Role of \( \text{L-Ca}^{2+} \) channels in intestinal pacing in wild-type and \( \text{W/W}^V \) mice

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Boddy, Geoffrey, and E. E. Daniel. Role of \( \text{L-Ca}^{2+} \) channels in intestinal pacing in wild-type and \( \text{W/W}^V \) mice. \textit{Am J Physiol Gastrointest Liver Physiol} 288: G439–G446, 2005. First published October 14, 2004; doi:10.1152/ajpgi.00255.2004.—Rhythmic contractions generating transit in the digestive tract are paced by a network of cells called interstitial cells of Cajal (ICC) found in the myenteric plexus (MP). ICC generate cyclic depolarizations termed “slow waves” that are passively transmitted to the smooth muscle to initiate contractions. The opening of \( \text{L-Ca}^{2+} \) channels are believed to be primarily responsible for the influx of calcium generating a contraction in smooth muscle. However, \( \text{L-Ca}^{2+} \) channels are not thought to be important in generating the pacing current found in ICC. Using intact segments of circular (CM) and longitudinal (LM) muscle from wild-type mice and mice lacking c-kit kinase (\( \text{W/W}^V \)), we found that \( \text{L-Ca}^{2+} \) channel currents are required for pacing at normal frequencies to occur. Application of 1 \( \mu \text{M} \) nicardipine caused a significant decrease in contraction amplitude and frequency in LM and CM that was successfully blocked with BAY K 8644. Nicardipine also abolished the pacing gradient found throughout the intestines, resulting in a uniform contraction frequency of 30–40/min. Stimulating \( \text{L-Ca}^{2+} \) channels with BAY K 8644 neither removed nor recovered the pacing gradient. \( \text{W/W}^V \) mice, which lack ICC-MP, also exhibited a pacing gradient in LM. Application of nicardipine to LM segments of \( \text{W/W}^V \) mouse intestine did not reduce pacing frequency, and in jejunum, resulted in a slight increase. BAY K 8644 did not affect pacing frequency in \( \text{W/W}^V \) tissue. In conclusion, we found that \( \text{L-Ca}^{2+} \) channel activity was required for normal pacing frequencies and to maintain the pacing frequency gradient found throughout the intestines in wild-type but not in \( \text{W/W}^V \) mouse intestine.

Interstitial cells of Cajal; gradient of pacing frequency; nicardipine

STUDIES BASED ON ISOLATED, dissected circular muscle (CM) and isolated cultured interstitial cells of Cajal (ICC) have led to a model of pacing in the mouse intestine (19, 24, 28, 34, 36). This model hypothesizes that the network of ICC in the myenteric plexus (MP) provides the currents to pace slow waves in both the longitudinal muscle (LM) and CM layers through low-resistance gap junctions. Much of the evidence for the essential role of ICC-MP in pacing comes from studies of genetically modified mice lacking c-kit kinase (\( \text{W/W}^V \) mice) and, as a consequence, having no network of ICC-MP and no slow waves in CM (7, 13, 35). Currents in ICC are believed to be generated by Ca\(^{2+}\) release from the endoplasmic reticulum (ER) of ICC mediated through the inositol 1,4,5-trisphosphate \([\text{Ins(1,4,5)P}_3]\) receptor, leading to Ca\(^{2+}\) uptake by mitochondria and subsequent depletion of Ca\(^{2+}\) near the plasma membrane (22, 25, 31, 34, 36). This is thought to initiate an inward current through nonspecific cation channels. What keeps the system producing regular spontaneous inward currents is unclear. Others studies (14) have suggested that an inward current carried by Cl\(^-\) is involved. The role of \( \text{L-Ca}^{2+} \) channels has been considered negligible (12, 19, 21, 28, 37) except in mouse proximal colon (38).

