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DEPENDENCE OF ELECTRICAL SLOW WAVES OF CANINE COLONIC SMOOTH MUSCLE ON CALCIUM GRADIENT

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SUMMARY

1. The ionic dependence of the upstroke and plateau components of slow waves of canine colonic circular muscles was studied.

2. Reduced extracellular Ca^{2+} caused a decrease in the amplitude of the upstroke and plateau components, a decrease in the depolarization velocity, and a decrease in frequency. The reduction in the upstroke phase per 10-fold reduction in external Ca^{2+} was close to the value predicted by the Nernst relationship, suggesting that the membrane permeability to Ca^{2+} increases steeply during this phase.

3. Nifedipine (10^{-9}–10^{-6}) reduced the plateau component, but concentrations of 10^{-6} M did not abolish the upstroke component. The data suggest that a nifedipine-resistant component of Ca^{2+} current may be involved in the upstroke.

4. Inorganic Ca^{2+} channel blockers (Mn^{2+} and Ni^{2+}) blocked spontaneous slow waves at concentrations of 1·0 mM or less.

5. The upstroke component was more sensitive to Ni^{2+} than to Mn^{2+}; a concentration of 0·040 mM-Ni^{2+} caused more than a 50% reduction in upstroke velocity. Ni^{2+} also reduced the plateau phase of slow waves.

6. The results suggest that the upstroke and plateau components of slow waves are dependent upon activation of voltage-dependent Ca^{2+} currents. The current responsible for the upstroke is partially resistant to dihydropyridines (at least at 10^{-6} M). The current responsible for the plateau component is nifedipine-sensitive.

INTRODUCTION

Electrical slow waves are rhythmic changes in membrane potential that initiate excitation–contraction coupling in many gastrointestinal smooth muscles (Christensen, Caprilli & Lund, 1969; Morgan & Szurszewski, 1980; Sanders, 1983; Bauer & Sanders, 1985; Sanders & Smith, 1986*b*; Barajas-Lopez & Huizinga, 1989). These events consist of an upstroke depolarization that occurs with a velocity of approximately 1 V/s (El-Sharkawy, Morgan & Szurszewski, 1978; Smith, Reed & Sanders, 1987). The maximum level of depolarization reached by the upstroke depolarization is about −20 mV. After reaching a peak, the upstroke partially repolarizes and is followed by a sustained plateau phase that persists for several

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seconds at potentials in the range of −40 to −30 mV. Repolarization of the plateau phase returns membrane potential to the 'resting potential' which averages between −80 and −60 mV in most gastrointestinal muscles (see reviews by Szurszewski, 1987; Sanders & Smith, 1989; Sanders & Publicover, 1989). Slow waves, and the contractions they generate, are reduced in amplitude by lowering external Ca^{2+} or by Ca^{2+} channel blocking agents (Sanders & Publicover, 1989; Barajas-Lopez, Den Hertog & Huizinga, 1989). These observations suggest that Ca^{2+} channels participate in the generation of slow waves; it is likely that the inward current resulting from Ca^{2+} influx depolarizes membrane potential and increases cytoplasmic Ca^{2+} above the threshold for contraction. We have recently reported that a dihydropyridine-sensitive Ca^{2+} current is activated in the range of the plateau phase of the slow wave in colonic and gastric myocytes (Langton, Burke & Sanders, 1989; Vogalis, Publicover, Hume & Sanders, 1991), and this current is sustained throughout the plateau phase. Although the plateau current is small in magnitude, its duration of several seconds leads to sufficient entry of Ca^{2+} to cause a significant increase in cytoplasmic Ca^{2+} and contraction (Vogalis *et al.* 1991).

Although the involvement of Ca^{2+} current in the plateau phase of the slow wave is suggested from previous studies, the ionic mechanism of the upstroke depolarization has not been clarified. From voltage clamp experiments performed at 23 °C, it would appear that essentially all of the inward current can be blocked by nifedipine (Langton *et al.* 1989); yet treatment of intact muscles with nifedipine or D600 does not block the upstroke component of slow waves (Barajas-Lopez *et al.* 1989; Ward & Sanders, 1990). Barajas-Lopez *et al.* (1989) studied the ionic mechanism of the slow wave in a series of ion replacement studies using intact colonic muscles and intracellular electrical recording techniques. Under the conditions of their studies, a number of treatments expected to block Ca^{2+} current failed to abolish slow waves. These observations and others led these authors to conclude that the upstroke depolarization is due to the deactivation of a K^+ conductance, not the activation of a Ca^{2+} conductance. This conclusion, based on intracellular microelectrode recordings, neglected some rather compelling arguments to the contrary: (i) voltage clamp studies of isolated colonic myocytes have failed to find a K^+ current of the type (inward rectifier) proposed by Barajas-Lopez *et al.* (1989) (Cole & Sanders, 1989; Burke & Sanders, 1990); (ii) the inward rectifier that has been identified in gastrointestinal muscles is non-selective for potassium *vs.* sodium ions and has a reversal potential of −24·5 mV (Benham, Bolton, Denbigh & Lang, 1987). Although a conductance of this type could provide pacemaker current (DiFrancesco & Ojeda, 1980; DiFrancesco, 1981), it may not be capable of producing the upstroke depolarization of slow waves because it would tend to deactivate with depolarization (i.e. its activation occurs at potentials negative to −50 to −60 mV; DiFrancesco, 1981). An 'i_f-type' current would be better suited to providing enough depolarization to activate a voltage-dependent inward current.

