Pacemaker potentials generated by interstitial cells of Cajal in the murine intestine

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Kito, Yoshihiko, Sean M. Ward, and Kenton M. Sanders. Pacemaker potentials generated by interstitial cells of Cajal in the murine intestine. Am J Physiol Cell Physiol 288: C710–C720, 2005. First published November 10, 2004; doi:10.1152/ajpcell.00361.2004.—Pacemaker potentials were recorded in situ from myenteric interstitial cells of Cajal (ICC-MY) in the murine small intestine. The nature of the two components of pacemaker potentials (upstroke and plateau) were investigated and compared with slow waves recorded from circular muscle cells. Pacemaker potentials and slow waves were not blocked by nifedipine (3 μM). In the presence of nifedipine, mibefradil, a voltage-dependent Ca2+ channel blocker, reduced the amplitude, frequency, and rate of rise of upstroke depolarization (dV/dt max) of pacemaker potentials and slow waves in a dose-dependent manner (1–30 μM). Mibefradil (30 μM) changed the pattern of pacemaker potentials from rapidly rising, high-frequency events to slowly depolarizing, low-frequency events with considerable membrane noise (unitary potentials) between pacemaker potentials. Caffeine (3 mM) abolished pacemaker potentials in the presence of mibefradil. Pinacidil (10 μM), an ATP-sensitive K+ channel opener, hyperpolarized ICC-MY and increased the amplitude and dV/dt max without affecting frequency. Pinacidil hyperpolarized smooth muscle cells and attenuated the amplitude and dV/dt max of slow waves without affecting frequency. The effects of pinacidil were blocked by glibenclamide (10 μM). These data suggest that slow waves are electrotonic potentials driven by pacemaker potentials. The upstroke component of pacemaker potentials is due to activation of dihydropyridine-resistant Ca2+ channels, and this depolarization entrains pacemaker activity to create the plateau potential. The plateau potential may be due to summation of unitary potentials generated by individual or small groups of pacemaker units in ICC-MY. Entrainment of unitary potentials appears to depend on Ca2+ entry during upstroke depolarization.

Pacemaker activity; slow waves; gastrointestinal motility; calcium channel

Small intestinal smooth muscles display rhythmic electrical activity known as slow waves (28, 32). Interstitial cells of Cajal in the plane of the myenteric plexus (ICC-MY) serve as the pacemakers for slow-wave activity because slow waves are absent from the small intestinal muscles of animals lacking ICC-MY (8, 33, 34). The ionic basis for the generation of slow waves has been studied in short-term, primary cell cultures of ICC-MY isolated from murine small intestine using the patch-clamp technique (18, 35). ICC-MY generate pacemaker currents that appear to be due to periodic activation of a nonselective cation conductance (18), although the involvement of a Cl− conductance also has been suggested (9, 31). Thus the precise ionic mechanism responsible for the generation and propagation of pacemaker currents in ICC-MY in situ remains controversial.

Pacemaker potentials of in situ ICC-MY were first recorded in the guinea pig gastric antrum (3). Gastric pacemaker potentials have two components: a primary component and a plateau component (5, 13). Pacemaker potentials conduct electrotonically to smooth muscles cells (3, 14) and activate voltage-dependent conductances in these cells. Responses to pacemaker potentials recorded from smooth muscle cells have been termed slow waves. Slow waves have a secondary depolarization (regenerative component) superimposed on the electrotonic remnant of the pacemaker potential (see also Ref. 32). Intramuscular interstitial cells of Cajal (ICC-IM), which are distributed between smooth muscle cells (1), may contribute to the regenerative component (2). Recently, pacemaker potentials also were recorded in situ from ICC-MY in the murine small intestine (15). These potentials have at least two components: a rapidly rising upstroke component and a plateau component. The rate of rise of the upstroke depolarization (dV/dt max) was greatly reduced by Ni2+, Ca2+-free solution, and membrane depolarization with high-K+ solution. The plateau component was inhibited by DIDS and low-Cl− solution (15). Therefore, different mechanisms appear to be responsible for the two components of pacemaker potentials.

Recent studies have suggested that a dihydropyridine-resistant Ca2+ conductance may be important for the active propagation of pacemaker potentials within ICC networks (12). Such a conductance exists in cultured ICC-MY from murine small and large intestine (12) and in freshly dispersed ICC from the canine colon (20). Activation of this conductance was proposed to increase Ca2+ influx that phase advances (or entrains) Ca2+ release from inositol 1,4,5-trisphosphate (IP3) receptors. Mibefradil, an inhibitor of T-type Ca2+ channels, blocked the dihydropyridine-resistant Ca2+ conductance but did not block the primary pacemaker current (12). Thus this agent may be helpful in determining the role of the dihydropyridine-resistant Ca2+ conductance in pacemaker potentials recorded from ICC-MY in situ. We hypothesize that the upstroke transient in pacemaker potentials may be a reflection of activation of the dihydropyridine-resistant Ca2+ conductance. Ca2+ entry during the upstroke depolarization may entrain the activity of multitudes of active pacemaker units in ICC-MY, causing the unitary potentials generated by these active sites to summate and form the plateau component.

