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UPSTROKE COMPONENT OF ELECTRICAL SLOW WAVES IN CANINE COLONIC SMOOTH MUSCLE DUE TO NIFEDIPINE-RESISTANT CALCIUM CURRENT

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SUMMARY

1. Electrical slow waves of gastrointestinal smooth muscles are not abolished by organic Ca^{2+} channel blocking drugs, such as nifedipine or D600. These compounds reduce the amplitude and duration of the plateau phase, but the upstroke phase of slow waves persists.

2. Voltage clamp experiments were performed on isolated circular muscle cells from the canine proximal colon to characterize the dihydropyridine-resistant component of inward current. Inward currents were measured at 25 and 35 °C. The higher temperature increased the amplitudes of the transient and sustained phases of the inward current. The voltage dependence of activation and inactivation of the inward current was not significantly changed at 35 *vs.* 25 °C.

3. At 35 °C the transient phase of the inward current was reduced but not blocked by nifedipine (10^{-6} M). The sustained phase was blocked by nifedipine.

4. The block by nifedipine was voltage dependent, increasing with depolarization. At voltages reached during the upstroke depolarization about 35% of the inward current persisted in the presence of nifedipine (10^{-6} M). This may be sufficient inward current to sustain the upstroke depolarization in intact muscles.

5. Nifedipine caused a 20 mV negative shift in the voltage dependence of inactivation suggesting that dihydropyridines may preferentially bind to Ca^{2+} channels in an inactivated state.

6. Ni^{2+} (< 100 μM) significantly decreased the transient phase of inward current. A combination of Ni^{2+} (40 μM) and nifedipine (10^{-6} M) blocked all of the inward current at 35 °C. Combination of nifedipine (10^{-6} M) and Ni^{2+} (40 μM) blocked slow waves in intact muscles.

7. Bay K 8644 (10^{-6} M) increased the amplitude of the transient and sustained components of inward current. On a percentage basis the increase in the sustained component was greater than the increase in the transient component with test potentials in the range of -50 to -20 mV. This may explain why Bay K 8644 preferentially increases the plateau component of slow waves *vs.* the upstroke component.

8. The findings of this study suggest that the nifedipine resistance of the upstroke depolarization could be due to the voltage dependence of the block of Ca^{2+} channels

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by dihydropyridines. Thus a single class of voltage-dependent Ca^{2+} channels could be responsible for the upstroke and plateau phases of slow waves.

INTRODUCTION

Previous studies have suggested a role of Ca^{2+} current in the generation of electrical slow waves (Ward & Sanders, 1992) and in the plateau phase of slow waves (Papasova, Nagai & Prosser, 1968; Szurszewski, 1975; El-Sharkawy, Morgan & Szurszewski, 1978; Fujii, Inoue, Yamanaka & Yoshitomi, 1985; Barajas-Lopez, Den Hertog & Huizinga, 1989; Lanton, Burke & Sanders, 1989; Sanders, Burke, Carl, Cole, Langton & Ward, 1990). Although it seems quite clear that the plateau phase is dependent upon activation of dihydropyridine-sensitive Ca^{2+} current, the involvement of Ca^{2+} current in the upstroke phase is less certain. Nifedipine and D600 greatly reduce the amplitude and duration of the plateau phase without abolishing the upstroke component (Fujii *et al.* 1985; Barajas-Lopez *et al.* 1989; Ward & Sanders, 1992). Barajas-Lopez *et al.* (1989) concluded that the upstroke is dependent upon deactivation of a K^+ current which they referred to as an inward rectifier. We have shown (Ward & Sanders, 1992) that a reduction in the driving force on Ca^{2+} ions by reducing the Ca^{2+} gradient or inorganic Ca^{2+} blocking agents reduced or abolished the upstroke component of slow waves. Therefore, it appears that a portion of the Ca^{2+} current that contributes to the generation of the upstroke depolarization is insensitive to block by dihydropyridines.

Dihydropyridine-resistant Ca^{2+} currents have been observed in a variety of tissues (for review, see Bean, 1989). One class of channels, referred to as 'T-channels', have small conductances, activate at relatively negative potentials, and inactive rapidly. A current with 'T-like' characteristics has been identified in vascular smooth muscle (Bean, Sturek, Puga & Hermsmeyer, 1986). If a dihydropyridine-insensitive Ca^{2+} current is responsible for the upstroke component of slow waves in colonic muscles, it has not been readily apparent in previous voltage clamp studies on isolated colonic cells; nifedipine blocked virtually all of the inward current in these cells (Langton *et al.* 1989).