In the present study we have re-examined the role of Ca^{2+} in the generation of slow waves by studying the effects of altering the Ca^{2+} gradient, the effects of organic and inorganic Ca^{2+} channel blocking agents, and the effects of the Ca^{2+} channel agonist, Bay K 8644. These experiments suggest that the upstroke and plateau phases of the slow wave result from activation of Ca^{2+} channels, but part of the Ca^{2+} current may

be resistant to complete block by dihydropyridines at the voltages attained during upstroke depolarizations. Part of these results were presented at the American Motility Symposium in October 1990 (Ward, Blondfield & Sanders, 1990).

METHODS

Mongrel dogs of both sexes were killed with sodium pentobarbitone (45 mg/kg). The abdomen was opened and a segment of proximal colon, 5–15 cm from the ileo-caecal sphincter, was removed. The colonic segment was opened along the mesenteric border and strips of muscle were prepared for electrophysiological studies as previously described (Ward & Sanders, 1990). The muscles were transferred to an electrophysiological chamber with a Sylgard floor and pinned in cross-section to allow impalement of cells at any point through the muscularis externa (Bauer, Reed & Sanders, 1985; Smith *et al.* 1987). The chamber was perfused with warmed, oxygenated Krebs–Ringer buffer (KRB) and the temperature was maintained at 37·5 ± 0·5 °C with a thermistor probe placed near the muscle. After dissection and pinning, the muscles were allowed to equilibrate for about 2 h before experiments were begun. Cells near the submucosal surface of the circular layer were selectively impaled in this study. Electrical recordings were made and analysed as described previously (see Sanders & Smith, 1986*a*; Smith *et al.* 1987). Data are expressed as means ± standard errors of the mean and plotted with Sigmaplot software.

Solution and drugs. The standard KRB solution contained (mM): Na^+, 137·4; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 134; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2; dextrose, 11·5. This solution had a pH of 7·3–7·4 at 37·5 °C when bubbled with 97 % O_2–3 % CO_2. Solutions with reduced Ca^{2+} were made by equimolar substitution with Na^+ or replacement of Ca^{2+} with Mg^{2+} as noted in the text. Solutions containing Mn^{2+} were made in phosphate-free KRB (pH was adjusted to 7·3 with NaOH). In control experiments we found that phosphate-free KRB had no significant effect on electrical activity over the time periods required for experiments. $NiCl_2$ was added directly to KRB to make the solutions containing Ni^{2+}. Nifedipine (Boehringer Mannheim) and Bay K 8644 (Miles) were dissolved in ethanol at a stock concentration of 10^{-2} M and diluted to the desired concentration in KRB immediately before testing. Experiments using nifedipine and Bay K 8644 were performed in the dark. Tetrodotoxin (Sigma) was dissolved in distilled water at 3×10^{-4} M and diluted to 3×10^{-6} M in KRB.

RESULTS

Effects of low Ca^{2+} on slow waves

Removal of extracellular Ca^{2+} (and buffering with 1 mM-EGTA) was previously reported to abolish slow waves in the canine proximal colon (Barajas-Lopez *et al.* 1989). The 23 mV depolarization that accompanied this effect can affect electrical rhythmicity in these muscles (Sanders, Burke & Stevens, 1989). We tested the effects of reduced extracellular Ca^{2+} ($[Ca^{2+}]_o$) on electrical activity to determine whether slow waves depend upon $[Ca^{2+}]_o$ in the absence of appreciable depolarization. Control slow wave activity was recorded in normal KRB containing 2·5 mM-Ca^{2+}. Then the KRB solution was changed to solutions containing reduced amounts of Ca^{2+}. After 15 min exposures, the test solution was replaced with normal KRB to restore control conditions. This cycle was repeated, and muscles were exposed to several $[Ca^{2+}]_o$ (2·5–0·1 mM) while maintaining impalement of the same cell. In some experiments the divalent ion concentration decreased as $[Ca^{2+}]_o$ was reduced; in others Ca^{2+} was replaced by Mg^{2+}. There were not significant differences noted in the responses.

