (CM). There were differences in electron density between the various ICC cells and processes, suggesting the presence of two distinct cell types comprising the network of these cells in the myenteric plexus. One cell with greater electron density made a gap junction-like contact with outer circular muscle; similar contacts between profiles of ICC cells were common. Less electron-dense ICC cells and their processes had numerous mitochondria, intermediate filaments, little rough endoplasmic reticulum, and numerous close contacts with one another. More electron-dense cells and their processes had more rough endoplasmic reticulum, and intermediate filaments were less obvious. Cells and processes of each type were in close relationship with other processes of both types, consistent with the participation of both in a pacemaking network.

Figure 2B shows at higher magnification the relationships among the processes of the ICC and with circular smooth muscle. A more electron-dense process is in close contact with a muscle cell. The less electron-dense processes were in contact with both dense processes and processes like themselves. Figure 2C demonstrates an example that numerous gap junctions as well as close and intermediate contacts were present between cells of circular smooth muscle.

Interstitial cells within circular muscle. Figure 3A shows the general organization of the deep muscular plexus region, i.e., intermittent nerve bundles associated with ICC cells partially separating the outer circular muscle layer from an inner single layer of small, smooth muscle cells lining the inner border of the circular muscle and separating it from the submucosa. A nerve bundle with associated ICC profiles is shown at higher magnification in Fig. 3B. Different axon profiles had different kinds of synaptic vesicles, some only small agranular vesicles and some a mixture of large and small granular vesicles as well as small agranular vesicles. The presence of a variety of synaptic vesicles appears to be consistent with release of multiple mediators in the deep muscular plexus. The ICC profiles were near but never very close (<40 nm) to or surrounding nerve profiles in a glial-like fashion; the ICC profiles had fewer actin filaments compared with adjacent smooth muscle and had intermediate filaments. No gap junction contacts of ICC to outer circular muscle cells were observed, and a continuous network of ICC cannot be deduced from these ultrastructural observations. Gap junction contacts between outer circular smooth muscle cells were frequently observed, as shown in Figs. 2C, 3, and 4A.

Figure 4A also depicts the region of the deep muscular plexus. It shows that the inner circular smooth muscle layer, like that of the intestine of other species, has no gap junctions between smooth muscle cells and, like that of guinea pig, rat, and mouse (26, 42, 43, 49–51) but unlike other large mammals such as dogs (17, 18) or humans (33), consists of only one cell layer. An ICC cell with nucleus was present unconnected to smooth muscle or other ICC by gap junctions. The ICC cell in Fig. 4A makes close contact with what seems to be an ICC profile on the right and with profiles of what are either other ICC or smooth muscle profiles on the left. ICC cells were not close (<50 nm), as in the canine intestine, to nerve axons. One such bundle, partially surrounded by glial cytoplasm, had axons with large granular vesicles and small granular vesicles. Gap junctions between smooth muscle profiles were present, as can be seen in Fig. 4A, top left.

Figure 4B depicts a bundle of axons associated with a glial cell and a rare ICC within the circular smooth muscle. The glial cell has more rough endoplasmic reticulum, no cavedae, no close contact with circular smooth muscle, and surrounds axons compared with ICC. The adjacent ICC cell has cavedae and is closer to muscle than the glial cell, but makes no close contact with it.

Electrophysiological Properties of Circular Muscle Cells

Microelectrode recordings from the circular muscle of the pig ileum showed cyclic slow waves with a mean frequency of 9.9 ± 1.1 counts/min (cpm; Fig. 5). The mean resting membrane potential was −61 ± 7 mV (n = 18). Slow waves showed a typical shape with a depolarization, a plateau, and a repolarization (Fig. 5). In 18 independent observations, the mean amplitude (5.2 ± 2 mV) and duration (4.6 ± 0.7 s) were consistent, but the amplitude was smaller than in other intestinal muscles. Electrotonic potentials were recorded in cells impaled at different distances (0.5 to 3 mm) from the partition (recording chamber) and at different strengths (0.3 to 1.2 V/cm) of electrical field stimulation (Fig. 5). For each distance, the amplitude of the electrotonic potential (steady state) was plotted as a function of the strength of stimulation (Fig. 6A). The amplitude increased linearly with the field strength, indicating a purely passive response. When the microelectrode was out of the cell, no voltage deflection could be detected. This observation showed that no current spread into the recording compartment occurred dur-
ing stimulation. The logarithm of the amplitude of the electrotonic potential decayed linearly with the distance (Fig. 6B). The average length constant was calculated from the slope ($l = 2.0 \pm 0.3 \text{ mm}$, $n = 4$). The relationship between the time to reach half the value of the steady state and the distance was linear (Fig. 6C). With the use of this procedure, the time constant ($t_m = 111 \pm 37 \text{ ms}$, $n = 4$) was calculated from the slope and the length constant (Fig. 6C).

