Evidence supporting presence of two pacemakers in rat colon

LÍDIA PLUJÀ,1 ELENA ALBERTÍ,1 ESTER FERNÁNDEZ,1 HANNE BIRTE MIKKELSEN,2 LARS THUNEBERG,2 AND MARCEL JIMÉNEZ1
1Department of Cell Biology, Physiology, and Immunology, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Catalunya, Spain; and 2Institute of Medical Anatomy, University of Copenhagen, DK-2200 Copenhagen, Denmark

Received 16 December 1999; accepted in final form 28 February 2001

Evidence supporting presence of two pacemakers in rat colon. Am J Physiol Gastrointest Liver Physiol 281: G255–G266, 2001.—Intracellular microelectrodes and organ bath techniques were used to study spontaneous cyclic electrical and mechanical activity in the rat colon. Electron microscopy and immunohistochemical studies showed two major populations of interstitial cells of Cajal (ICC): one associated with Auerbach’s plexus (ICC-AP) and one with the submuscular plexus (ICC-SMP). The ICC-SMP network partly adhered to the submucosa when removed and was generally strongly damaged after separation of musculature and submucosa. Similarly, longitudinal muscle removal severely damaged AP. Two electrical and mechanical activity patterns were recorded: pattern A, low-frequency (0.5–1.5 cycles/min), high-amplitude oscillations; and pattern B, high-frequency (13–15 cycles/min), low-amplitude oscillations. Pattern A was recorded in preparations with intact AP but absent in those without intact AP. Pattern B was recorded in preparations with intact SMP but was absent in those lacking SMP. With full-thickness strips, the superimposed patterns A and B were recorded in circular muscle. When longitudinal muscle mechanical activity was recorded, only pattern A was present. We conclude that two pacemakers regulate rat colonic cyclic activity: the ICC-SMP network (responsible for cyclic slow waves and small-amplitude contractions) and the ICC-AP network (which may drive the cyclic depolarizations responsible for high-amplitude contractions). This is the first report showing consistent slow wave activity in the rodent colon.

THE MEMBRANE POTENTIAL OF smooth muscle cells from the gastrointestinal tract usually displays rhythmic slow waves. These slow waves correlate with cyclic contractions in many gastrointestinal smooth muscles. For a long time, the cyclic activity was believed to be “myogenic” in origin because neural blockers were not able to modify rhythmicity. Interstitial cells of Cajal (ICC) were described for the first time by Cajal (3). In 1982 (28), it was suggested that ICC were probably pacemaker cells that mediate the input to smooth muscle cells to oscillate cyclically. For the last 20 years, many physiologists have used different methodologies to correlate ICC and slow waves, including dissection experiments, cytotoxic chemicals to lesion ICC, ICC isolation, and more recently, genetic and developmental models (for review, see Refs. 4, 11, 25, and 29). The discovery that ICC express c-kit, the protooncogene that encodes the receptor tyrosine kinase Kit, allowed many immunohistochemical studies (12, 17, 18, 30, 31) to be performed with Kit antibodies, improving knowledge about ICC.

The distribution of ICC within muscle layers differs between regions of the gastrointestinal tract. In the small intestine, ICC are located near Auerbach’s plexus (ICC-AP) and near the deep muscular plexus (ICC-DMP) (22, 24, 28). The main pacemaker regulating peristalsis is probably the ICC-AP network (4a, 10). However, the small intestine DMP might also contribute to rhythmicity and be involved in segmentation (13, 28a). In the colon, ICC are located near the AP (ICC-AP) and submuscular plexus (ICC-SMP) (1, 2, 23). In the canine colon, the slow wave activity (5–6 cycles/min) depends on the presence of an intact ICC-SMP network (6, 14, 26). Similarly, myenteric potential oscillations (MPO; 16–20 cycles/min) appear to depend on the presence of ICC-AP (27). Accordingly, two pacemakers might be working simultaneously, and the final electrical activity results from the combined activities of both pacemakers (27). In the human colon, the slow wave activity probably depends on the interaction between ICC-SMP and ICC-AP. Slow electrical oscillations lasting 9 s occur at a frequency of 3 cycles/min. These oscillations were described as slow waves generated near the SMP (21). Near the myenteric border of the circular muscle, MPO similar to those described in the canine colon have also been reported (21).