Reduced $[Ca^{2+}]_o$ decreased the amplitude and duration of slow waves (Fig. 1*A*). The velocity of the upstroke depolarization also decreased, suggesting a decrease in the current driving the depolarization. The lowest $[Ca^{2+}]_o$ tested caused a dramatic decrease in slow wave frequency or disappearance of slow wave activity. Figure

1*B–E* summarizes the effects of $[Ca^{2+}]_o$ on slow waves in six experiments. Decreasing $[Ca^{2+}]_o$ depolarized membrane potential; this averaged 12±3 mV at 0·1 μM-Ca^{2+}. This magnitude of depolarization would be expected to decrease upstroke velocity, but it would not abolish slow wave activity (see Ward & Sanders, 1990).

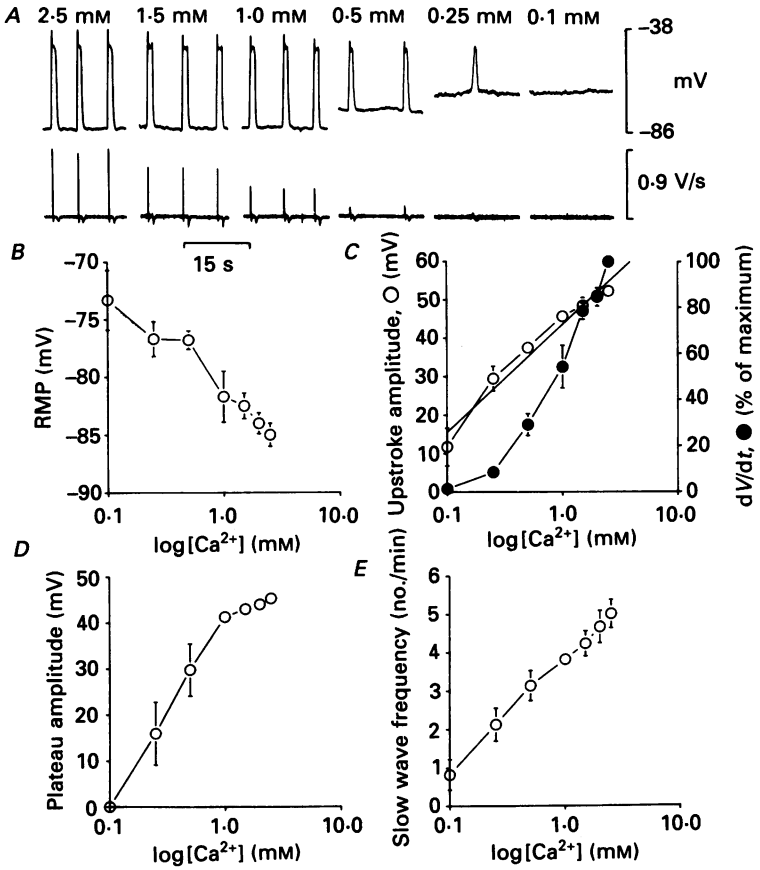

Fig. 1. Effect of external Ca^{2+} on slow waves. Muscle was exposed to several concentrations of external Ca^{2+} and slow waves (top trace each panel, *A*) and the first derivative of voltage (dV/dt; bottom trace each panel, *A*) were recorded. The concentration is shown above segments of recording in panel *A*. Reduced Ca^{2+} decreased the amplitude, velocity and duration of slow waves (see text for specific details). Slow waves were abolished in this cell at 0·1 mM-Ca^{2+}. Panels *B–E* show summary effects of reduced Ca^{2+} on electrical parameters (resting membrane potential (RMP; *B*), upstroke amplitude (*C*), dV/dt (*C*), plateau amplitude (*D*), and frequency (*E*) as a function of external Ca^{2+} concentration). Data are means±standard error of the mean from six experiments. The upstroke depolarization was highly dependent upon external Ca^{2+}. Data in *C* were fitted with lines, by the least-squares method, having a slope of 28 mV per decade change in $[Ca^{2+}]_o$. This is close to the theoretical dependence on the Ca^{2+} gradient if the membrane behaved as a Ca^{2+} electrode during the upstroke phase.