However, this theory is not supported by other studies (5, 6, 30) that have found an absence of gap junctions connecting ICC-MP to muscles and the absence of inhibition of pacing after gap junction uncouplers using small segments of LM or CM. Furthermore, the role of Ca\(^{2+}\) release from ICC ER has been questioned by evidence that putative blocking of Ins(1,4,5)P\(_3\)-mediated release from ER, such as by 2-aminoethoxyphenyl borate is likely nonspecific, whereas other agents, such as xestospongin C, are ineffective (1). Also, the sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) pump blockers cyclopiazonic acid or thapsigargin have not completely blocked pacing in intact muscle segments, as reported in cultured and dissected intestine (1). Inhibition of mitochondrial Ca\(^{2+}\) uptake by carbonyl cyanide-p-(triflouromethoxy)phenylhydrazone also was questioned, because of its serious effects on cellular function.

Studies of segments of intestine from \( \text{W/W}^V \) mice revealed that the LM segments had a robust pacing function not abolished by block of \( \text{L-Ca}^{2+} \) channels or affected by depletion of ER Ca\(^{2+}\) stores (4). There appear to be no records in the literature of intracellular recording from intestinal LM cells in wild-type or \( \text{W/W}^V \) mice. These findings suggest that models of pacing in mouse intestine by ICC-MP, based on studies of highly reduced systems, need to be reevaluated by using more intact systems.

The aims of this study were to evaluate the role of \( \text{L-Ca}^{2+} \) channels in pacing in segments of wild-type and \( \text{W/W}^V \) mice, and to compare the mechanisms of pacing in LM segments of \( \text{W/W}^V \) mice with those of LM and CM segments from wild-type mice.

MATERIALS AND METHODS

Preparation of Tissue

Male, 6- to 12-wk-old Balb/C, \( \text{W/W}^+ \), or \( \text{W/W}^V \) mice (from Jackson Laboratories, Bar Harbor, ME) were killed by cervical dislocation following the guidelines of our Institutional Animal Ethics Committee. After the opening of the abdominal wall, the digestive tract, beginning from the level of the stomach to the rectum, was removed from the mouse. Tissue was immediately placed into a beaker of Krebs Ringer solution that had been bubbled at room temperature (21–22°C) with carbogen (95% O\(_2\)-5% CO\(_2\)) for ~5 min before the dissection. In a petri dish filled with Krebs solution, continuously bubbled with carbogen, ileal (considered to be the distal third of the intestine) or jejunum (considered to be half of the intestine and located proximal to the ileum) tissue was isolated and cut into ~0.5 (for CM) or 1–1.5 cm (for LM) segments in preparation to mount onto glass holders with electrodes.

To study LM contractions, a tissue segment was placed between two platinum concentric circular electrodes and tied to a hook at the...

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bottom of the electrode holder with silk suture thread. The top of the tissue was also tied with thread and attached to a strain gauge. To study CM contractions, the open side of a thin metal triangle was slid through the lumen of the tissue segment. The triangle was then hooked together. A stainless steel rod attached to the bottom of the electrode holder was inserted into the lumen of the tissue under the metal triangle to secure the triangle and provide slight tension. Suture thread, attached at the apex of the triangle opposite to the tissue, was tied to the strain gauge. Two thin platinum rods, situated parallel to and on either side of the tissue, were used to stimulate the tissue electrically.

Two LM and two CM preparations were mounted and placed in muscle baths, filled with 10 ml Krebs solution and bubbled continuously throughout the experiment with 95% O2-5% CO2 and maintained at a temperature of 37°C. The thread connected to each tissue segment was then tied to a strain gauge (Grass FT-03). Tissues were subjected to slight tension, just sufficient to remove slack in the thread, and allowed to stabilize for 10 min. Tissue contractile activities were recorded on a Beckman Dynograph 611.