In small rodents, the pacemaker activity of the colon is not well understood and might involve cyclic depolarizations and slow waves. In mouse colon, cyclic de-

Address for reprint requests and other correspondence: M. Jiménez, Dept. of Cell Biology, Physiology, and Immunology, Veterinary Faculty, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Catalunya, Spain (E-mail: marcel.jimenez@uab.es).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
polarizations occur every 3 min. These depolarizations allow the muscle to reach the threshold to open L-type Ca\textsuperscript{2+} channels, giving rise to smooth muscle action potentials. However, the origin of the cyclic activity is controversial. One hypothesis involves a neurogenic mechanism that is partially resistant to L-type Ca\textsuperscript{2+} channel blockers (16) and a second hypothesis derived from developmental studies (32) involves ICC probably located near the myenteric plexus. In the rat colon, we (20) have reported the presence of cyclic depolarizations similar to those described in the colon of the mouse. It is very difficult to record slow waves in isolated mouse circular muscle (15, 32). Small slow waves (15–18 cycles/min) 3–10 mV in amplitude can only be recorded in a small percentage of the strips (15). This study aimed to characterize the ICC from the rat colon and to investigate their relation to the recorded spontaneous electrical and mechanical activity of rat colonic muscle.

MATERIALS AND METHODS

Tissue Preparation

Male Sprague-Dawley rats (weighing 300–350 g), fasted overnight (18 h) but allowed ad libitum access to water, were killed by decapitation and bled (this procedure was approved

![Organ bath recordings showing the spontaneous cyclic mechanical activity displayed by the circular (A) and longitudinal muscles (B) in a rat colonic preparation with the submucosa kept intact and therefore with both the submuscular plexus (SMP) and Auerbach’s plexus (AP) intact. C: mechanical recording showing the spontaneous contractile activity of the circular muscle in a colonic preparation devoid of submucosa, but with AP intact. D: mechanical recording showing the spontaneous contractile activity of the circular muscle in a colonic preparation devoid of AP, but with the SMP intact.]

Fig. 1. Organ bath recordings showing the spontaneous cyclic mechanical activity displayed by the circular (A) and longitudinal muscles (B) in a rat colonic preparation with the submucosa kept intact and therefore with both the submuscular plexus (SMP) and Auerbach’s plexus (AP) intact. C: mechanical recording showing the spontaneous contractile activity of the circular muscle in a colonic preparation devoid of submucosa, but with AP intact. D: mechanical recording showing the spontaneous contractile activity of the circular muscle in a colonic preparation devoid of AP, but with the SMP intact.
by the Ethics Committee of the Universitat Autònoma de Barcelona). The colon was then removed and placed in Krebs solution consisting of (in mM) 10.10 glucose, 115.48 NaCl, 21.90 NaHCO₃, 1.14 NaH₂PO₄, 2.5 CaCl₂, and 1.16 MgSO₄ bubbled with a mixture of 5% CO₂-95% O₂ (pH 7.4). Microelectrode and organ bath studies were carried out under nonadrenergic noncholinergic conditions (1 μM of atropine, propranolol, and phentolamine). To perform these studies the following three preparations were used: full-thickness strips; muscle strips without submucosa (circular and longitudinal muscle with AP); and muscle strips without the longitudinal layer (circular muscle with SMP). In all cases, muscle strips were ~1 cm long and 0.3 cm wide.

**Spontaneous Mechanical Activity Recordings**

Muscle strips were attached with silk threads to a stable mount in the bottom of a 10-ml organ bath filled with carbogenated Krebs solution at 37 ± 1°C. The upper end was tied to an isometric force transducer (Harvard UF-1) connected to an amplifier and then to a computer. Data were digitized (25 Hz) and simultaneously displayed and collected using Datawin2 software (Panlab-Barcelona) coupled to an ISC-16 A/D card installed in a personal computer.