In the present study, we investigated the nature and role of the upstroke and plateau components of pacemaker potentials recorded in ICC-MY in situ of the murine small intestine using

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conventional microelectrode recording techniques. The frequency of pacemaker potentials was decreased when the upstroke component was inhibited by mibebradil. Reducing the upstroke caused resolution of unitary potentials, suggesting reduced entrainment of pacemaker activity. The results suggest that there is a causal relationship between the generation of the upstroke depolarization and the frequency of pacemaker potentials. The possible ionic mechanisms generating the upstroke and plateau components of pacemaker potentials are discussed.

METHODS

BALB/c mice between the ages of 30 and 60 days postpartum were obtained from The Jackson Laboratory (Bar Harbor, ME). Animals were anesthetized by administering isoflurane (Baxter, Deerfield, IL) inhalation and were exsanguinated after cervical dislocation. The use and treatment of animals was approved by the Institutional Animal Use and Care Committee at the University of Nevada. Segments of terminal ileum were removed from animals and opened along the mesenteric border in Krebs-Ringer buffer (KRB; see below for composition). The mucosal layers, the serosal layers, and a part of the longitudinal layers were carefully peeled away under a dissecting microscope. A tissue segment (~0.5 mm wide and 0.5 mm long) was pinned out with the serosal side uppermost in a recording chamber. Preparations were constantly perfused with KRB solution warmed to 37°C. Experiments were performed in the presence of 3 μM nifedipine throughout, which conveniently minimized the movement of the muscles without blocking pacemaker potentials.

Conventional microelectrode techniques were used to record intracellular electrical responses from smooth muscle tissues, and the glass capillary microelectrodes filled with 3 M KCl had tip resistances ranging from 90 to 150 MΩ. Signals were amplified with an Axoprobe amplifier, low-pass filtered (cutoff frequency 1 kHz), digitized, and stored on a computer for later analysis.

The bath chamber was constantly perfused with oxygenated KRB of the following composition (in mM): 118.5 NaCl, 4.5 KCl, 1.2 MgCl₂, 23.8 NaHCO₃, 1.2 KH₂PO₄, 11.0 dextrose, and 2.4 CaCl₂. The pH of the KRB solution was 7.3–7.4 when bubbled with 97% O₂-3% CO₂ at 37°C. Experiments were performed in the presence of 3 μM nifedipine (data not shown). When ICC-MY were impaled, the membrane potential dropped to between 65 mV (data not shown). When circular muscle cells were impaled, the membrane potential dropped sharply to a maximum negative potential of ~68 mV developed and pacemaker potentials had peak negative membrane potentials of ~68.7 ± 2.5 mV (n = 12; each n value represents the number of animals used). Pacemaker potentials occurred at a frequency of 24.2 ± 1.5 min⁻¹ (n = 12) and had peak amplitude of 58.9 ± 3.0 mV (n = 12), dV/dt max of 1.85 ± 0.29 V s⁻¹ (n = 12), and half-widths (measured at 50% peak amplitude) of 0.91 ± 0.09 s (n = 12). Thus pacemaker potentials depolarized ICC-MY to approximately ~9 mV.

Cells in which slow waves were recorded had peak membrane potentials of ~67.4 ± 2.7 mV (n = 12). Slow waves occurred at a frequency of 25.2 ± 1.4 min⁻¹ (n = 12) and had peak amplitudes of 30.0 ± 2.3 mV (n = 12), dV/dt max of 0.52 ± 0.08 V s⁻¹ (n = 12), and half-widths of 0.90 ± 0.08 s (n = 12). The peak amplitudes and dV/dt max measured from pacemaker potentials differed significantly from those of slow waves, while membrane potentials between the spontaneous depolarizations were not significantly different in the two types of cells. Comparison of the electrical properties of the spontaneous depolarizations with those reported previously (15) suggests that pacemaker potentials and slow waves were recorded from ICC-MY and circular smooth muscle cells, respectively.

When circular muscle cells were impaled, the membrane potential dropped sharply to a maximum negative potential of about ~65 mV (data not shown). When ICC-MY were impaled, membrane potential dropped to between ~20 and ~50 mV initially (Fig. 1A) and then increased gradually to nearly ~70 mV (Fig. 1B). In more than one-half of the cells tested...
(63.6%), unitary potentials (see Refs. 4 and 16 for definition and description of these events) were resolved during the intervals between pacemaker potentials just after impalement of ICC-MY (Fig. 1A). However, resolvable unitary potentials decreased as the resting membrane potential stabilized (Fig. 1B).