Experimental Protocol

Tissues were electrically field stimulated to test nerve activity (parameters: 50 V/cm, 0.5 ms, and 5 pps). LM from wild-type mouse intestines always responded with contraction, whereas CM usually responded with relaxation. TTX (10^{-6} M) and N^\text{6}-nitro-L-arginine (L-NNA; 10^{-4} M) were added to the baths to eliminate enteric nerve function and NO production. Within 1 min, CM contraction amplitudes increased. After 5 min, nerve activity was tested again by electrical field stimulation. If there was any response in the tissue, TTX was added again until all enteric nerve function was blocked. Frequencies of slow-wave-driven contractions were measured before and after TTX and L-NNA. After nerve activity was blocked by TTX and L-NNA, tension was adjusted to provide maximum spontaneous contractions and the experiment was carried out by using the pharmacological agents under investigation. In some experiments, electrical field stimulation was carried out at 50 V/cm, 10- or 30-ms pulses, and 60 Hz to pace segments electrically. At the end of each experiment, all tissues were washed twice with 10 ml Ca^{2+}-free Krebs with 1 mM EGTA to relax tissues to basal passive tension and abolish spontaneous contractions.

Experimental Procedures

Several types of procedures were used, and they will be described in depth in RESULTS. In general, for each experiment, one LM and one CM segment was used as experimental tissue and another of each as time control. Frequency and amplitude measurements were made every 5–10 min. Frequencies were measured over a period of at least 20 s. Contractions were regular and stable in time control over time (see RESULTS). Amplitudes of contractions were determined as the values above the passive tension determined at the end of the experiment.

Amplitude was measured by evaluating individual contractions over at least nine contractions and calculating a mean ± SE. In many cases, only the data before each experimental intervention and final measurements are presented. In the case of contraction amplitudes, results were normalized to the mean values in the control periods set as 100%. The n values represent the number of mice whose intestine provided segments for study.

Analysis of Data

Measurements were entered into Graph Pad Instat and analyzed by paired comparison, using Student’s paired t-test or ANOVA as appropriate. Figures 1–7 were plotted in Prism 3. Statistical significance
was \(*P \leq 0.05\), \(**P \leq 0.01\), \(***P \leq 0.001\), and \(****P \leq 0.0001\).

Comparisons between experimental tissues and time controls are 

\(#P \leq 0.05\), \(##P \leq 0.01\), \(###P \leq 0.001\), and \(####P \leq 0.0001\).

Drugs and Solutions

**Pharmacological agents.** TTX was from Alomone Laboratories (Jerusalem, Israel); L-NNA, BAY K 8644, and nicardipine were from Sigma-Aldrich (St. Louis, MO). Because some drugs were dissolved in DMSO, the solvent was added to time control tissues in equivalent amounts. The final concentration of DMSO did not exceed 0.1%, and had no significant effects on control tissues. DMSO was from Sigma-Aldrich.

**Solutions.** Krebs solution was (in mM) 115.5 NaCl, 21.9 NaHCO3, 11.1 glucose, 4.6 KCl, 1.16 MgSO4\(\cdot\)H2O, 1.16 NaH2PO4\(\cdot\)H2O, and 2.5 CaCl2\(\cdot\)H2O. Ca2\(+\)-free Krebs solution was (in mM) 115.5 NaCl, 21.9 NaHCO3, 11.1 glucose, 4.6 KCl, 1.16 MgSO4\(\cdot\)H2O, 1.16 NaH2PO4\(\cdot\)H2O, and 1.0 EGTA.

**RESULTS**

Application of Nicardipine

Nicardipine is a dihydropyridine, L-Ca2\(+\) channel antagonist that binds with highest affinity to the depolarized, inactivated state of the channel, causing the channel to remain inactivated (11, 33). It has the advantage over some other L-Ca2\(+\) channel antagonists in that it is not light sensitive. We have previously demonstrated the effect of 1 \(\mu\)M nicardipine on intestinal smooth muscle pacing to reduce the frequency and amplitude in both LM and CM. In this study, we examined the effect of 0.3, 1, and 3 \(\mu\)M nicardipine added consecutively and cumulatively to LM and CM strips from ileum and jejunum independently (Figs. 1 and 2). Final measurements of frequency and amplitude were taken 15 min after application of nicardipine at each concentration. A time control was also measured for comparison. In all cases, the time controls showed no significant change in frequency. However, time-control amplitudes decreased significantly in most experiments over time. All statistical comparisons of amplitude were done by using the time controls.