**Resting Membrane Potential Recordings**

Muscle strips were placed in a Sylgard-coated chamber and continuously perfused with carbogenated Krebs solution at 37 ± 1°C. In all cases, preparations were allowed to equilibrate for ~1 h before experiments started. Circular muscle cells were impaled with glass microelectrodes (resistance = 40–60 MΩ) filled with 3 M KCl. Membrane potential was measured using a standard electrometer Duo773 (WPI). Data were displayed on a digital storage oscilloscope 4026 (Racal-Dana) and simultaneously digitized (100 Hz) and collected using EGAA software coupled to an ISC-16 A/D card (RC Electronics) installed in a 486 personal computer.

In the preparations without submucosa (circular muscle with AP) the tissue was pinned with the circular muscle facing up. In the preparations with submucosa (circular muscle without the longitudinal layer and full-thickness strips), cells were impaled from the longitudinal side. We used this procedure because it was impossible to penetrate the submucosa without damaging the microelectrode. In some experiments, we measured the electrical and mechanical activity of the preparation simultaneously. In these experiments, one end was pinned for intracellular recordings and the opposite end was attached to an isometric transducer and preloaded with 1 g.

**Immunohistochemistry**

Specimens from the colon were quick-frozen in isopentane cooled in liquid nitrogen and stored at −80°C until freeze...
sectioning. The immunohistochemical techniques were as described previously (19). To demonstrate the c-Kit receptor polyclonal rabbit antibodies, c-kit (C-19, Santa Cruz Biotechnology) was used. Before incubation with the antibodies, all sections were covered with a solution of either swine or goat serum (1:5 or 1:10) for 20 min. Experimental sections were incubated overnight with the specific antibody (1:500). Control sections were incubated with rabbit IgG (Dako X 903). Immunoreactivity was demonstrated with the streptavidin-biotin (avidin-biotin complex) method or by the indirect fluorescence technique. To detect primary antibodies, the second and third layers were biotinylated swine anti-rabbit (Dako E 353) and streptavidin-avidin-biotin complex-horse-radish peroxidase (Dako K377) (incubation time, 1 h). For secondary antibodies with the indirect fluorescence technique, we used goat anti-rabbit IgG (heavy and light chain) conjugated with rhodamine (Jackson ImmunoResearch Laboratories). For light and fluorescence microscopy, we used a Zeiss Axioplan 2. Both intact tissue and specimens in which the submucosa or the longitudinal muscle had been fully or partially removed were examined as described above.

Electron Microscopy

The colonic tissue was prepared by two methods. In method A, colonic segments were cut and immersed in a fixative solution containing 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.1, at 4°C for 4 h. After immersion, pieces (1 x 2 mm) were cut, rinsed with 0.1 M phosphate buffer containing 6% saccharose, and postfixed in 1% OsO4 in 1 M phosphate buffer, pH 7.1 for 1.5 h. In method B, the entire colon was isolated and immediately immersed in 2% OsO4 in 0.1 M phosphate buffer, pH 7.1, for 1 min with constant agitation, followed by addition of 4–5 vol aldehyde fixative (2% glutaraldehyde, 2% formaldehyde, 0.1% picric acid, and 0.1 M phosphate buffer, pH 7.3). After 2–3 h at room temperature, the colon was rinsed with and transferred to fresh aldehyde fixative of the same composition. Small pieces of tissue (1 x 2 mm) were cut and postfixed in 2% OsO4 for 1 h. Tissue prepared by either method was dehydrated in a graded series of alcohol, block stained in uranyl acetate (2% in ethanol 70%) for 1 h at 4°C, and embedded in Epon 812 R (Merck). Thin sections were stained with toluidine blue for light-microscopy investigation. Suitable areas were selected for transmission electron microscopy, and ultrathin (50–70 nm) sections were cut, mounted on copper grids, and contrasted with uranyl acetate and lead citrate. The grids were examined in Philips 300 or 400 electron microscopes (Eindhoven, The Netherlands).