Effects of mibefradil on pacemaker potentials and slow waves. Previous studies have suggested that the initial upstroke depolarization of pacemaker potentials in ICC-MY of the murine intestine in situ may be due to activation of voltage-dependent Ca$^{2+}$ channels because NiCl$_2$, Ca$^{2+}$-free solution, and membrane depolarization with high K$^+$ solution greatly reduced dV/dt$_{\text{max}}$ (15). The voltage-dependent Ca$^{2+}$ channels hypothesized must be dihydropyridine-resistant, because this activity occurs in micromolar concentrations of nifedipine. A dihydropyridine-resistant conductance is expressed by murine intestinal ICC-MY, and current through these channels is blocked by mibefradil (12). To examine the possibility that a dihydropyridine-resistant Ca$^{2+}$ conductance is involved in the generation of the upstroke component of pacemaker potentials, we tested mibefradil on activity recorded from ICC-MY in situ. Mibefradil (1 $\mu$M) had no significant effect on any electrophysiological properties of pacemaker potentials (Figs. 2Ab and 5). In the presence of 10 $\mu$M mibefradil, the amplitude, frequency, and dV/dt$_{\text{max}}$ of pacemaker potentials were decreased without alteration in the half-width (Fig. 2, Ac and B, and Fig. 5). Subsequent application of 30 $\mu$M mibefradil further decreased the amplitude, frequency, and dV/dt$_{\text{max}}$, and increased the half-width of pacemaker potentials (Figs. 2Ad and 5). Resolution of unitary potentials was increased in the presence of 30 $\mu$M mibefradil in all preparations (Fig. 2Ad).

The period of depolarization between pacemaker potentials was greatly increased by treatment with mibefradil (Fig. 2, Ac and 2Ad). To compare the effects of mibefradil with control recordings, we fitted the diastolic depolarizations with single-exponential functions. Figure 3 shows an example of the effects of mibefradil on the time constants (\(\tau\)) of the fitted diastolic depolarizations. The average \(\tau\) value during control recordings was 0.59 $\pm$ 0.20 s \((n = 7)\). Mibefradil greatly increased the \(\tau\) values for the diastolic depolarization to 2.88 $\pm$ 1.53 s \((n = 7; P < 0.01; \text{Fig. 3B})\). These results suggest that activation of the mibefradil-sensitive current contributes to the initiation of the upstroke phase of pacemaker potentials. Mibefradil shifted the apparent threshold for initiation of the upstroke to more positive potentials. We tabulated this apparent shift in threshold by determining the voltage at which a sharp transition in dV/dt occurred. In control recordings, ICC-MY needed to depolarize by only a few millivolts before the upstroke was initiated. Thus, under control conditions, the take-off potential for the upstroke phase averaged $-62.0 \pm 2.0$ mV \((n = 7)\). In the presence of mibefradil (30 $\mu$M), the take-off potential shifted significantly to $-43.3 \pm 2.3$ mV \((n = 7; P < 0.01)\).

Mibefradil also depolarized membrane potential by 3.8 $\pm$ 1.2 mV \((n = 7)\) at 10 $\mu$M and by 6.1 $\pm$ 2.3 mV \((n = 7)\) at 30 $\mu$M. This depolarization may have been due to mibefradil’s effects on voltage-dependent K$^+$ channels (e.g., Ref. 21). The effects of 30 $\mu$M mibefradil on pacemaker potentials were not fully reversed 2 h after removal of the drug \((n = 3)\). The results of experiments with mibefradil are summarized in Fig. 5.

The effects of mibefradil on slow waves (i.e., events recorded from smooth muscle cells) were similar to its effects on pacemaker potentials. Mibefradil, at concentrations of 10 and 30 $\mu$M but not 1 $\mu$M, reduced the amplitude, frequency, and dV/dt$_{\text{max}}$ of slow waves (Fig. 4). Membrane potential between slow waves was depolarized by 3.7 $\pm$ 2.2 mV at 10 $\mu$M.

Fig. 2. Effects of mibefradil on pacemaker potentials recorded in the murine small intestine. Pacemaker potentials were recorded before (Aa) and during (Ab, 1 $\mu$M; Ac, 10 $\mu$M; Ad, 30 $\mu$M) exposure to mibefradil. B: superimposed high-speed traces of pacemaker potentials recorded in the absence (Bo) and presence (Bb) of 10 $\mu$M mibefradil. All traces were recorded from the same cell, which had an initial resting membrane potential of $-67$ mV.
mibebradil \((n = 8)\) and by \(8.4 \pm 0.7 \text{ mV}\) at \(30 \mu\text{M}\) mibebradil \((n = 8)\) (Figs. 4 and 5). As with pacemaker potentials, mibebradil converted slow waves into slowly developing changes in potential, indicating that modulation of pacemaker potentials is closely reflected in slow-wave activity. The transient repolarization at the end of the upstroke, which is distinctive behavior recorded from smooth muscle cells, was abolished in the presence of \(10 \mu\text{M}\) mibebradil (Fig. 4B). The effects of mibebradil on slow waves are summarized in Fig. 5.