It is possible that the enzymatic dispersion of cells, dialysis of cells with pipette filling solutions, or other experimental conditions could have resulted in loss of some component of current in previous studies (Langton *et al.* 1989). For example, it is possible that Ca^{2+} currents are affected by temperature, and therefore previous voltage clamp experiments (performed at room temperature) may have missed a portion of the inward current. In the present study we have tested the hypothesis that a portion of the inward current expressed in colonic myocytes is resistant to dihydropyridines, and this current can be better resolved at physiological temperatures. Part of these results were presented at the American Motility Symposium in October of 1990 (Ward, Blondfield & Sanders, 1990).

METHODS

Colonic muscles were obtained from adult dogs as previously described (Ward & Sanders, 1992). Muscle strips were isolated from the submucosal region of the circular muscle layer, and smooth muscle cells were enzymatically dispersed as previously described (Langton *et al.* 1989). After

dispersion, the cells were plated into plastic culture dishes and incubated at 37 °C (90% humidity and 95% O_2–5% CO_2) for 30 min. Cells were stored at 4 °C and used within 6 h.

The isolated cells were bathed in a perfusion solution buffered with HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2'ethanesulphonic acid) and containing (mM): Na^+, 89·9; K^+, 5·8; TEA, 40; Cl^-, 135; HCO_3^-, 4·16; HPO_4^{2-}, 0·34; $H_2PO_4^{2-}$, 0·4; Ca^{2+}, 2·5; Mg^{2+}, 0·9; SO_4^{2-}, 0·4; dextrose, 10; sucrose, 2·9; HEPES, 10. The solution pH was adjusted to 7·4 with NaOH. Patch pipettes were prepared as previously described (Langton *et al.* 1989). The pipette solution used for isolating inward calcium currents contained (mM): caesium aspartate, 110; caesium chloride, 20; Na_2ATP, 4; phosphocreatine, 2; $MgCl_2$, 1; HEPES, 5; EGTA, 1; Na_2GTP, 1. The pH was adjusted to 7·2 with CsOH, and the solution was filtered and stored frozen. The pH of both solutions decreased by about 0·1 pH unit when solutions were warmed from 25 to 35 °C. In some experiments, as noted, pipette solutions contained 0·1 mM-EGTA.

After obtaining gigaseals and access to the cell interior, 3–5 min were allowed for dialysis (Langton *et al.* 1989). Voltage clamp protocols were delivered by an Axopatch 1A patch clamp amplifier (Axon Instruments). Membrane currents were recorded with a 12 bit A/D converter (Lab Master, Scientific Solutions) interfaced to an AT-type computer running pClamp software (Axon Instruments). Currents were recorded at a sampling rate of 10 and 20 kHz and records were low-pass filtered at 2 kHz.

Data were analysed with pClamp software, and hard copies were made, after leak subtraction, with a Hewlett Packard digital plotter (Colorpro) and Sigmaplot (Jandel Scientific). Averaged data are expressed as the means ± S.E.M., and 'n' represents the number of cells. Recordings were made at either 25 or at 35 °C.

Solutions and drugs used in voltage clamp experiments. Ethyleneglycol-bis-(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA) and tetraethylammonium chloride (TEA) were obtained from Sigma, and nifedipine was obtained from Boehringer Mannheim. Nifedipine and Bay K 8644 (Miles, Inc.) were dissolved in 100% ethanol in a stock solution of 10^{-2} M and stored at −20 °C until immediately before use. Ni^{2+} was made as a 1 M stock solution and added to the desired concentration directly to the perfusion solution. Tetrodotoxin (TTX; Sigma) was dissolved in distilled water at 3×10^{-4} M and then further diluted in perfusion solution to 3×10^{-6} M.