Effects of nifedipine on slow waves

Barajas-Lopez and colleagues (1989) found that D600 (10^{-6} M) reduced upstroke amplitude and velocity, plateau amplitude, and overall slow wave duration without affecting resting potential. We studied the effects of the dihydropyridine, nifedipine,

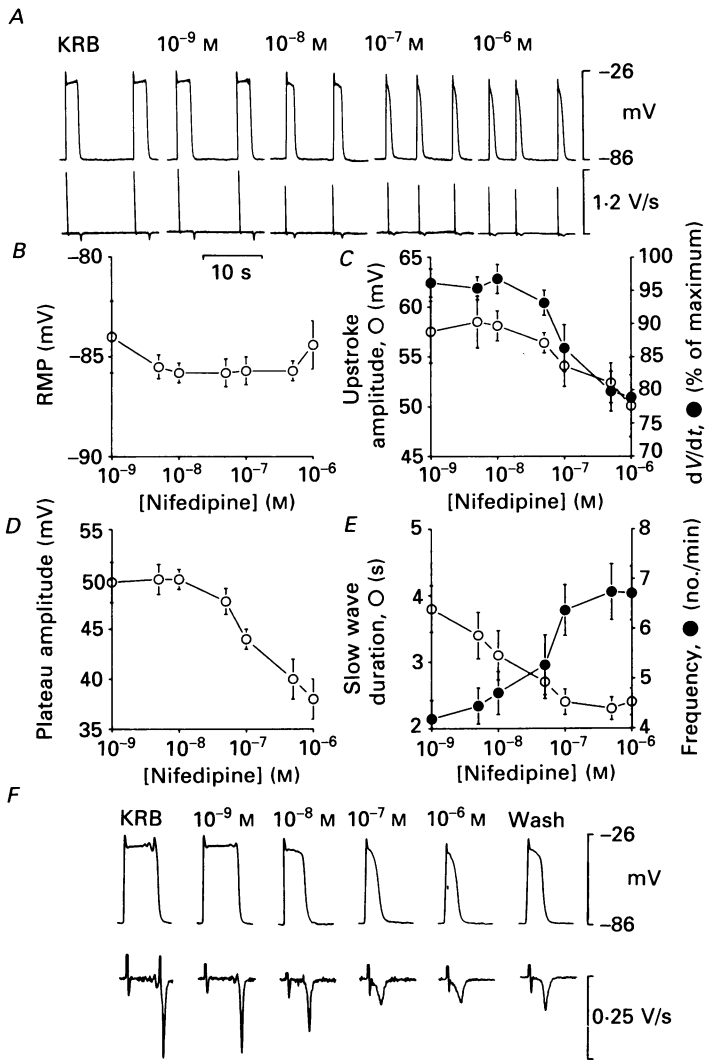

Fig. 2. Effects of nifedipine on slow wave activity. Panel *A* shows excerpts of a recording during which a muscle was exposed to several concentrations of nifedipine (10^{-9}–10^{-6} M). Each portion of the record shows membrane potential (top traces) and dV/dt (bottom traces). At 10^{-8} M, nifedipine reduced the amplitude and duration of the slow wave. This effect reached a maximum at 10^{-7} M. At 10^{-6} M, nifedipine produced a clear inhibitory effect on the upstroke velocity. The effects of nifedipine were difficult to wash out, and after more than 1·5 h in normal KRB, control slow wave activity was not restored (data not shown). Panels *B*–*E* show summary plots of resting potential (*B*), upstroke amplitude (*C*), dV/dt (*C*), plateau amplitude (*D*), frequency (*E*) and slow wave duration (*E*) as a function of nifedipine concentration. Data are means ± standard error of the mean from six experiments. Closer inspection of the dV/dt records showed that nifedipine also reduced the rate of repolarization. Top trace in panel *F* shows individual slow waves recorded in KRB and during exposure to several concentrations of nifedipine (10^{-9}–10^{-6} M; dose noted above each slow wave). Bottom trace in panel *F* shows dV/dt at increased gain to amplify the repolarization rates (at this gain the depolarization phase is cut off). Note the reduction in repolarization velocity, suggesting that this phase may have some Ca^{2+} dependence (cf. Carl *et al.* 1990).

which blocks a large fraction of the Ca^{2+} current in colonic myocytes (Langton *et al.* 1989). Exposure of six muscles to nifedipine (10^{-9}–10^{-6} M) caused dose-dependent reduction in slow wave upstroke velocity and amplitude, plateau amplitude and slow wave duration (Fig. 2). At 10^{-6} M, nifedipine also increased the frequency of slow waves from 4·2±0·4 to 6·7±0·5 cycles/min.