Effect of Hyperpolarization and Depolarization on Slow Waves and IJP

We measured the effect of conditioning hyperpolarization or depolarization of smooth muscle cells on slow wave parameters using square electrical pulses (Fig. 7). Slow waves were recorded during both depolarization and hyperpolarization. The frequency or duration of slow waves was not modified during electrical field-induced depolarization or hyperpolarization (Fig. 7). However, the amplitude of slow waves was affected by membrane potential. For example, 10 mV depolarization decreased the slow wave amplitude by 60%. However, slow wave amplitude was less sensitive to hyperpolarizing pulses. Up to 20 mV hyperpolarization (to a membrane potential of about −80 mV) did not modify markedly the amplitude of slow waves. Strong pulses (>20 mV hyperpolarization) decreased the slow wave amplitude (Fig. 7). To illustrate the relationship between slow wave amplitude ($y$) and conditioning membrane potential ($x$), data were fitted with a third-order linear regression ($r^2 = 0.943$; $y = 99.258 - 2.096x - 0.1788x^2 - 0.0023x^3$).

To check that smooth muscle cells were appropriately current clamped in the preceding studies, we stimulated the preparation to elicit IJP. Supramaximal IJP were elicited before, during, and after electrical field-induced hyperpolarization (Fig. 8A). The amplitude of the IJP progressively decreased with hyperpolarization. The relationship between membrane potential and amplitude of the junction potential was linear (Fig. 8B). The IJP reversed in a positive deflection when the imposed membrane potential reached −90 ± 9 mV ($n = 4$).

**DISCUSSION**

This study shows that the pig intestine circular muscle has slow waves with some, but not all, properties similar to slow waves of other species and that there probably exists a network of ICC at the level of the myenteric plexus with two structurally different cells that may provide pacemaking activity for the circular muscle. Other ICC, but no continuous network of ICC, exist at the deep muscular plexus. These structural findings may help explain the electrophysiological findings.

**ICC and Slow Waves in Gastrointestinal Tract of Other Species**

In the canine colon, ICC near the submucous plexus are the main pacemaker cells (2, 5, 35–38). In this tissue, a low-threshold Ca\(^{2+}\) current has been described (31) in ICC cells (but not in smooth muscle cells), which might trigger slow waves through coupling pathways to the muscle layers. Part of the upstroke and the plateau phase of the slow wave are dependent on L-type Ca\(^{2+}\) currents, which are blocked by dihydropyridine derivatives such as nifedipine (21, 47). In the small intestine from different species, slow waves (but not the associated mechanical contractions) are insensitive to nifedipine (6, 8, 19), suggesting that L-type Ca\(^{2+}\) channels might not be involved in the ionic basis of intestinal slow waves. In the small intestine, ICC are distributed near the myenteric plexus and deep muscular plexus (32–34, 42, 43, 46). In the canine ileum, typical slow waves (fast depolarization, plateau, and repolarization) are only recorded in smooth muscle cells near the myenteric plexus, and both networks of ICC are putative pacemakers (8, 24). Gap junctions are present (17, 42, 43) between myenteric plexus ICC, rarely between ICC and circular smooth muscle cells, but never between ICC and longitudinal muscle cells in electron microscopy (17). In this case, ICC of the myenteric plexus form a network, but smooth muscles appear poorly coupled to this network. It has been suggested (17) that, as in the cardiac sinus node (25, 29), poor coupling is necessary to isolate pacemaking currents in the ICC network from dissipation in the well-coupled circular muscle syncytium.

In contrast, in the deep muscular plexus of dog small intestine, gap junctions are common between ICC and the outer smooth muscle (but not the inner smooth muscle) as in other species (34, 42, 43). Moreover, circular muscles in all species recorded are well coupled through gap junctions and are expected to and do display cable properties (1, 10, 13, 14, 28, 30, 37).