RESULTS

Temperature effects on inward current

Thirteen cells were studied under whole-cell recording conditions using Cs^+-containing pipettes and TEA in the external solution (see Methods). Seals were obtained at 25 °C, and at least 3–5 min were allowed for dialysis (Langton *et al.* 1989). A series of test potentials ranging from −90 to 0 mV were applied from a holding potential of −85 mV. Depolarization positive to −50 mV caused activation of inward currents consisting of a transient phase which partially inactivated, leaving a sustained inward current that persisted for the duration of the 900 ms test pulses. After *I–V* data were collected at 25 °C, the cells were warmed (within 2 min) by increasing the temperature of the perfusion buffer. The change in temperature did not significantly affect the holding current; currents averaged 29·5 ± 5·6 pA at 25 °C and 26·0 ± 3·6 pA at 35 °C at −85 mV ($P > 0·05$; $n = 13$). After warming to 35 °C, *I–V* protocols were repeated. The increased temperature enhanced the magnitude of the transient and sustained phases of the inward current. For example at a test potential of −20 mV the transient current increased by an average of 448 ± 92% and the sustained current increased by 377 ± 84% ($P < 0·001$). Figure 1 compares a family of currents from one cell and average *I–V* curves from thirteen cells at 25 and 35 °C.

Temperature also increased the rate and degree of inactivation of the inward current. For example, with test potentials from −85 to −20 mV, inactivation of the

inward current was fitted with two exponentials having time constants averaging $21 \pm 1{\cdot}8$ and $216 \pm 15{\cdot}9$ ms at 25 °C and $8{\cdot}3 \pm 0{\cdot}6$ and $174 \pm 19{\cdot}2$ ms at 35 °C (r values were each 0·99). These values were significantly different at the $P < 0{\cdot}001$ level. In a separate series of experiments on ten cells pipette solutions containing 0·1 mM-

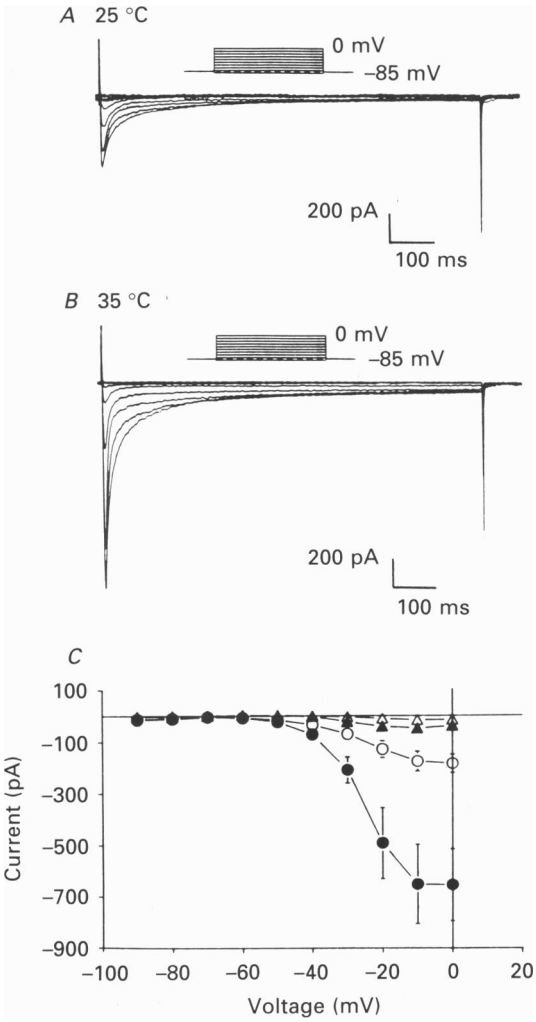

Fig. 1. Effects of temperature on inward current. Inward currents were recorded at 25 °C (*A*) in response to test pulses spanning the physiological range of potentials (from −90 to 0 mV); holding potential −85 mV). Then cells were warmed to 35 °C and voltage clamp protocol was repeated (*B*). Inward currents were greatly increased at the higher temperatures. *C*, averaged *I–V* curves for inward currents (peak (circles) and sustained (triangles) components) at 25 (open symbols) and 35 °C (filled symbols) ($n = 13$ cells). Note that transient component is more sensitive to temperature than sustained component.

EGTA were used. At 35 °C the inactivation of the Ca^{2+} current was still well fitted by a double exponential ($r = 0{\cdot}99$), but the rate of the second component of inactivation increased with the lower EGTA concentration. The averages of the

inactivation time constants were $8{\cdot}9 \pm 0{\cdot}5$ and 107 ± 10 ms. The second time constant was significantly less than in experiments in which 1·0 mM-EGTA was used ($P < 0{\cdot}01$). These data suggest that intracellular Ca^{2+} concentration affects the inactivation process, as described previously (cf. Tillotson, 1979; Plant, Standen & Ward, 1983; Chad & Eckert, 1986).