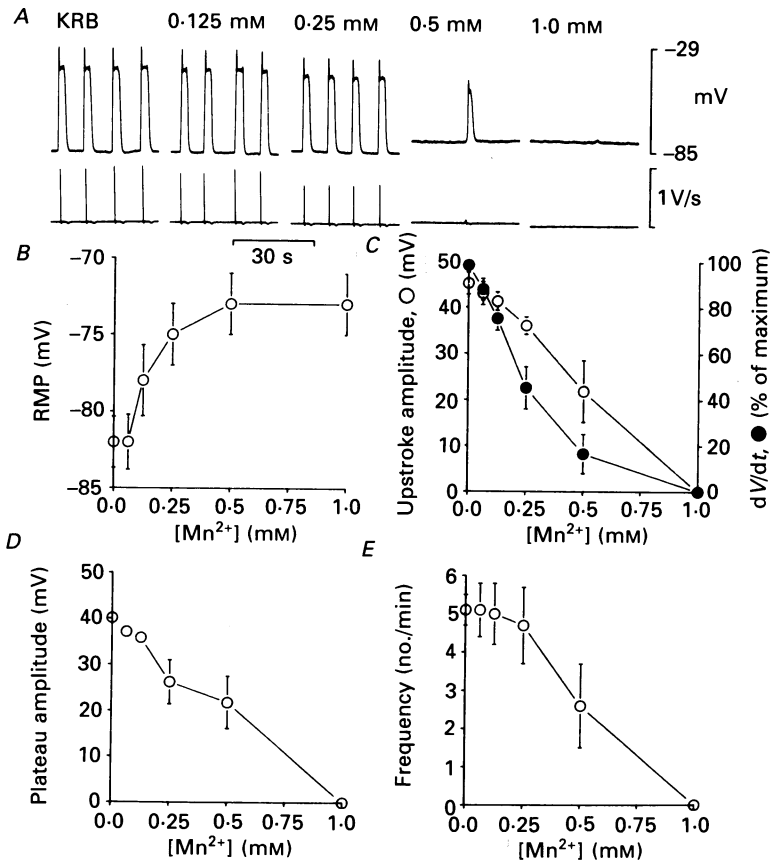

Fig. 3. Effects of Mn^{2+} on slow wave activity. In panel *A* muscle was exposed to several concentrations of Mn^{2+} (0·125–1·0 mM; dose shown above each excerpt) added to the KRB (phosphate free). Panel *A* shows membrane potential (top traces) and dV/dt (bottom traces). Mn^{2+} decreased the amplitude and duration of slow waves, the velocity of the upstroke component, and the frequency. At 1·0 mM, Mn^{2+} abolished spontaneous slow wave activity. Panels *B–E* show plots of resting potential (*B*), upstroke amplitude (*C*), dV/dt (*C*), plateau amplitude (*D*), and frequency (*E*) as a function of Mn^{2+} concentration. Data are means±standard error of the mean from six experiments.

The effects of nifedipine were time dependent and increased during 15 min exposures. In three experiments muscles were exposed to a high concentration of nifedipine (10^{-5} M) for up to 2 h. This altered the frequency and waveforms of slow waves and eventually disrupted normal rhythmicity (data not shown). KRB solutions containing 0·1 % ethanol (final concentration of ethanol in 10^{-5} M-nifedipine solution)