**Interstitial Cells of Pig Intestine Compared With Other Species**

ICC are located in the intestinal myenteric plexus like those described in the dog (17) and in other species (33, 44, 46). One type of ICC was distinguished from fibroblasts by their nuclear structures, their relatively small amount of rough endoplasmic reticulum, the occasional presence of cavedae, and their close contacts...
(definitive gap junctional contacts were not seen) with muscle cells. They had no myoid features (no thin filaments, no dense bodies or bands), and they had very scant basal lamina unlike smooth muscle cells. Another class of ICC was more electron dense and had more rough endoplasmic reticulum, very few caveolae, and no myoid features. They resemble the fibroblast-like cells found in the myenteric plexus of guinea pig and rat small intestine and were considered not to be ICC by Komuro and colleagues (26) because they lack immunoreactivity for the c-kit antigen. These cell studies by Komuro et al. (26) were stained by the zinc iodide osmium method and did make close or gap junction contacts with smooth muscle. These workers also described a second type of “fibroblast-like” cell that was recognized by the c-kit antibody but that had a less electron-dense cytoplasm with more smooth endoplasmic reticulum, mitochondria, and electron lucent processes with many intermediate filaments and interconnected by gap junctions. These may correspond with the less electron-dense cells in the myenteric plexus of the pig. The presence of two cell types within the putative ICC network in the myenteric plexus of the pig intestine thus differs from the descriptions in mouse, dogs, and humans (17, 33, 44–46) but may correspond to the arrangement in guinea pig and rat intestine (26).

Fig. 4. A: deep muscular plexus and ICM and OCM. ICC cell is present along with some ICC processes with close contacts (double ▲ at left or arrow at right), identified by the absence or paucity of actin filaments and dense bodies (arrowheads in a smooth muscle cell). ICC is not electron dense and does not have close contact with axons (some of which have LGV and SGV) or gap junction contact with OCM. Intermediate contacts between a cell of OCM and an ICC process are labeled by short open arrows. Some intermediate filaments are within the circle, and there is rare intermittent basement membrane. Note the gap junction between two cells (thick arrow) of OCM. B: nerve bundle with axon profiles, some with small granular vesicles (labeled A) with a glial cell (G) and an ICC cell within the CM. Circle shows a bundle of neurofilaments.

Fig. 5. Slow waves and electrotonic potential in the circular muscle of the pig ileum. Recordings from different distances from partition (top: 0.5 mm; middle: 2 mm; bottom: 3 mm). A, B, C, and D are hyperpolarizing pulses (square pulses) of 0.3, 0.6, 0.8, and 1.2 V/cm, respectively. Notice that the amplitude of electrotonic potential increases with field strength, and note how the electrotonic potential decreases with distance from partition. Slow waves show a depolarization, a plateau, and a repolarization.

Fig. 6. Example of data to analyze passive properties of circular muscle of pig ileum. A: relationship between field strength (I) and amplitude of electrotonic potential at different distances from partition. B: relationship between distance from partition and amplitude of electrotonic potential (log scale) induced by applications of different I. Slope is equal to 1/length constant (l). C: relationship between distance from partition and time to reach half of steady state (t1/2). Slope is equal to t1/2/l, where t1/2 is the time constant. In each curve, data were fitted with a linear regression, and length and time constants were calculated for each animal.
We consider both cell types to participate in the ICC network of the pig intestine. The close connections of both cell types to one another and to nerves and muscle in pig ileum suggest to us that they both participate in pacemaking, perhaps with different roles. ICC were close to longitudinal muscle cells, but our recordings of slow waves were entirely from circular muscle, and the existence of slow waves in longitudinal muscle, while likely, has not been established. Bundles of ICC processes were frequently found in the myenteric plexus, and they were sometimes connected by gap junctions. Although it was usually not possible to establish in single micrographs that the various ICC near longitudinal muscle or circular muscle were interconnected, the density and common occurrence of ICC processes and bundles of these processes in close contact and sometimes in gap junction contact allow the suggestion that there was a network of ICC in the myenteric plexus. This putative network was in close and possible gap junctional contact with circular smooth muscle and was positioned to provide pacemaking to this layer. We have to consider the possibility suggested by Komuro and colleagues (26, 49–51) that fibroblasts or fibroblast-like cells participate in pacemaking. Clearly, both types of cells were part of a network with many regions of close contact in the studies of these authors.

A Network of ICC at the Deep Muscular Plexus?

There were also some fibroblast-like putative ICC cells within the circular muscle, but these were rarely found. ICC of the deep muscular plexus of the pig occurred intermittently and without visible interconnections to one another. ICC of the deep muscular plexus had lower electron density and had much less rough endoplasmic reticulum compared with those of the myenteric plexus. They had some thin filaments, no dense bodies, intermediate filaments scattered or in bundles, and occasional caveolae. In contrast to the canine intestine deep muscular plexus (17, 18) and that of other species (26, 33, 42, 43, 49–51), these ICC made few or no very close (<50 nm) contacts with nerves.