Drugs

The following drugs were used: nifedipine and phentolamine (Sigma Chemical, St. Louis, MO), and TTX, atropine.

CIRCULAR MUSCLE (SMP-AP)

Fig. 3. Microelectrode recording from a full-thickness strip. A: overview of the electrical activity showing the presence of two rhythmic cyclic activities. B: detail of the low-frequency oscillation triggering muscular action potentials. C: detail of the slow wave activity.
sulfate, and propranolol (Research Biochemicals International, Natick, MA). Stock solutions were prepared by dissolving drugs in distilled water, except for nifedipine, which was dissolved in ethanol.

Data Analysis and Statistics

Data are expressed as means ± SE. A paired Student’s t-test was used to compare mechanical activity in the absence and presence of drugs. P < 0.05 was considered to be statistically significant.

RESULTS

Spontaneous Mechanical Activity

The circular muscle of the rat colonic strips with the submucosal layer kept intact showed spontaneous cyclic mechanical activity composed of two types of phasic contractions (Fig. 1A): low-frequency and high-amplitude contractions with superimposed high-frequency and small-amplitude contractions (n = 9; Table 1). In contrast, the longitudinal muscle in all the full-thickness preparations just showed the low-frequency and high-amplitude cyclic contractions exclusively (n = 30; Fig. 1C and Table 1). The circular muscle strips devoid of submucosa showed the low-frequency and high-amplitude cyclic contractions exclusively (n = 30; Fig. 1C and Table 1). The circular muscle with submucosa and without the longitudinal muscle layer displayed the high-frequency and low-amplitude contractions (n = 5; Fig. 1D and Table 1).

In the presence of 1 μM TTX (n = 9), the low-frequency contractions increased in amplitude (1.81 ± 0.19 vs. 2.72 ± 0.47 g; P < 0.05) and frequency (0.61 ± 0.07 vs. 0.79 ± 0.07 contractions/min; P < 0.005) whereas the duration was not affected. In contrast, the high-frequency contractions were not modified in the presence of this neural blocker (n = 9) (Fig. 2A). In the presence of 1 μM nifedipine, both types of contraction were abolished (n = 20; Fig. 2B). Similar results for TTX and nifedipine were obtained when both types of activities were studied separately using the circular muscle without AP or SMP, respectively (n = 4 each).

Spontaneous Electrical Activity

Full-thickness strips. In this preparation, the electrical activity consisted of two types of cyclic depolarizations (n = 6; Fig. 3A). Cyclic depolarizations (9.3 ± 1.6 mV; 19 ± 4 s) at a frequency of 1.4 ± 0.2 cycles/min triggered several muscular action potentials (20 ± 3) (Fig. 3B). In between the cyclic depolarizations, slow waves at a frequency of 15 ± 1 cycles/min were recorded (Fig. 3C). These slow waves lasted 3.3 ± 0.4 s and showed an amplitude of 4.3 ± 0.4 mV. The minimum resting membrane potential measured at the bottom of the slow waves was -53 ± 4 mV. This electrical activity, with two putative pacemakers, was related to the mechanical activity described before when the circular muscle with AP and SMP was studied (Fig. 1A).

Strips without SMP. The resting membrane potential of circular muscle cells from the rat colon was -52.1 ± 0.6 mV (n = 6). The circular muscle cells showed cyclic depolarizations (4.9 ± 0.3 mV) of the membrane potential that triggered a number of action potentials at their peaks (17.4 ± 3.2 action potentials/depolarization). Each episode of depolarization with associated action potentials correlated with a low-frequency and high-amplitude contraction and appeared at a frequency of 0.78 ± 0.11 episodes/min (Fig. 4A). These cyclic depolarizations and the corresponding action potentials were abolished in the presence of 1 μM nifedipine (n = 5; Fig. 4B).