Application of 0.3, 1, and 3 \(\mu\)M nicardipine to jejunal LM caused concentration-dependent decreases in frequency (\(n = 10\)) (Fig. 1). The time controls had no significant changes in frequency over time (\(n = 10\)). Decreases in frequency after 1 and 3 \(\mu\)M nicardipine were significantly larger than 0.3 \(\mu\)M. Contraction amplitude in LM decreased after all concentrations of nicardipine were added (\(n = 5\)) (Fig. 1). These changes in amplitude, none significant from one another, likely represent direct actions of the drug on the muscle. Application of 0.3, 1, and 3 \(\mu\)M nicardipine to jejunal CM, like LM, caused decreases in frequency that were concentration dependent (\(n = 11\)) (Fig. 1). Amplitude in CM was also significantly decreased after all concentrations of nicardipine (\(n = 8\)) (Fig. 1). Effects of nicardipine were comparable between LM and CM.

To determine whether there were major differences in L-Ca2\(+\) activity between the jejunum and ileum, we conducted all experiments separately on muscle from each location. Application of 0.3, 1, and 3 \(\mu\)M nicardipine to ileum had the same effect as in the jejunum (\(n = 5\)) (Fig. 1), except for one
important difference. The application of nicardipine to jejunal and ileal smooth muscle resulted in similar contraction frequencies, regardless of initial frequency. Faster-paced jejunal muscle, and slower-paced ileal muscle all slowed to between 30 and 40 contractions/min after 3 μM of nicardipine was added. This occurred in LM and CM. A linear regression analysis showed the relationship between initial frequency and change in frequency (Fig. 3) for individual segments. In LM, we found an $R^2$ of 0.88 ($P \leq 0.01$) and a slope of 0.91, and in CM, a $R^2$ of 0.86 ($P \leq 0.01$) and a slope of 0.94 between the initial frequency and the change in frequency 15 min after 3 μM nicardipine. In comparison, there was no linear relationship found between initial and final frequencies (not shown).

**Application of BAY K 8644 and Nicardipine**

To determine whether L-Ca$^{2+}$ channel activity in smooth muscle was directly responsible for the pacing gradient found throughout the intestines, we applied the L-Ca$^{2+}$ channel agonist BAY K 8644 to muscle strips to determine whether pacing frequencies, particularly in the ileum, could be increased in this way. BAY K 8644 is a dihydropyridine, like nicardipine, but it acts as a L-Ca$^{2+}$ channel agonist by increasing the mean open time of the channel (11, 33). Furthermore, BAY K 8644 and dihydropyridine antagonists like nicardipine are thought to interact allosterically, which could account for some of our results outlined below. We applied BAY K 8644 at an initial concentration of 10$^{-7}$ M. After 10 min, we measured the frequency and amplitude and added more BAY K 8644 to a final concentration of 10$^{-6}$ M. After 10 min, we again measured frequency and amplitude and added 1 μM nicardipine. This was to determine whether the effects of nicardipine could be blocked by prior addition of an L-Ca$^{2+}$ channel agonist. Measurements were recorded 10 min after nicardipine application. These experiments were carried out in LM and CM strips from the jejunum and ileum (Fig. 4).

Application of 10$^{-7}$ M BAY K 8644 to jejunal LM strips caused no significant change in frequency. However, subsequent application of 10$^{-6}$ M BAY K 8644 caused a small increase in frequency ($n = 10$). Subsequent application of nicardipine caused no significant change in frequency ($n = 5$) (Fig. 4). Application of BAY K 8644 (10$^{-7}$ and 10$^{-6}$ M) did not significantly change amplitude ($n = 10$) but successfully blocked the inhibitory effect of nicardipine ($n = 5$) (Fig. 4). Application of BAY K 8644 (10$^{-7}$ and 10$^{-6}$ M) to jejunal CM strips did not significantly change frequency or amplitude ($n = 5$). Subsequent application of 1 μM nicardipine also had no significant effect on frequency or amplitude ($n = 5$). BAY K 8644 successfully blocked the effects of nicardipine in the jejunum, but had little effect on normal spontaneous contractions.