In the canine ileum, the ICC of the deep muscular plexus provide a secondary pacemaking network for slow waves and impose a different configuration on slow waves recorded near the network (8, 9, 24). Such slow waves lacked plateaus and did not arise from a stable baseline membrane potential. In the pig intestine, no evidence suggested that slow waves of different configuration existed near the deep muscular plexus, consistent with a lack of structural evidence that an ICC pacemaking network existed there.

In other species such as the mouse, in which the deep muscular plexus network does not appear to be able to drive slow waves (46), it has been suggested that the deep muscular plexus is the site of origin of IJPs. It is unclear whether the nerve varicosities concentrated near the deep muscular plexus of the pig intestine are the main source of mediator of IJ Ps, which in this case is not NO (20).

The small amplitude of the slow waves in circular muscle of the pig intestine compared with other species may relate in part to the absence of a network of ICC at the deep muscular plexus (DMP) coupled to outer circular muscle. In other species with thick intestinal walls, such as the dog, the ability of the ICC network at the DMP to generate slow waves, phase-locked in the normal case to those initiated by the ICC network of the myenteric plexus, may amplify the slow waves generated from the dominant pacemaker. The absence of such amplification from the ICC of the DMP in the pig intestine may help explain the slow wave amplitudes of 10 mV compared with 20 mV in the dog (17, 24). The mouse intestine generates large slow waves, and no contribution from ICC of the deep muscular plexus has been identified or eliminated by showing that removal of the DMP leaves slow waves unaffected (23, 46). In any case, the need for amplification may be much less in a thin compared with a thick intestinal circular muscle.
ICC and Inhibitory Nerves

Our studies suggest that ATP rather than NO is the main mediator of IJPs in this tissue (20) by acting on apamin-sensitive K^+ channels. The ultrastructural characterization of synaptic vesicles releasing ATP has not been established (16). However, several classes of vesicles were present in the area of the deep muscular plexus, with large granular vesicles usually associated with peptide neurotransmitters, small granular vesicles usually associated with adrenergic neurotransmitters, and small agranular vesicles usually associated with acetylcholine and other neurotransmitters, including possibly ATP (16). ICC were not very close to bare nerve endings, and the transmitter producing IJPs was not NO (20). These ICC were not regularly connected to outer circular muscle by gap junctions. An essential role of ICCs for IJPs in the pig seems unlikely (7, 35).

Comparative Electrophysiology of Pacemaking in Pig Intestine

The outer circular muscle itself was a syncytium, as demonstrated by its passive electrical properties (discussed in Passive Electrical Properties of Pig Ileal Circular Muscle), in which cells were connected by gap junctions. The electrophysiological experiments show that the circular muscle of pig intestine has slow waves similar in configuration and frequency to those described in other species, such as rabbits, dogs, and humans (10, 13, 19, 24, 35). They consisted of regular depolarizations followed by a plateau and a repolarization at \( \frac{10}{10} \) cpm. They were smaller than those of the other species, less than one-half the amplitude of slow waves of rabbits (10, 19) or dogs (8, 9, 24). Their amplitude varied with the local membrane potential, maximal near the resting potential and falling off with strong hyperpolarization or small depolarization. This is similar to findings in rabbit intestine (41) or in the

**Fig. 8.** Effect of conditioning hyperpolarization on inhibitory junction potential (IJ P). A: recordings showing that the amplitude of the IJP decreased when the cell was hyperpolarized and the junction potential reversed near the K^+ equilibrium potential. Notice the effect of hyperpolarization on the amplitude, frequency, and duration of slow waves. (a) And (b) refer to IJPs initiated at hyperpolarized (a) and control (b) membrane potentials. B: relationship between membrane potential and amplitude of IJP.
circular muscle of the guinea pig stomach (28) where small hyperpolarization pulses increased the slow wave amplitude and strong hyperpolarization decreased but did not abolish the amplitude of slow waves. Our interpretation of this similar data differs from that in the guinea pig stomach (see Fig. 2 from Ref. 28), which did not consider a role of pacemaker cells, such as the ICC network.