**Effects of caffeine on pacemaker potentials and slow waves.** The plateau component of pacemaker potentials is abolished in ICC-MY of the guinea pig antrum by \(3 \text{ mM}\) caffeine (5, 13). We tested the effects of caffeine on pacemaker potentials and slow waves in the murine small intestine. Caffeine (3 mM) had...
biphasic effects on membrane potentials in ICC-MY. Initially, caffeine caused a transient hyperpolarization (4.8 ± 1.1 mV; n = 9), followed by depolarization (3.1 ± 2.2 mV; n = 9). After 5-min exposure to caffeine (3 mM), the frequency (control, 24.1 ± 1.6 min⁻¹; in caffeine, 20.3 ± 1.9 min⁻¹; n = 9; P < 0.05) and half-width (control, 0.86 ± 0.04 s; in caffeine, 0.65 ± 0.07 s; n = 9; P < 0.01) of pacemaker potentials were reduced, with no changes in amplitude (control, 56.4 ± 7.7 mV; in caffeine, 54.0 ± 9.1 mV; n = 9; P > 0.05) and dV/dt max (control, 1.82 ± 0.62 V s⁻¹; in caffeine, 1.71 ± 0.55 V s⁻¹; n = 9; P > 0.05) (Fig. 6, A and B). Caffeine (3 mM) also transiently hyperpolarized membrane potentials of slow-wave-producing cells (i.e., by 3.4 ± 2.0 mV, n = 11), followed by depolarization (by 5.7 ± 3.6 mV, n = 11). The

Fig. 6. Effects of caffeine on pacemaker potentials and slow waves recorded from the murine small intestine. Pacemaker potentials were recorded before (Aa) and during (Ab) application of 3 mM caffeine. B: superimposed high-speed traces of pacemaker potentials recorded in the absence (Ba) and presence of 3 mM caffeine (Bb). C: slow waves were recorded before (Ca) and during (Cb) application of 3 mM caffeine. D: superimposed high-speed traces of slow waves recorded in the absence (Da) and presence (Db) of 3 mM caffeine. Resting membrane potentials were −70 mV in A and −67 mV in C. Traces in A and C were recorded from different tissues.
amplitude (control, 27.9 ± 2.3 mV; in caffeine, 14.0 ± 2.4 mV; n = 11; P < 0.01), frequency (control, 23.3 ± 1.0 min⁻¹; in caffeine, 19.7 ± 2.0 min⁻¹; n = 11; P < 0.05), half-width (control, 0.87 ± 0.07 s; in caffeine, 0.69 ± 0.07 s; n = 11; P < 0.01), and dV/dt_max (control, 0.51 ± 0.08 V s⁻¹; in caffeine, 0.17 ± 0.06 V s⁻¹; n = 11; P < 0.01) of slow waves were decreased after 5-min exposure to 3 mM caffeine (Fig. 6, C and D).

To further examine the effects of caffeine on pacemaker potentials, caffeine (3 mM) was applied in the presence of mibefradil (30 μM). As above, this concentration of mibefradil decreased the amplitude, frequency, and dV/dt_max of pacemaker potentials and increased the resolution of unitary potentials (Fig. 7B). Under these conditions, caffeine (3 mM) caused a transient hyperpolarization (by 3.3 ± 2.0 mV; n = 6) (Fig. 7C) and abolished pacemaker potentials in four of six preparations. Unitary potentials continued in the presence of caffeine (Fig. 7, D and E). In two preparations, caffeine (3 mM) did not fully block pacemaker potentials but greatly reduced the frequency from 8.04 min⁻¹ in mibefradil to 3.62 min⁻¹ in mibefradil and caffeine. The effects of caffeine were reversible within 15 min of removing the drug (Fig. 7F). In all preparations (n = 6), the generation of pacemaker potentials accelerated after caffeine was removed (Fig. 7G). Taken together, these results demonstrate that caffeine (3 mM) inhibits the plateau component of pacemaker potentials.