Strips without longitudinal muscle. When the longitudinal muscle was removed, the slow-frequency cyclic depolarization disappeared (n = 5). Only slow waves at a frequency of 14.2 ± 1.93 cycles/min were recorded. Figure 5 shows an example from three different recordings. Some slow waves carried one to three muscular action potentials (Fig. 5, A and B). The resting membrane potential measured at the bottom of the slow waves was -52 ± 2 mV. The mean amplitude of the slow waves was 5.3 ± 0.3 mV, and the duration was 3.8 ± 0.5 s. This electrical rhythm was similar to the...
mechanical rhythm of the circular muscle when the longitudinal muscle was removed (Fig. 1D). When 1 μM nifedipine \((n = 4)\) was added to the bath, a residual cyclic activity was still recorded but action potentials at the top of the slow waves were abolished.

**Immunohistochemistry**

In the rat colon, Kit immunoreactivity was present in cells that enveloped the ganglia and fascicles of AP and in cells at the border between the circular muscle layer and the submucosa \((n = 3; \text{Fig. } 6A)\). Weaker staining was observed in subserosal and ramified cells running parallel with circular muscle cells (Fig. 6B). Removal of submucosa resulted in abolition of Kit immunoreactivity at the submucosal border of the musculature \((n = 3; \text{Fig. } 6C)\).

**Electron microscopy**

ICC were identified throughout the colon at the sites of the AP between the main muscle layers and the SMP at the submucosal surface of the circular muscle (Figs. 7, 8, and 9). These ICC were referred to as ICC-AP and ICC-SMP, respectively. Both populations of ICC were arranged in cellular networks with branching processes in close contact with smooth muscle cells and nerves. ICC have a cytoplasm rich in cell organelles, including smooth and granular endoplasmic reticulum, Golgi apparatus, and a large number of mitochondria mainly in the branching processes. ICC displayed a basal lamina, most prominent at the submuscular site, and many caveolae in both sites. Bundles of thin filaments were prominent in all parts of the cytoplasm and attachment plaques were seen, whereas thick filaments were not identified. In contrast, fibroblast-like cells did not show close contact with muscle cells, nerves, or ICC and were located close to collagen bundles. Although the fibroblasts also showed a differentiated cytoplasm, they generally had larger, dilated cisternae of granular endoplasmic reticulum and formed long, thin (in 3 dimensions: sheet-like) exten-

---

**Fig. 5.** Slow wave activity in circular smooth muscle with intact submucosal border from 3 different preparations (with different time scales). Notice the presence of a few inhibitory junction potentials in B.
sions. They were devoid of basal lamina and caveolae, and filaments were less prominent (Fig. 8). In addition to ICC-AP and ICC-SMP, some intramuscular ICC were also identified (by similar criteria) among circular fibers.

In those preparations where the submucosa had been stripped off, no intact ICC could be identified at the submucosal surface of the circular muscle. However, several unidentified dead cells, possibly ICC, with a broken plasma membrane remained at the submucosal border of the circular muscle. Some ICC and dead cells remained attached to the submucosa (Fig. 10).

Tissue strips, from which the longitudinal muscle had been removed and in which the electrical activity from the circular muscle had been recorded, were processed and sectioned. Examination by light and electron microscopy of the central region of the strips (the region used for recording) confirmed the successful removal of longitudinal muscle. A few ganglia still adhered to the circular muscle. The majority of adhering interstitial cells were grossly damaged, and very few ICC-AP could be recognized.

**DISCUSSION**

In the present study, we show that two pacemakers are involved in the electrical activity of the rat colon. When the full-thickness strip was studied, a low-frequency electrical rhythm that provokes muscular action potential and high-amplitude contractions is superimposed with slow waves corresponding to the low-amplitude mechanical activity. The interaction between these two pacemakers determines the electrical and mechanical activity of the smooth muscle. The electrical activity is correlated to the ICC distribution.