Although the inward current was sustained at a low amplitude throughout test depolarizations of 900 ms, we observed a slow rate of inactivation of this component. Figure 2 shows the sustained current at 25 and 35 °C in thirteen experiments at potentials spanning the range of potentials observed physiologically during slow waves in intact muscles. Note that the rate of the inactivation increased with potential.

Effects of nifedipine on inward current

Temperature greatly enhanced the magnitude of the inward current, and it is possible that this effect was due to: (i) the activation of additional components of inward current; (ii) faster activation of the inward current. We tested the effects of nifedipine to determine whether a dihydropyridine-resistant component could be detected at 35 °C. *I–V* relationships were characterized in seven cells before and in the presence of nifedipine (10^{-6} M). Nifedipine reduced the amplitude of the transient component of inward current but did not abolish this component, as previously reported (Langton *et al.* 1989). The sustained component was completely blocked, and often a small outward current was recorded in the presence of nifedipine suggesting that the outward current was not entirely blocked by Cs^+ dialysis and external TEA. Figure 3 shows a family of currents from a typical cell before and in the presence of nifedipine and average *I–V* curves from seven cells studied.

Inward currents were fitted with double exponentials before and in the presence of nifedipine. Nifedipine had no effect on the fast time constant of inactivation ($8{\cdot}3 \pm 0{\cdot}7$ and $9{\cdot}6 \pm 1{\cdot}3$ ms; $P > 0{\cdot}4$; control *vs.* nifedipine), but decreased the slower time constant ($167 \pm 6{\cdot}8$ and $89 \pm 13{\cdot}3$ ms; $P < 0{\cdot}005$; control *vs.* nifedipine).

The magnitude of the block of the transient current was dependent upon voltage; with more positive test potentials, a greater percentage of the transient inward current was blocked (see Fig. 3). Note that at −30 mV (near the level of depolarization reached during the upstroke potential of slow waves), nifedipine blocked only 66% of the transient current leaving an average of $67 \pm 13{\cdot}9$ pA of inward current.

In one experiment several concentrations of nifedipine ranging from 10^{-9} to 10^{-6} M were studied in a single cell. The reduction in both phases of the inward current was concentration dependent, with complete block of the sustained component occurring at 10^{-7}–10^{-6} M. This was the concentration that had a maximal effect on the plateau phase of the slow waves in intact muscles (see Fig. 2 of Ward & Sanders, 1992). The transient component was more resistant to nifedipine at all doses. With 10^{-6} M-nifedipine, 71, 30 and 14% of the transient current persisted with test depolarizations to −40, −30 and −20 mV (i.e. potentials experienced during upstroke depolarization of slow waves). Figure 4*A* shows the effects of several doses of nifedipine on currents elicited by test depolarizations of −40 to −20 mV, and Fig. 4*B* shows *I–V* relationships for the two components of inward current in the presence of several concentrations of nifedipine.