**Effects of pinacidil on pacemaker potentials and slow waves.**

In gastric antral muscles, ATP-sensitive K⁺ channel (K_ATP channel) openers have the opposite effects on the amplitude of pacemaker potentials and slow waves. These drugs increase the amplitudes of pacemaker potentials and decrease the amplitudes of slow waves (14). In the present study, we examined the effects of pinacidil on pacemaker potentials and slow waves in murine small intestine. Pinacidil (10 μM) hyperpolarized ICC-MY by 9.6 ± 2.0 mV (n = 8), increased the amplitude and dV/dt_max of pacemaker potentials, and decreased the half-width without affecting the frequency (Figs. 8A and 9). The effects of 10 μM pinacidil on pacemaker potentials were blocked by 10 μM glibenclamide (Figs. 8A and 9), suggesting that pinacidil exerts its effects via activation of K_ATP channels (19). The effects of pinacidil are summarized in Fig. 9.

Application of pinacidil (10 μM) hyperpolarized slow-wave-producing cells by 10.3 ± 1.0 mV (n = 11) and decreased the amplitude, duration, and dV/dt_max of slow waves without affecting frequency (Figs. 8Bb and 9). The effects of pinacidil on slow waves were blocked by glibenclamide (10 μM; Figs. 8Ac and 9). The inhibitory effects of pinacidil on the slow-wave amplitude may be due to the decrease in input resistance caused by the opening of K_ATP channels in smooth muscle cells. This observation is consistent with the hypothesis that slow waves conduct electrotonically from ICC-MY to smooth muscle cells and that there is no active regeneration of slow waves by smooth muscle cells (3, 6).

Figure 10 shows a comparison of the configuration of pacemaker potentials with the configuration of slow waves before (Fig. 10, Aa and Ba) and during (Fig. 10, Ab and Bb) exposure to 10 μM pinacidil after normalization of the amplitudes of slow waves to the amplitudes of pacemaker potentials. Pinacidil had no appreciable effect on the waveforms of pacemaker potentials (Fig. 10Ab). In contrast, there was a distinct change in the configuration of slow waves in the presence of pinacidil. The transient repolarization after the upstroke of slow waves was reduced by pinacidil (Fig. 10Bb).

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**Fig. 7. Effects of caffeine on pacemaker potentials in the presence of mibefradil.**

Pacemaker potentials were recorded before (A) and during (B) application of 30 μM mibefradil, followed by 3 mM caffeine at 2 min (C), 4 min (D), and 6 min (E). F, G, and H show the responses of pacemaker potentials recorded during washout of caffeine in the presence of 30 μM mibefradil for 2, 4, and 15 min, respectively. All traces are excerpts from a continuous recording. The resting membrane potential was initially ~72 mV and is indicated by dotted lines.
DISCUSSION

The nature of pacemaker potentials of ICC-MY in the murine intestine in situ was explored in the present study. Generation of pacemaker potentials had at least three distinct phases: diastolic depolarization, upstroke depolarization, and plateau potential. Mibefradil reduced the frequency of pacemaker potentials, increased the time constant of the depolarization between slow waves, and greatly reduced the $dV/dt_{\text{max}}$ of the upstroke component. Mibefradil also caused a small depolarization of resting potential. The effects of mibefradil on pacemaker potentials were unlikely to be secondary to simple depolarization, because the effects that we noted were far greater than those of increasing the external $K^+$ concentration to 10.6 mM, which depolarized cells by $\sim 12$ mV (15). Thus activation of dihydropyridine-resistant $Ca^{2+}$ channels may generate inward current involved in the initial “foot” depolarization of ICC-MY during propagation of pacemaker potentials and in the upstroke depolarizations of pacemaker potentials. Caffeine and pinacidil reduced the plateau component of pacemaker potentials. Pinacidil also increased the amplitude and

Fig. 8. Effects of pinacidil on pacemaker potentials and slow waves recorded from the murine small intestine. Pacemaker potentials were recorded before (Aa) and during (Ab) exposure to 10 $\mu$M pinacidil, followed by 10 $\mu$M glibenclamide (Ac). Slow waves were recorded before (Ba) and during (Bb) application of 10 $\mu$M pinacidil, followed by 10 $\mu$M glibenclamide (Bc). The initial resting membrane potentials were $\sim 69$ mV in A and $\sim 70$ mV in B. Traces shown in A and B were recorded in different tissues.

Fig. 9. Summary of the effects of pinacidil on the pacemaker potentials and slow waves recorded from the murine small intestine. A: effects of pinacidil on the amplitude of pacemaker potentials recorded from ICC-MY and slow waves recorded from circular smooth muscles (CM). The amplitudes were measured before (control) and during application of 10 $\mu$M pinacidil and during coapplication of 10 $\mu$M glibenclamide. B: effects of mibefradil on the frequency of pacemaker potentials and slow waves. The frequencies were measured before (control) and during application of 10 $\mu$M pinacidil and during coapplication of 10 $\mu$M glibenclamide. C: effects of mibefradil on the duration of pacemaker potentials and slow waves. The half-widths were measured before (control) and during application of 10 $\mu$M pinacidil and during coapplication of 10 $\mu$M glibenclamide. D: effects of mibefradil on the $dV/dt_{\text{max}}$ of pacemaker potentials and slow waves. The $dV/dt_{\text{max}}$ were measured before (control) and during application of 10 $\mu$M pinacidil and during coapplication of 10 $\mu$M glibenclamide. Data are means $\pm$ SD. *$P < 0.05$ vs. control. **$P < 0.01$ vs. control.


\[ dV/dt_{\text{max}} \] of pacemaker potentials without changing frequency.