Independence of Slow Wave Frequency From Membrane Potential

The frequencies and durations of the slow waves were unchanged at all membrane potentials similar to the case in the guinea pig stomach in which frequencies changed <20% with large membrane potential changes (28). One explanation of this is that the applied voltage changes affected only the responding circular smooth muscle cells and not the pacemaking cells, which comprise the ICC network of the myenteric plexus. The cardiac sinus node is poorly coupled electrically to the surrounding atrial muscle, and this may allow pacemaking currents to behave largely independent of the surrounding well-coupled muscle (25, 29). With good coupling between pacing and paced tissues, the pacemaking currents could easily be dissipated in the large capacitance of the atrial muscle syncytium. Furthermore, a sufficient, but relatively high resistance, coupling from the pacemaking network to the responding syncytium allows the pacemaking network to produce voltage changes for pacemaking in the responding cells (30). The structural arrangements described here are consistent with that explanation. Small numbers of junctions, possibly gap junctions and close contacts, between the ICC network and the circular muscle syncytium were found. The cells of the ICC network had multiple close and some gap junction contacts and were probably closely coupled to another, as were the circular muscle cells. Thus the pacemaking activity within the ICC network may have functioned independently of the applied potential in the circular muscle if the coupling between the two systems did not allow significant electrotonic spread between them. A similar structural arrangement between the myenteric plexus region ICC network and the circular muscle syncytium has recently been described for the canine ileum (17, 44).

An alternate possibility is that the gap junctions or other junctions between the two coupled networks are rectifying and do not pass currents when the circular muscle is more polarized. No evidence about the nature of these gap junctions as to the connexins involved or their current-passing properties is available. Moreover, there is a possibility that other junctions besides gap junctions can contribute to coupling (3, 39). Finally, two obvious and important possibilities are that the mechanisms initiating pacemaking are relatively voltage insensitive or that, in the Abe and Tomita (1) partitioned bath, voltage changes do affect pacemaker cells but not cells several length constants distant from the partition and that these pacemaker cells drive impaled cells near the partition. These possibilities are not mutually exclusive.

Variation of Slow Wave Amplitude With Membrane Potential

If the slow wave amplitude variation with applied potentials is a property of circular muscle, how can the responses to polarizing and depolarizing currents be explained? No change was found in slow wave duration or in their general shapes associated with these membrane potential changes. This result differs from that in the guinea pig stomach (28) in which the slow wave seemed to involve two components, a basal one unaffected by membrane potential and another component superimposed on the basal one and lost with strong hyperpolarization. One might expect that hyperpolarization would increase the size of depolarizing events such as slow waves if they are caused by opening of voltage-dependent ion channels, either because the driving forces increased or because the ion channels involved recovered more fully from partial inactivation. Small increases in amplitude may have occurred when small hyperpolarizing pulses were applied (<20 mV), but all other membrane polarizations reduced slow wave amplitude.

However, hyperpolarization was effective in reducing and reversing the $I_{JP}$, which are apamin sensitive (20) and dependent on $K^+$ channel opening. This established that the hyperpolarizing electrotonic currents were effective in changing membrane potentials within recorded cells, and smooth muscle $K^+$ channels responded to them. Either slow wave characteristics were not dependent on active responses of smooth muscle and pacemaking activity was unaffected by current clamping, or smooth muscle cells had unusual $K^+$ channels. Clearly, further study of ionic mechanisms present in ileal circular muscle of pig intestine and their contributions to slow waves is required. The changes in conductance associated with slow waves, although difficult to measure because of the long time constant common to this and other circular smooth muscles, would be helpful to know.

The small amplitude (mean = $5 \text{ mV}$) of pig ileum slow waves raises an additional question. Were these slow waves large enough to control contractile activity? Their amplitude was too small to reach a high probability of opening of L-type $Ca^{2+}$ channels (major increases occur at about $-40 \text{ mV}$) from resting potentials of near $-60 \text{ mV}$; the maximum depolarization in pig ileum in various experiments was $-50$ to $-43 \text{ mV}$. Also, action potentials were not found superimposed on them. In other species, these are mediated primarily by $Ca^{2+}$ entrance through L-type $Ca^{2+}$ channels and are initiated at about $-40$ to $-35 \text{ mV}$. Thus it is unlikely that either of these slow waves directly control contractions of the pig intestine. Direct experiments simultaneously comparing slow waves and contraction are needed. The
possible reasons for their small amplitude were discussed above.

Passive Electrical Properties of Pig Ileal Circular Muscle

The passive properties of the circular muscle syncytium resembled those in other species. The circular muscle of the rabbit duodenum has a length constant of 1.02 mm and a time constant of 107 ms (10). The smooth muscle of the rabbit duodenum has a length constant of 1.02 mm and a time constant of 107 ms (10). The smooth muscle of pig ileum circular muscle behaves like a cable along its long axis, consistent with the morphological data about gap junctions.

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