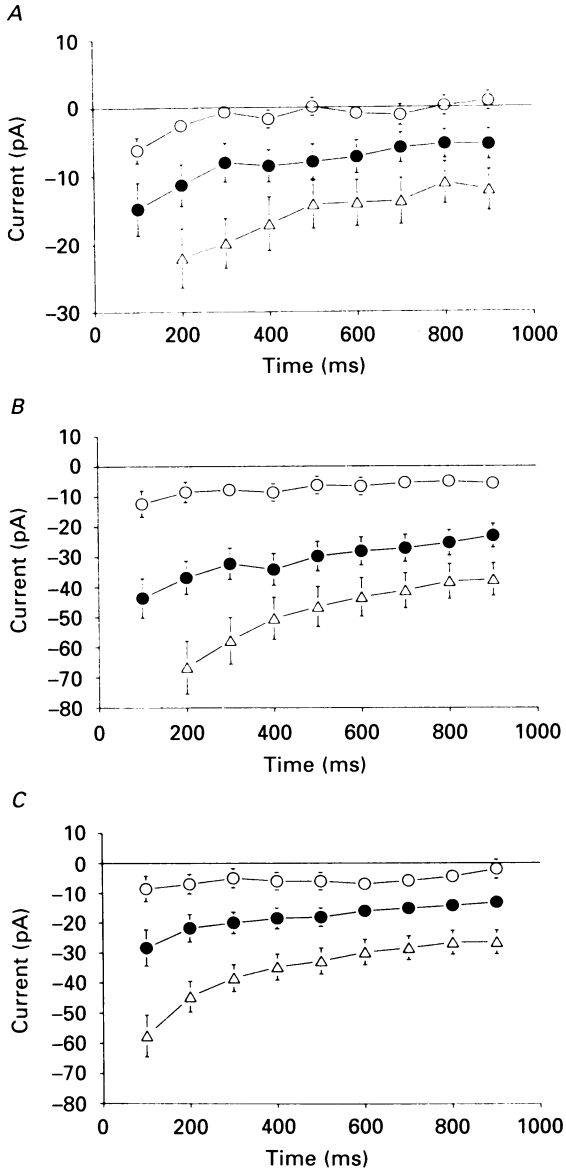

Fig. 2. Slow inactivation of inward current during sustained depolarization. After the initial rapid phase of inactivation there was slow relaxation of the inward current over the next several hundred milliseconds. Panel *A* shows slow relaxation at 25 °C with test potentials spanning range of potentials reached during upstroke and plateau phase of slow waves. Panel *B* shows relaxations at 35 °C. Note that the rate of relaxation increases with depolarization. In experiments depicted in panels *A* and *B* ($n = 13$) pipette solutions contained 1 mM-EGTA. Panel *C* shows slow inactivation in cells dialysed with solutions containing 0·1 mM-EGTA at 35 °C ($n = 10$). This slow relaxation of inward current during sustained depolarization (which simulates the plateau phase of slow waves) may contribute to repolarization of slow waves in intact muscles. Test potentials: −40 mV, ○; −30 mV, ●; −20 mV, △.

Effects of nickel ions on inward currents

Inorganic Ca^{2+} channel blockers were found to affect both phases of slow waves in intact muscles (Ward & Sanders, 1992). In comparing the effects of Mn^{2+} and Ni^{2+} it was noted that the upstroke component was affected at lower concentrations of

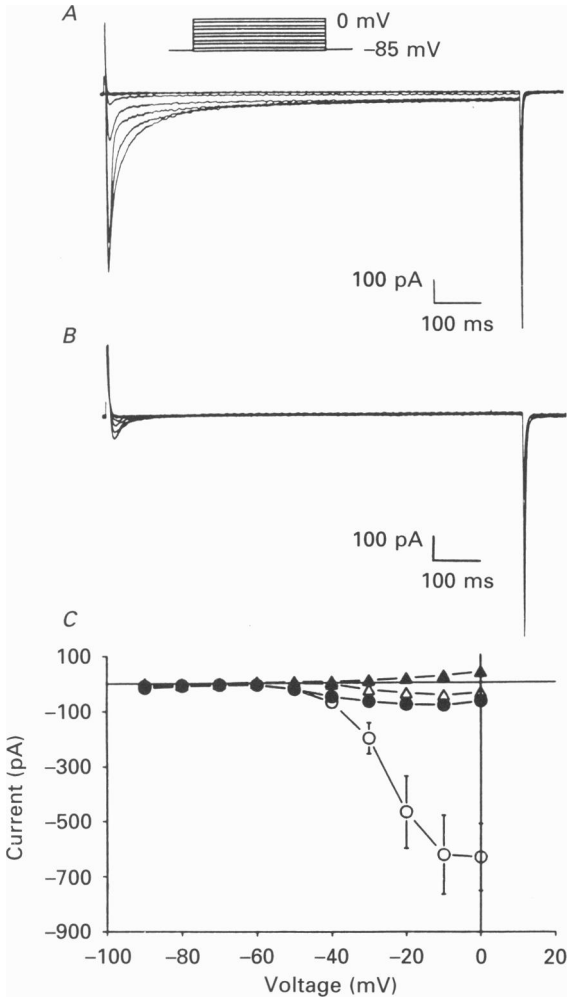

Fig. 3. Effect of nifedipine on inward current. Panel *A* shows family of currents generated at 35 °C by test depolarizations from a holding potential of −85 mV (protocol at reduced time scale shown in inset). Panel *B* shows repeat of voltage clamp protocol in the presence of nifedipine (10^{-6} M). Nifedipine failed to block all inward current. Small portion of transient phase remains; all sustained current blocked. Panel *C* shows averaged *I–V* curve for seven cells exposed to nifedipine. Peak (circles) and sustained (triangles) components are plotted before (open symbols) and in the presence of nifedipine (filled symbols). Data are means ± standard error of the mean. Note that block of transient phase increases with depolarization.