It was shown previously that Ni\(^{2+}\) and reduced extracellular Ca\(^{2+}\) decreased the \( dV/dt_{\text{max}} \) and frequency of pacemaker potentials recorded in ICC-MY in the murine small intestine (15). The depolarization during the diastolic period was also slowed by Ni\(^{2+}\) and reduced extracellular Ca\(^{2+}\), suggesting the involvement of Ca\(^{2+}\) entry during the initial and upstroke depolarizations. To further explore this possibility, we tested the effects of mibefradil, a blocker of T-type Ca\(^{2+}\) channels (24), on pacemaker potentials, because this compound has been shown to inhibit a dihydropyridine-resistant Ca\(^{2+}\) current in ICC-MY of the murine small intestine without affecting the primary pacemaker conductance (12). Mibefradil decreased the \( dV/dt_{\text{max}} \) and frequency of pacemaker potentials. The inhibitory effects of mibefradil were strikingly similar to the effects of Ni\(^{2+}\) and reduced extracellular Ca\(^{2+}\), thus strengthening the hypothesis that a dihydropyridine-resistant Ca\(^{2+}\) conductance contributes to the depolarization during the diastolic period and to the upstroke depolarization of pacemaker potentials.

At 30 \( \mu \text{M} \), mibefradil dramatically increased the resolution of unitary potentials during the intervals between pacemaker potentials (see Figs. 2 and 7). Unitary potentials may represent the basic pacemaker events of ICC-MY. Unitary potentials are also a common feature of small bundles of antral circular muscle (containing ICC-IM), and these events can summate to produce events termed regenerative potentials (22, 26). Unitary potentials also occur in ICC-MY of the gastric antrum (5, 16), and summation of these events has been suggested as the basis for the plateau component of pacemaker potentials (i.e., “driving potentials” in the terminology of Edwards and colleagues). In the present study, the shape of pacemaker potentials in the presence of 30 \( \mu \text{M} \) mibefradil was similar to that of the regenerative potentials recorded from single bundles of circular muscle from the guinea pig gastric antrum (22, 26). Thus it is reasonable to suggest that pacemaker potentials in the presence of mibefradil (or low concentrations of Ni\(^{2+}\) or extracellular Ca\(^{2+}\); see Ref. 15) may similarly result from the summation of unitary potentials. We also noted a buildup of unitary potentials before the discharge of pacemaker potentials in the presence of mibefradil (see Figs. 2 and 7). A basic difference between ICC-MY (which generate regular pacemaker potentials with fast upstroke depolarizations) and ICC-IM (which also have an intrinsic capacity for generation of unitary potentials, but such events are typically uncoordinated and entrained by the pacemaker activity of ICC-MY in situ) may be the channel density of voltage-dependent, mibefradil-sensitive Ca\(^{2+}\) channels.

ICC-MY contain an abundance of intracellular structures (e.g., sarcoplasmic reticulum and mitochondria) that are arranged in close apposition to the plasma membrane (25). The plasma membrane contains ionic conductances necessary for generation and propagation of pacemaker potentials. We have referred to these complexes (i.e., intracellular organelles and plasma membrane conductances) as “pacemaker units” and have suggested that these complexes are responsible for the generation of pacemaker currents by regulating submembrane Ca\(^{2+}\) concentration (12). Each pacemaker unit is capable of spontaneous generation of inward current, and random activation of these currents from a multitude of pacemaker units is likely to be the basis for unitary potentials. Thus a mechanism must exist to entrain the activity of pacemaker units throughout ICC-MY networks. Our data suggest that a dihydropyridine-resistant Ca\(^{2+}\) conductance is required for entrainment of the activity of pacemaker units and active regeneration of pacemaker potentials (see Refs. 12 and 15). Thus voltage-dependent, dihydropyridine-resistant Ca\(^{2+}\) channels appear to be a critical participant in the pacemaker unit complex. On the basis of this realization, we interpret our results in the following manner. Under control conditions, activation of dihydropyridine-resistant Ca\(^{2+}\) channels facilitates entrainment of unitary potentials, which provides the basis for regeneration of pacemaker potentials. When the availability of dihydropyridine-resistant Ca\(^{2+}\) channels (as occurs in the presence of mibefradil, Ni\(^{2+}\), or elevated external K\(^{+}\); see Ref. 15) or the driving force for Ca\(^{2+}\) entry is reduced (as occurs when extracellular Ca\(^{2+}\) is depleted), the probability of entrainment is reduced. Under these conditions, the activity of pacemaker units is effectively uncoupled, and the tendency to resolve unitary potentials is therefore increased. A further observation consistent with previous studies (12) is that manipulations that reduce current through dihydropyridine-resistant Ca\(^{2+}\) channels do not appear to affect the intrinsic pacemaker activity of...
ICC-MY (at least for a period of many minutes), and thus the generation of unitary potentials persists.