Application of 10$^{-7}$ M BAY K 8644 to ileal LM strips did not significantly affect frequency ($n = 9$). Subsequent application of 10$^{-6}$ M BAY K 8644 increased frequency, but still well below the frequency of jejunum ($n = 9$) (Fig. 4). Subsequent application of 1 μM nicardipine did not significantly change the frequency ($n = 4$). BAY K 8644 also blocked the effects of nicardipine successfully in the ileum. However, different from jejunal muscle, application of 10$^{-7}$ and 10$^{-6}$ M
BAY K 8644 to ileal LM caused an increase in contraction amplitude ($n = 8$) (Fig. 4). Subsequent application of nicardipine decreased the amplitudes back to control levels ($n = 4$). Application of BAY K 8644 ($10^{-7}$ and $10^{-6}$ M) and nicardipine to ileal CM did not significantly change frequency, and amplitude was increased only after $10^{-6}$ M. Subsequent application of 1 μM nicardipine caused a slight decrease in contraction amplitude ($n = 4$) (Fig. 4). However, this decrease is much less than that observed with nicardipine alone. The major difference observed between jejunum and ileum was the occurrence of increased contraction amplitude in ileum after BAY K 8644. In both tissues (LM and CM), the actions of nicardipine were blocked by prior application of BAY K 8644, but BAY K 8644 had little or no effect on pacing frequency.

In a separate series of experiments, we added nicardipine before BAY K 8644 to determine whether BAY K 8644 could successfully reverse the effects. In all cases, BAY K 8644 was unable to reverse the effects of nicardipine (not shown).

**W/W⁺⁺ and W/W⁺⁻ Frequency Comparison**

Strips from W/W⁺⁺ mice were placed into muscle baths and their contraction frequencies were recorded after nerve block. Ileum LM and CM, having respective frequencies of $43.0 \pm 1.0$ and $39.3 \pm 1.1$ contractions/min, were found to be significantly slower than jejunal LM and CM, having frequencies of $49.6 \pm 1.9$ and $49.1 \pm 0.9$ (Fig. 5A). Furthermore, ileal LM had a higher average frequency than ileal CM. LM strips from W/W⁻⁻ mice were also studied, and jejunal LM was found to have a frequency of $50.2 \pm 2.3$ contractions/min (Fig. 5B), compared with ileal LM paced at an average frequency of $37.0 \pm 1.5$ contractions/min ($P \leq 0.0001$, $n = 21$), similar to wild-type intestine despite the absence of ICC-MP.

**W/W⁺⁺ Compared With Balb/C mice**

In this series of experiments, similar to previous experiments on Balb/C mice, nicardipine and BAY K 8644 were added to LM and CM muscle tissue preparations from W/W⁺⁺ mice. These experiments successfully replicated those done previously in Balb/C tissue and described in Application of Nicardipine (not shown).

**W/W⁻⁻, Application of Nicardipine, and BAY K 8644**

We have previously reported that W/W⁻⁻ LM displays robust pacing that is not susceptible to blockade by cyclopiazonic acid or nicardipine. In this study, we examined more closely the role of L-Ca²⁺ channels in W/W⁻⁻ LM pacing. Unlike wild-type tissue, application of 1 μM nicardipine to jejunal LM increased contraction frequency slightly ($n = 8$) (Fig. 6). Subsequent application of $10^{-6}$ M BAY K 8644 lowered frequency back down to control levels. Effects on amplitude were not different from previous experiments on Balb/C or W/W⁺⁺ mice (Fig. 6).

In ileal segments, application of 1 μM nicardipine and subsequent BAY K 8644 did not significantly change frequency ($n = 8$) (Fig. 6). Also, application of BAY K 8644 and subsequent nicardipine did not significantly change frequency from controls, and effects on amplitude were not different from those observed in wild-type tissue ($n = 8$). Overall, W/W⁻⁻ LM tissue responded with no change or an increase in frequency after nicardipine. This is in contrast to the consistent decrease in frequency observed in Balb/C and W/W⁺⁺ intestine (Fig. 7).