The pacemaker activity of the colon has been well studied (6, 14, 26, 27) in the dog. In the canine colon, the ICC network located near the SMP is responsible for cyclic slow waves (5–6 slow waves/min) whereas high-frequency cyclic depolarizations may originate from the ICC network located at the site of AP (6, 14, 26, 27). In small rodents, the pacemaker activity is not so well characterized. In the mouse, cyclic depolarizations occur at a frequency similar to the one we found in the rat colon (15, 16, 32). However, the origin of this
cyclic activity is controversial. When the whole colon of the mouse was studied (16), cyclic depolarizations seemed to be neurogenic because TTX and hexamethonium abolished the cyclic activity. In contrast to these results, the cyclic activity of the mouse colon was well correlated to the development of ICC (32), suggesting that interstitial cells might be the pacemaker of this activity. In small strips from the circular muscle of the rat colon, cyclic depolarizations insensitive to TTX have been described (Ref. 20 and present study). These results suggest that the pacemaker activity is not neurogenic when small strips are studied. Cyclic depolarizations can be recorded in strips without intact ICC-SMP. This result shows that the ICC-SMP is not the pacemaker of this cyclic activity but does not exclude the ICC-AP network as the origin. In agreement with this hypothesis, Ward and co-workers (32) suggested that the pacemaker responsible in mouse colon for the cyclic activity is the ICC network found near the myenteric plexus. Another possibility is that the cyclic activity originated in the smooth muscle itself. However, previous data from our laboratory, using the patch-clamp technique, show that isolated myocytes do not oscillate cyclically (unpublished data). It is interesting to notice that the cyclic activity found in small rodents is very similar to nonneurogenic cyclic depolarizations (2–4 cycles/min) found in the circular muscle of the human colon (21). In the latter study, these cyclic depolarizations were described as slow waves although it is not clear if the cyclic activity was sensitive to L-type Ca\(^{2+}\) channel blockers. In the rat colon, cyclic depolarizations are abolished in the presence of nifedipine and consequently they are not classical slow waves. In contrast to what we found in the rat, the low-frequency cyclic depolarizations might originate near the SMP in the human colon (21). Another interesting finding was that the cyclic contractions we found in the circular muscle were also observed in the longitudinal muscle. As we discovered in the circular muscle, this cyclic mechanical activity was increased by TTX and abolished by nifedipine. This shows that this cyclic activity is not neurogenic. An interesting hypothesis is that the cyclic activity found in both the circular and the longitudinal muscle might have the same origin. Accordingly, the ICC network between the two layers might be responsible for the cyclic activity. In this case, coupling between the ICC and both muscular layers should be expected. In agreement with this hypothesis, MPO originating in the ICC-AP network of canine colon spread into the longitudinal and circular muscle (26). It is interesting to notice that electromyographic recordings display cyclic spike bursts that have the same frequency as the cyclic mechanical activity found in either the circular or longitudinal layer (9). According to our results, these spike bursts originate near the myenteric plexus and are probably related to action potentials from both circular and longitudinal muscles that might contract simultaneously.

In the dog colon, high-amplitude low-frequency (5–6 cycles/min) slow waves can be recorded in smooth muscle cells near the submucosal border. We were able to record slow waves and cyclic contractions when we used strips with an intact submucosal border region. In contrast, when we used preparations devoid of intact ICC-SMP this cyclic electrical and mechanical activity was not recorded. These results suggest that the ICC-SMP network is probably the pacemaker that generates slow waves and the corresponding mechanical activity. It is important to notice that it is extremely difficult to record slow waves in small rodents (15, 32). This might well be a purely technical problem. Our results demonstrate that it is important to preserve the submucous plexus area to record slow waves. Obviously, it is very hard to impale circular muscle cells by penetration of the submucosa because the microelectrode breaks. To avoid this problem, we impaled circular muscle cells in strips that had the longitudinal muscle removed by fine dissection. Using this procedure, we exposed the circular muscle with the submu-
cosal border intact, and recordings displayed slow waves and the corresponding mechanical activity. Electrical slow waves were partially resistant to nifedipine, although the mechanical activity and action potentials triggered at the top of the slow waves were nifedipine sensitive.