Previous studies of pacemaker activity in ICC-MY of the gastric antrum have shown that shortly after repolarization of pacemaker potentials, the occurrence of unitary potentials is low but the probability of these events increases steadily during the diastolic period (5). It is likely that the net depolarization caused by the summation of unitary potentials is responsible for bringing membrane potential to the point of threshold. Threshold in ICC-MY appears to be due to the inward current generated by activation of voltage-dependent, dihydropyridine-resistant Ca\textsuperscript{2+} channels. Reducing the availability of these Ca\textsuperscript{2+} channels would tend to shift threshold away from the maximum diastolic (i.e., resting) potential between slow waves and increase the time required for sufficient recovery of unitary potentials from refractoriness and summation of these events to reach threshold. This would result in a slowing of the frequency of pacemaker potentials, which is what we observed in response to mibefradil. This concept is also in agreement with our findings that mibefradil slowed the rate of the diastolic depolarization and caused a positive shift in the potential at which the upstroke depolarization developed (take-off potential). Reduction in the current from dihydropyridine-resistant Ca\textsuperscript{2+} channels also resulted in slower upstroke depolarizations, suggesting that this current contributes significantly to the charge movement responsible for this phase of pacemaker potentials. This conclusion is consistent with findings in a long history of experiments on slow waves and pacemaker activity in gastrointestinal muscles in which several authors have concluded that the upstroke potential fundamentally depends on voltage-dependent, dihydropyridine-resistant Ca\textsuperscript{2+} entry (4, 7, 15, 36).

Caffeine is thought to have multiple effects on intracellular Ca\textsuperscript{2+} mobilization, such as enhancing Ca\textsuperscript{2+} release from ryanodine receptors (10), blocking IP\textsubscript{3} receptors (23), and inhibiting IP\textsubscript{3} production (30). These effects are related to Ca\textsuperscript{2+} release from internal Ca\textsuperscript{2+} stores. Pinacidil inhibits the production of IP\textsubscript{3} (11). A previous study showed that BAPTA-AM abolished pacemaker potentials and unitary potentials recorded from ICC-MY in murine small intestine (15). In the present study, pinacidil reduced the duration of the plateau potential. It seems reasonable to hypothesize that the generation of unitary potentials and the plateau component of pacemaker potentials are related to Ca\textsuperscript{2+} release from internal Ca\textsuperscript{2+} stores. Indeed, it is known that intracellular Ca\textsuperscript{2+} handling mechanisms mediated by IP\textsubscript{3}-induced Ca\textsuperscript{2+} release from internal stores (27, 35) and Ca\textsuperscript{2+} uptake into mitochondria (35) play a key role in generating spontaneous activity in gastrointestinal muscles and ICC. Furthermore, our results support the idea that pacemaker potentials result from at least two conductances: an initial Ca\textsuperscript{2+} influx (upstroke component) and a second conductance activated via Ca\textsuperscript{2+} release from IP\textsubscript{3} receptor-operated stores and mitochondrial Ca\textsuperscript{2+} uptake (plateau component) (see Ref. 15).

Pinacidil increased the dV/dt\textsubscript{max} of the upstroke of pacemaker potentials. This is likely caused by the increase in driving force for Ca\textsuperscript{2+} ions caused by hyperpolarization due to activation of K\textsubscript{ATP} channels. While significant hyperpolarization would be expected to stabilize membrane potentials and decrease the likelihood of activation of voltage-dependent Ca\textsuperscript{2+} channels, it appears that the 10-mV hyperpolarization caused by pinacidil was not sufficient to block pacemaker potentials. Hyperpolarization would also be likely to increase the amplitude of unitary potentials, because these are also due to inward currents with equilibrium potentials positive to the resting potential. In fact, previous studies of unitary potentials in gastric tissues confirms the increase in amplitude in response to hyperpolarization (4, 16). The dihydropyridine-resistant (mibefradil sensitive) Ca\textsuperscript{2+} current activates and inactivates at potentials more negative than those of dihydropyridine-sensitive current (12). In our previous characterization of the dihydropyridine-resistant Ca\textsuperscript{2+} current in ICC-MY, we found that only \approx 50% of channels were available at the resting potentials recorded from ICC-MY in situ in the present study. Hyperpolarization by 10-mV increased the availability of these channels to \approx 80%. Thus, as these cells hyperpolarize, there may be accommodation to the hyperpolarization that results in a negative shift in threshold. The increase in dihydropyridine-resistant Ca\textsuperscript{2+} channel availability would help to preserve the frequency and would tend to increase the amplitude and dV/dt\textsubscript{max}. While the pacemaker current underlying unitary potentials and the plateau component of pacemaker potentials are apparently independent of membrane potential (17, 29), the contributions of the voltage-dependent, dihydropyridine-resistant Ca\textsuperscript{2+} conductance to the process of entrainment and generation of the upstroke potential lend voltage dependency to the integrated phenomenon of pacemaker activity in ICC.