**DISCUSSION**

In this study, we demonstrated that L-Ca²⁺ channels are involved in intestinal smooth muscle pacing and that they may be a necessary component of pacing responsible for the differences in frequency found between the jejunum and ileum. We also demonstrated that pacing in W/W⁻⁻ LM lacking an ICC-MP network is not controlled by the same L-Ca²⁺ channel-dependent mechanism as wild-type tissue. Previous work done on cultured and dissected tissue has suggested that L-Ca²⁺ channels do not make a significant contribution to pacing in mouse intestinal smooth muscle (12, 21, 28, 37). There have also been a number of studies done in intact rabbit, guinea pig, and porcine intestine, which suggest L-Ca²⁺ channels are only involved in smooth muscle contraction and not in pacing (2, 8,
Our findings show that a closer look must be taken at the role of L-Ca\textsuperscript{2+} channels in ICC-driven pacing.

We found that the effects of nicardipine were dependent on concentration. Nicardipine (0.3 \textmu M) had a smaller effect than 1 or 3 \textmu M nicardipine, which were similar. Blockade of L-Ca\textsuperscript{2+} channels consistently reduced the frequency of pacing in intestinal smooth muscle to 30–40 contractions/min. This is likely due to the effect on ICC-MP, because contractions

Fig. 6. Nicardipine (1 \textmu M) and BAY K 8644 (10–6 M) were added to W/W\textsuperscript{+} jejenum (A) and ileum (B). Only recordings from the LM were made. Unlike in wild-type tissue, nicardipine did not attenuate contraction frequency, and in jejenum, LM caused a slight increase. Statistical significance was *\(P \leq 0.05\), **\(P \leq 0.01\), ***\(P \leq 0.001\).

Fig. 7. A: examples of individual traces from Balb/C, W/W\textsuperscript{++}, and W/W\textsuperscript{V} mice tissue after application of 1 \textmu M nicardipine and BAY K 8644. All traces are from jejunal LM. The dashed lines indicate zero tone. Increases in amplification are shown above individual traces. Each trace is 20 s in duration. The scale bar is 1 g/2 s. B: average contraction frequencies from jejunal LM segments in Balb/C, W/W\textsuperscript{++}, and W/W\textsuperscript{V} mouse intestine after 1 \textmu M nicardipine and BAY K 8644. Statistical significance was *\(P \leq 0.05\), **\(P \leq 0.01\), and ***\(P \leq 0.001\).
remained regular although smaller, but effects on slow-wave transmission and smooth muscle contractility may also contribute. The cycling of calcium among the cytosol, ER, and mitochondria has been suggested to be required for ICC pacing (34, 36). It is likely that L-Ca²⁺ channels are also important in this process, as a source of calcium, either directly or to refill Ca²⁺ stores, and/or a signal to trigger the release of calcium from intracellular stores. An L-Ca²⁺ channel, dihydropyridine-sensitive current, has been found to account for ~50% of the voltage-dependent inward current in cultured ICC from the mouse small intestines (16). By influencing the influx of calcium through L-Ca²⁺ channels, the pacing mechanism can be regulated. For example, Jackson et al. (15) have found that an autoantibody, produced in type 1 diabetes, disrupts intestinal motility by activating smooth muscle L-Ca²⁺ channels at the dihydropyridine binding site. Furthermore, pacing abnormalities found in Crohn’s disease and some inflammatory bowel disorders may be partly caused by decreased ICC or smooth muscle L-Ca²⁺ channel activity (17, 23).

The blockade of L-Ca²⁺ channels appeared to have large, direct effects on smooth muscles, observed as decreases in contraction amplitude. We cannot be sure that the effects of nicardipine on smooth muscle account for this, because an effect on slow-wave transmission to muscle might be involved, but it is the most likely explanation. As described in the introduction, slow waves in mouse intestine are not considered to be affected by block of L-Ca²⁺ channels, but action potentials are abolished. We have observed a high level of L-Ca²⁺ channel expression in both LM and CM in the mouse intestine as well as ICC, and nicardipine at 1 μM blocked KCl-induced contractions in LM and CM, which are independent of ICC function (unpublished observations). These results support previous experiments demonstrating the importance of L-Ca²⁺ channels in smooth muscle contractility (2, 8, 10).