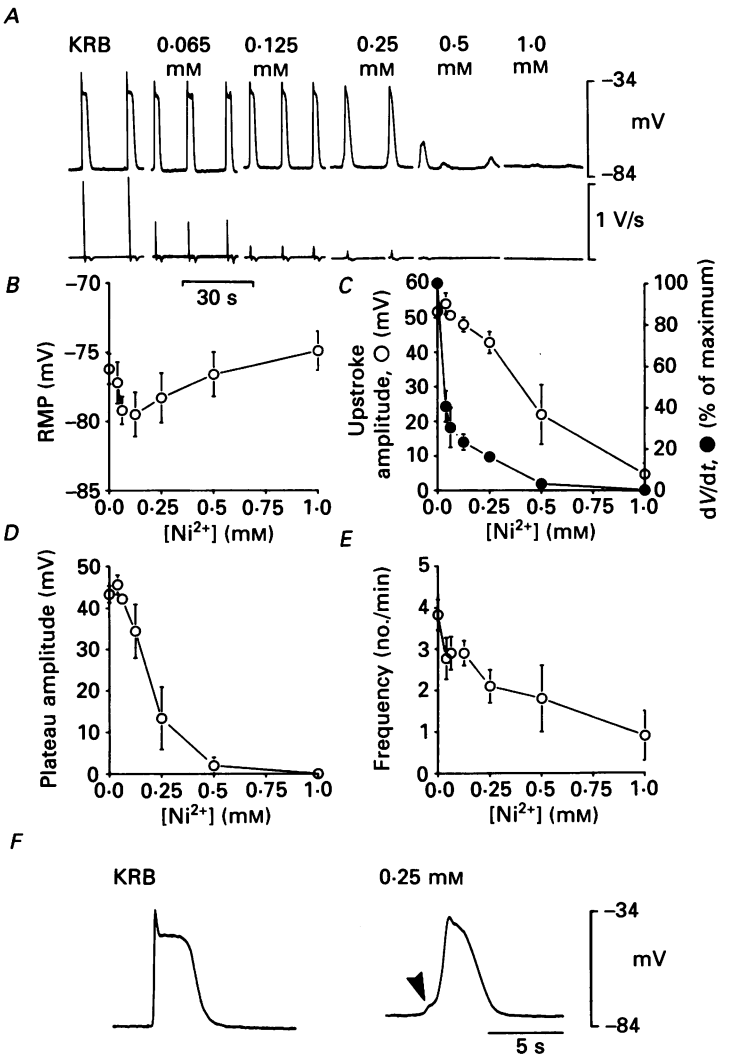

Fig. 4. Effects of Ni^{2+} on slow waves. In panel *A* muscle was exposed to several concentrations of Ni^{2+} ranging from 0·065 to 1·0 mM (dose shown above each excerpt) added to the KRB. Panel *A* shows membrane potential (top traces) and dV/dt (bottom traces). Ni^{2+} decreased the amplitude and duration of slow waves, the velocity of the upstroke component, and the frequency. At 1·0 mM, Ni^{2+} abolished spontaneous slow wave activity. The upstroke component was more sensitive to Ni^{2+} than to Mn^{2+}. Panels *B–E* show plots of resting potential (*B*), upstroke amplitude (*C*), dV/dt (*C*), plateau amplitude (*D*) and frequency (*E*) as a function of Ni^{2+} concentration. Data are means ± standard error of the mean from six experiments. In several experiments reduction of the upstroke depolarization by Ni^{2+} unmasked an underlying depolarization (or 'hump'; cf. Ohba *et al.* 1975). Panel *F* shows slow waves recorded at an expanded time scale in KRB and in presence of Ni^{2+} (0·25 mM). Note distinctive initial depolarization (arrow). In some experiments this 'hump' could also be seen during the repolarization phase.

did not affect slow waves. Nifedipine (10^{-6} M) also decreased the velocity of repolarization of slow waves (Fig. 2*F*), suggesting that the mechanism for slow wave repolarization may be dependent upon Ca^{2+} influx (i.e. perhaps due to activation of Ca^{2+}-dependent outward current as suggested in Carl, McHale, Publicover & Sanders, 1990).

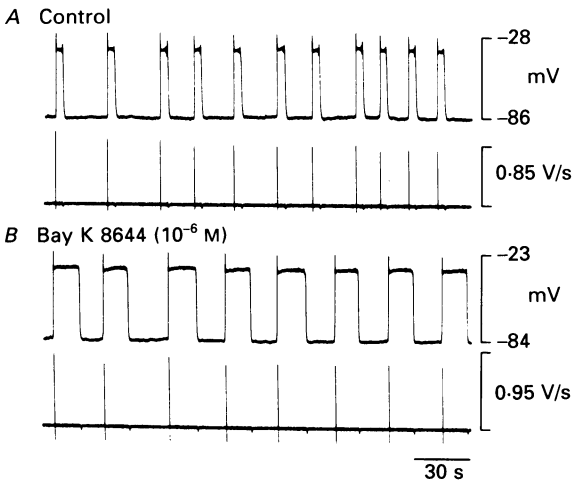

Fig. 5. Effects of Bay K 8644 on slow wave activity. Figure shows records of slow waves (top trace each panel) and dV/dt (bottom traces) in KRB (*A*) and in response to Bay K 8644 (10^{-6} M; *B*). Bay K 8644 caused a large increase in the duration of slow waves. This was matched by a reduction in frequency. Small increases were also noted in the amplitude and velocity of the upstroke depolarization, but these changes did not reach a level of significance in the five muscles studied. Bay K 8644 also increased the rate of repolarization of the plateau phase; this is opposite to the effect of nifedipine (see Fig. 2).

Effects of inorganic Ca^{2+} channel blockers on slow waves

The above results suggest: (i) the upstroke of slow waves depends upon $[Ca^{2+}]_o$; (ii) a portion of the inward current responsible for the upstroke is insensitive to block by nifedipine. Inorganic Ca^{2+} channel blockers were used as another test of the involvement of Ca^{2+} current in the upstroke depolarization. Six muscles were exposed to Mn^{2+} (0·0625 to 1 mM). As above, several concentrations were administered while maintaining impalements in a single cell. Mn^{2+} decreased the amplitude and duration of slow waves in a concentration-dependent manner, and 1 mM-Mn^{2+} abolished slow wave activity (Fig. 3).

Ni^{2+} also reduced slow wave amplitude and duration; however, Ni^{2+} was more potent than Mn^{2+} (Fig. 4). The effects of Ni^{2+} on the upstroke velocity were observed at much lower concentrations than Mn^{2+}. At 0·5 mM, Ni^{2+} caused an irregular pattern of small-amplitude depolarizations. We noted that when the upstroke velocity was significantly reduced another component of depolarization was apparent in the upstroke or repolarization phase (Fig. 4*F*). This component may be similar to the '1st component' of slow waves in the guinea pig stomach described by Tomita (1981).