To confirm our hypothesis suggesting the presence of two pacemakers, we tried to record both pacemakers simultaneously. According to our previous arguments, we attempted to impale circular muscle cells through the longitudinal muscle, which is extremely thin in the rat. With the electrode somewhat deep in the preparation we were able to record both pacemakers at the same time. It is likely that under these conditions we actually recorded from the circular muscle, because we never found similar mechanical activity when the longitudinal muscle was studied. This, together with the correspondence between the electrical and mechanical activities, supports our hypothesis that slow waves originate in the ICC at the submucosal border. The slow waves spread into the circular muscle but do not reach the longitudinal layer. Moreover, the slow-frequency cyclic oscillations producing high-amplitude contractions probably originate in the AP and spread into the circular and longitudinal layers.

Our results using microelectrodes and muscle bath experiments are consistent with the distribution of ICC. We were able to identify two networks of ICC associated with AP and the SMP. This distribution is very similar to those described in other species, including humans (8, 23), dogs (1, 2), and mice (7). The ICC-SMP network was not present in the preparations devoid of submucosa.

In conclusion, this study indicates that two pacemakers exist in the circular muscle of the rat colon. Cyclic depolarizations occur at a low frequency, inducing cyclic contractions. Both cyclic depolarizations and contractions are abolished in the presence of L-type Ca\(^{2+}\) channel blockers and consequently they are not classical slow waves (the pacemaker input to drive

Fig. 8. Medium-power electron micrograph. ICC-AP (at right) could be identified and distinguished from fibroblasts (at left) by the presence in ICC-AP of caveolae, a partial basal lamina, a higher number of mitochondria, and fewer and nondilated endoplasmic reticulum cisternae. The cytoplasmic density of the ICC may vary between preparations, depending on fixation methods. Magnification, \(\times23,000\).
Fig. 9. Medium-power electron micrograph. ICC-SMP were identified by position and cytoplasmic detail: numerous mitochondria, many caveolae (* indicates prominent areas), and a distinct and complete basal lamina (arrows). Magnification, ×16,000.

Fig. 10. Low-power electron micrograph. Before fixation, the submucosa had been carefully separated from the musculature up to the site marked by the full line (the cleavage marked by the dashed line). Note the disrupted border layer of the musculature at left, compared with the much less affected cells at right, including intact ICC-SMP (compare with Fig. 6C). Magnification: ×1,800.
slow waves in the intestine or colon is nifedipine insensitive). The ICC-SMP network is clearly not essential for this cyclic activity, because it is present in strips devoid of intact ICC-SMP. When the submucosal region is preserved and the longitudinal muscle removed, slow waves and muscular contractions (13–14/min), can be recorded. These slow waves are partially nifedipine insensitive. Consistent with our electrophysiological and mechanical studies, we identified two ICC networks, associated with the SMP and AP, using electron microscopy and immunohistochemistry. We suggest that these ICC constitute two networks of pacemaker cells responsible for the observed cyclic activities.

This coordination between two pacemakers is not all that different from what has been described in the dog (1, 2, 27) or cat (5): the ICC-SMP network is responsible for the 6–7/min slow waves and mechanical contractions as it has been described in these species. Although the ICC-AP is responsible for high-frequency contractions in the dog and cat, trains of MPO at 1–2 min intervals also exist in the cat (5). These trains of MPO induce cyclic phasic contractions similar to those we described in the rat. In this sense, MPO and cyclic depolarizations described in this study might have the same origin, spread to both circular and longitudinal layers, and cause similar cyclic contractions. However, two major questions about the origin of both cyclic activities remain to be answered: (1) which input, probably coming from ICC-SMP, allows the slow wave generation and (2) which input, probably coming from ICC-AP, allows the smooth muscle to reach the threshold to open L-type Ca$^{2+}$ channels? Answer to these questions should help us understand how the rat colon works and, as rat colon has been extensively used as a model of colitis, to pinpoint the origin of motility changes found in such conditions.

This work was supported by Direcció General de Ensenància Superior e Investigacion Científica Grant PM-98–0171, Generalitat found in such conditions.

REFERENCES