Frequency differentials in ICC pacing along the intestine are crucial for normal transit of gut contents. Typically the jejunum contracts at a higher frequency than the ileum (4, 32). Investigations in animals and humans have shown that electrical pacing at higher frequencies in distal sites drives the intestine slow waves in a reverse direction and delays or reverses transit. Thus the basis for the intestinal frequency gradients of slow waves is of major physiological significance (3, 9, 20, 29).

Our initial experiments suggested that the difference in pacing between the jejunum and ileum might be due to ICC-MP-based L-Ca²⁺ channel activity. From these results and previous reports that BAY K 8644 increases both cytosolic calcium levels in antral smooth muscle (26) and voltage-dependent inward currents in cultured ICC (16), we expected that the application of a L-Ca²⁺ channel agonist would increase pacing in the ileum to that of the jejunum. This was not the case. Instead, only a time-dependent decrease in pacing was prevented. However, the effects of nicardipine were reduced or completely blocked, indicating that BAY K 8644 was successfully agonizing the L-Ca²⁺ channels. Due to the inability of an L-Ca²⁺ channel agonist to increase pacing, our hypothesis that L-Ca²⁺ channels were alone responsible for the difference in pacing observed in the jejunum and ileum was weakened. Clearly, it is not simply differences in the level of activation of L-Ca²⁺ channels in different intestinal segments, which accounts for frequency differences. However, it remains certain that L-Ca²⁺ channel activity is required for intestinal muscle to be paced above a certain level.

In W/W¹ mouse intestine that does not have ICC-MP, we found LM muscle from the jejunum to be paced faster than the ileum, similar to wild-type tissue that has ICC-MP. This difference was originally thought to originate exclusively from ICC-MP, but this finding suggests that there is a conservation of the gradient of pacing frequency between W/W¹ and wild-type intestine that persists despite the absence of a network of ICC-MP. There are, however, major differences that exist between LM pacing in W/W¹ and wild-type intestine: they now include the lack of responses in frequencies of W/W¹ LM to SERCA pump and L-Ca²⁺ channel blockade (4). In this report, we also found that W/W¹ LM did not respond to L-Ca²⁺ channel block except in some instances in jejunal LM where pacing was found to increase after exposure to nicardipine. From these findings it is clear that pacing in W/W¹ LM is driven by a different pacemaker than in wild-type muscle. Possible sources for this pacemaker include serosal ICC, intramuscular ICC, and the LM itself.

We have observed a network of serosal ICC present in both wild-type and W/W¹ mouse intestine. Techniques currently used to isolate ICC for culture do not distinguish between various types of ICC or their location of origin (19). It is possible that serosal ICC may take on the role of pacemaker in W/W¹ intestine. This could account for the differences found, although it would also suggest that serosal ICC have a pacemaker mechanism that is drastically different than that of wild-type ICC-MP. So far, there is no information about the coupling of serosal ICC to LM, either structural or functional. The other possibility is that LM retains some pacemaker function, because LM originates from the same mesenchymal precursor cells as ICC-MP (18, 27, 39, 40).

In conclusion, we have demonstrated an important role for L-Ca²⁺ channels in pacing of mouse intestine. Most of the work done in this area has focused on cultured and dissected mouse intestine. Using intact muscle segments, we have found differences between jejunum and ileum that could not be studied by using current techniques of cell culture and dissection. A closer examination of the role of L-Ca²⁺ channels may help to uncover the basis for differences in spontaneous pacing found throughout the intestine. It also appears clear that pacing in W/W¹ LM is driven by a separate pacemaker. The nature of this pacemaker is not known, but the presence of intact networks of serosal ICC in W/W¹ intestine suggests they could be involved. Presently, ICC-MP have been the focus of study and the involvement of other ICC needs to be addressed.

REFERENCES


10. G446 ROLE OF L-Ca²⁺ CHANNELS IN INTESTINAL PACING