Effects of Bay K 8644 on slow waves

We have suggested that the plateau phase of slow waves is due to a balance between inward Ca^{2+} current and voltage-dependent outward current (Sanders *et al.* 1990). The inward Ca^{2+} current appears to be due to dihydropyridine-sensitive Ca^{2+} channels (Langton *et al.* 1989). We further tested the involvement of dihydropyridine-sensitive conductances in the plateau phase by using the Ca^{2+} channel agonist, Bay K 8644, which has been shown to increase the open time of Ca^{2+} channels in smooth muscles (Nelson & Worley, 1989).

Five muscles were exposed to Bay K 8644 (10^{-6} M; TTX, 3×10^{-6} M). This compound did not affect resting potential (mean -84 ± 1 mV in control and -84 ± 1 mV in the presence of Bay K 8644), but greatly enhanced the duration of the plateau phase of slow waves (i.e. from a mean of $4{\cdot}0 \pm 0{\cdot}8$ s to $11{\cdot}0 \pm 2{\cdot}3$ s; $P < 0{\cdot}05$; Fig. 5). There were increases in the amplitude of the upstroke and plateau phases (i.e. from 57 ± 3 to 60 ± 3 mV and from 50 ± 2 to 55 ± 3 mV, respectively). The upstroke velocity increased by an average of 25 ± 7 %. Bay K 8644 also decreased the frequency of slow waves by an average of 30 ± 6 %, which was likely to be the result of the increase in the duration of these events.

DISCUSSION

We have shown that slow waves of colonic circular muscle are sensitive to reduction in the transmembrane Ca^{2+} gradient and to blockers of voltage-dependent Ca^{2+} channels. This pharmacological approach allows certain inferences about the participation of Ca^{2+} currents in slow waves. (i) The amplitude and duration of slow waves depended on the Ca^{2+} gradient. At 2·5 mM-Ca^{2+} the equilibrium potential for Ca^{2+} (E_{Ca}) is theoretically +134 mV assuming an intracellular Ca^{2+} activity of about 100 nM (Becker, Singer, Walsh & Fay, 1989; Vogalis *et al.* 1991). Reduction in $[Ca^{2+}]_o$ to 0·1 mM would decrease the driving force on Ca^{2+} ions by about 32 % (i.e. to an E_{Ca} of about +92 mV). This was sufficient to block slow waves in most muscles, demonstrating the dependence of slow waves on the Ca^{2+} gradient. (ii) The reduction in the amplitude of the upstroke potential in response to decreased external Ca^{2+} was close to the theoretical dependence of the upstroke if the membrane behaved as a Ca^{2+} electrode during this phase. We calculated a 28 mV decrease in upstroke amplitude per decade change in external Ca^{2+} (see Fig. 2*B*; the theoretical change at 37·5 °C should be about 30 mV). (iii) The duration of the plateau phase of slow waves was reduced by nifedipine and enhanced by Bay K 8644, suggesting the involvement of 'L-type' channels in this component. (iv) The upstroke was reduced in amplitude and velocity by nifedipine, but this component persisted at concentrations of 10^{-6} M-nifedipine. (v) Inorganic Ca^{2+} channel blockers (Mn^{2+} and Ni^{2+}) reduced the velocity and amplitude of the upstroke depolarization, reduced the amplitude and duration of the plateau component, and blocked slow waves at 0·5–1 mM. Taken together, these data suggest that the upstroke component of slow waves may be due to a Ca^{2+} current that is partially resistant to dihydropyridines. The plateau phase appears to depend upon a dihydropyridine-sensitive Ca^{2+} current.

The role of Ca^{2+} conductances in slow wave generation in canine colonic muscles was considered previously (Barajas-Lopez *et al.* 1989), but different mechanisms for