Fig. 11. Schematic diagram of pacemaker potentials and slow waves in murine ICC-MY and smooth muscle cells. Pacemaker potentials were recorded in ICC-MY. These events were of large amplitude and had fast upstroke depolarizations. The phases of these events are labeled 0, diastolic phase between events; 1, upstroke depolarization sensitive to block by mibefradil; and 2, plateau phase sensitive to reduction by caffeine. During the diastolic phase (0), depolarization from propagating pacemaker potentials activated a voltage-dependent (mibefradil-sensitive) Ca\textsuperscript{2+} conductance and a threshold was reached for the upstroke depolarization (1). Mibefradil prolonged the diastolic period, shifted the threshold for the upstroke to more positive potentials, and slowed the upstroke depolarization. In the presence of mibefradil, unitary potentials were observed that were likely due to uncoordinated pacemaker currents that failed to reach threshold (see Figs. 2, 3, and 7). Entry of Ca\textsuperscript{2+} during the upstroke activated inositol 1,4,5-trisphosphate receptor-operated channels in Ca\textsuperscript{2+} stores, and, through intracellular Ca\textsuperscript{2+} handling mechanisms involving mitochondrial Ca\textsuperscript{2+} uptake, activated inward current responsible for the plateau phase (2). Caffeine, via an unknown mechanism, reduced the plateau phase. Passive conduction of pacemaker potentials to smooth muscle cells resulted in slow waves with slower upstroke depolarization and smaller amplitude. A pronounced repolarization phase occurred between the upstroke and plateau phases in smooth muscle cells, perhaps because of activation of voltage-dependent K\textsuperscript{+} conductances prominent in these cells. Slow waves recorded in smooth muscle cells were greatly reduced in amplitude by pinacidil (see Fig. 8), suggesting that input resistance of smooth muscle syncytium affected the length constant for conduction of these events.
The recordings in the present study show a contrast in the manifestations of slow waves in pacemaker cells (ICC-MY) and smooth muscle cells. Previous studies performed in gastric muscle cells suggest that slow waves passively conduct in smooth muscle cells (3). The results of the present study are consistent with this conclusion. Slow waves recorded from smooth muscle cells were always smaller in amplitude than the pacemaker potentials recorded in ICC-MY, and the rate of rise of the upstroke phase of the slow waves was slower in smooth muscle cells. Because slow waves are driven by the pacemaker potentials in ICC-MY, any treatment that affected specific parameters of events in ICC-MY would be expected to have analogous effects in smooth muscle cells. Our observations demonstrate that mibefradil reduced the dV/dtmax of the upstroke component in ICC-MY and had equivalent effects in smooth muscle cells and that caffeine reduced the plateau phase and had equivalent effects in smooth muscle cell recordings. Pinacidil, which did not block pacemaker potentials in ICC-MY (see previous paragraph), would be expected to increase K+ conductance (via activation of KATP channels) of the smooth muscle syncytium. Reduction in input resistance would tend to reduce the amplitude of voltage signals that conduct passively through smooth muscle cells. Consistent with this hypothesis, pinacidil greatly reduced slow-wave amplitude.

In consideration of previous results published by several authors, our observations in the present study support the following concept (see also Fig. 1). Pacemaker potentials recorded from ICC-MY in the murine small intestine are composed of a rapidly rising upstroke component due to Ca2+ influx via a dihydropyridine-resistant Ca2+ conductance. The upstroke triggers a sustained plateau component that represents a summation of primary pacemaker currents. These events are initiated by Ca2+ release from internal stores. The primary pacemaker currents (which result in unitary potentials) persist when the availability of the dihydropyridine-resistant Ca2+ channels decreases, but under these circumstances, entrainment of unitary potentials is reduced. Thus the upstroke component due to the entry of Ca2+ is critical for 1) entrainment of pacemaker activity in the multitudes of active pacemaker units in ICC-MY networks and 2) active regeneration of pacemaker potentials.

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