slow waves were suggested. Several differences in the experimental design of our experiments might explain the differences in our results and interpretations. For example, Barajas-Lopez *et al.* (1989) tested a Krebs solution without Ca^{2+} (background Ca^{2+} was buffered with 1 mM-EGTA). This solution abolished slow waves but also produced a 23 mV depolarization. It is difficult to know whether the cessation of slow wave activity was due to the absence of Ca^{2+}, the reduction in divalent ion concentration, or the depolarization. Certainly the latter is known to interfere with slow wave activity independent of Ca^{2+} concentration (Sanders *et al.* 1989). We tested several concentrations of extracellular Ca^{2+} and found that reduced Ca^{2+} decreased the upstroke amplitude and velocity. At 0·1 mM-Ca^{2+}, slow waves were abolished in several preparations in the absence of a depolarization large enough to produce this effect independently (Ward & Sanders, 1990). These data clearly demonstrate the dependence of the upstroke component on $[Ca^{2+}]_o$. In the previous study Co^{2+} reduced the amplitude and velocity of the upstroke but failed to abolish these events (Barajas-Lopez *et al.* 1989). Contractions were also blocked by Co^{2+}, and the authors concluded that Co^{2+} blocked Ca^{2+} entry without abolishing slow waves. There are two problems with this interpretation. (i) It is unclear how 1–3 mM-Co^{2+} was dissolved in the Krebs solution used in the previous study. Usually this concentration of Co^{2+} causes precipitation when added to Krebs solutions containing phosphate and bicarbonate ions, and the authors of the previous study acknowledged this problem. Therefore, it is unclear what concentration of divalent ions were left in their solutions after addition of Co^{2+}. (ii) There is extensive intracellular buffering of Ca^{2+} in gastrointestinal smooth muscle cells (Beker *et al.* 1989; Vogalis *et al.* 1990). Therefore, a large portion of the Ca^{2+} entering cells may be buffered and unavailable for activation of the contractile proteins. In the case of the previously described Co^{2+} experiments, Ca^{2+} influx may have been reduced below the level necessary for contraction, but there still may have been enough *charge* carried by Ca^{2+} ions to produce the upstroke depolarization. Our experiments with Mn^{2+} and Ni^{2+} demonstrated that inorganic Ca^{2+} channel blockers reduce upstroke amplitude and velocity in a concentration-dependent manner. A concentration of 1 mM or less of these ions was sufficient to block the upstroke depolarization. In the previous study D600 decreased the amplitude and velocity of the upstroke phase of slow waves (Barajas-Lopez *et al.* 1989). The authors explained this finding by suggesting that Ca^{2+} plays an essential role in the metabolic processes involved in slow wave generation, but they were not specific about what 'metabolic process' might be involved. We interpret the effects of D600 to be due to a reduction in Ca^{2+} current, and this is supported by our finding that nifedipine also reduced the amplitude and velocity of the upstroke depolarization in the present study. We feel our studies provide compelling reasons to suggest that Ca^{2+} serves as the charge carrier during the upstroke depolarization. It appears that the upstroke current is partially nifedipine-resistant, but this is not sufficient reason to hypothesize another class of Ca^{2+} channels because of the voltage dependence of the block of Ca^{2+} channels by dihydropyridines (Bean, 1984; Nelson & Worley, 1989; and see accompanying paper by Ward & Sanders, 1992).

There have been studies and speculation about the mechanism of electrical slow waves in the gastrointestinal tract for more than 30 years, and Tomita (1981)

summarized results of earlier studies in which the sucrose gap technique or extracellular 'pressure' electrodes were used. Tomita (1981) divided slow waves into two categories (see Fig. 6.6 of Tomita, 1981): (i) one type of slow wave consisted of an upstroke and plateau potential; (ii) in another type of slow wave a small voltage-independent pacemaker component was thought to underlie a second, much larger component that was dependent upon ionic conductance changes. In the first group the upstroke was a monophasic depolarization from the resting potential to the peak of the upstroke. Slow waves of the canine colon would fit into this category (see Smith *et al.* 1987*a*). Slow waves of the second type, such as those recorded from the guinea-pig stomach, usually had 'humps' at potentials 6–12 mV positive to the resting potential (Ohba, Sakamoto & Tomita, 1975). These 'humps' were often observed on the repolarization phase, but sometimes could also be visualized during the depolarization phase as well, suggesting the presence of two components. In the present study we observed events underlying colonic slow waves in the presence of Ni^{2+} (see Fig. 4) that were similar to the 'humps' described by Ohba *et al.* (1975). The unmasking of these events might have been due to the reduction in the velocity of the upstroke depolarization by Ni^{2+}. Ni^{2+} may decrease the upstroke velocity by reducing the availability of inward current responsible for the upstroke. Our data may provide some linkage between the two types of slow waves described by Tomita (1981). In many smooth muscles the activation of voltage-dependent currents may be fast enough and of sufficient magnitude to mask the underlying pacemaker events. By reducing the availability of voltage-dependent Ca^{2+} channels, it was possible to unmask what may be the underlying pacemaker activity. Reduction of Ca^{2+} current with Ni^{2+} and hyperpolarization also prolongs the interval between cardiac action potentials in rabbit sino-atrial node cells and reveals a distinctive 'hump' in the diastolic phase depolarization (Hagiwara *et al.* 1988). More experiments are necessary to determine the properties and importance of the pacemaker events in smooth muscles.

During the review of this manuscript, another paper appeared by Huizinga, Farraway & Den Hertog (1991) in which similar experiments were described. The conclusions of that study and the present paper differ in that we feel that from studies of intact muscles there is no solid evidence for a second, 'non-L-type' conductance in colonic smooth muscles. As discussed in the next paper, the resistance of the upstroke phase of slow waves to block by dihydropyridines could be due to the voltage-dependence of the block of a single class of Ca^{2+} channels.

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