Ni^{2+} (Ward & Sanders, 1992). The effects of Ni^{2+} (0·04–0·125 mM) on inward currents were studied in six cells. Ni^{2+} reduced both the transient and sustained phases of the inward current (Fig. 5).

The nifedipine-resistant component of inward current was blocked by addition of Ni^{2+}. Three cells were stepped from a holding potential of −85 mV to a test potential

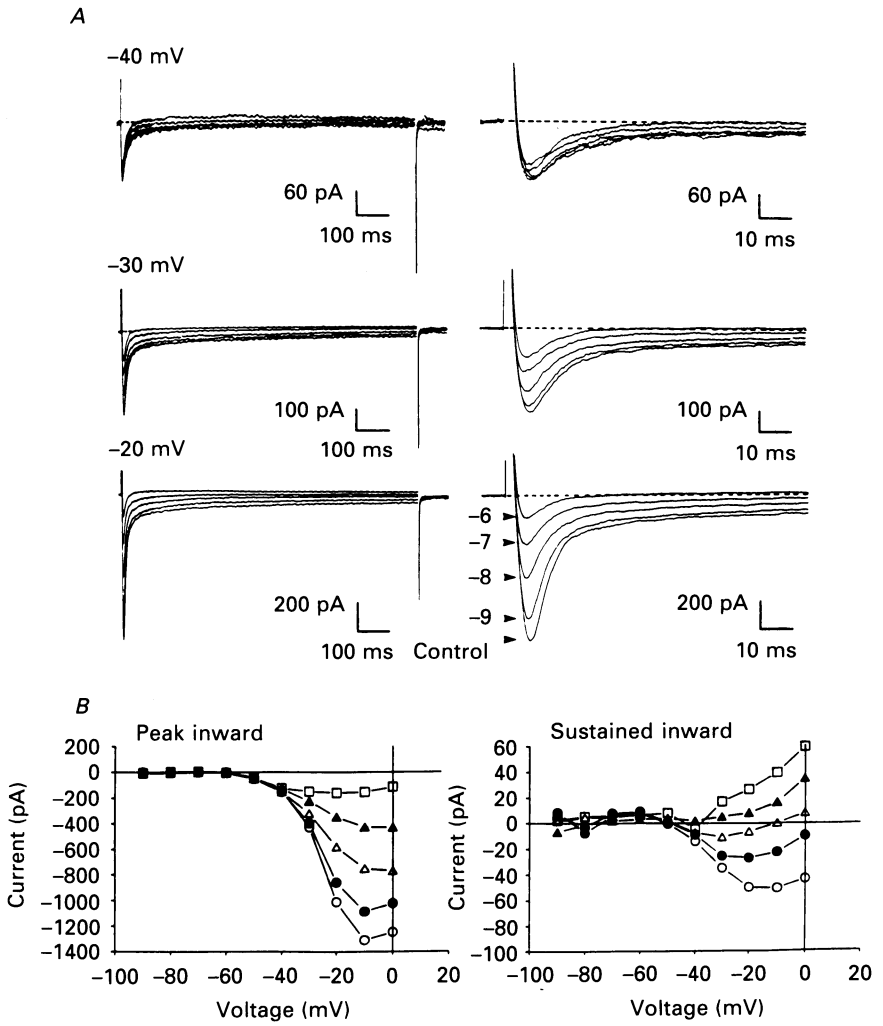

Fig. 4. Concentration effects of nifedipine. Panel *A* shows families of current generated with test potentials of −40, −30 and −20 mV in response to nifedipine (10^{-9}–10^{-6} M; as noted in record at bottom right). Records at left show complete responses to 900 ms depolarizations; records at right show transient phase of inward current at faster sweep speed. Note that degree of block increases with depolarization. At 10^{-6} M much of the transient phase persists at −40 mV and about 30% persists at −30 mV. Sustained phase of current blocked at all potentials by 10^{-6} M-nifedipine. Panel *B* shows plots of *I–V* curves obtained in the presence of several concentrations of nifedipine. ○, control; ●, 10^{-9} M-nifedipine; △, 10^{-8} M-nifedipine; ▲, 10^{-7} M-nifedipine; □, 10^{-6} M-nifedipine.

of −30 mV for 900 ms at a frequency of 0·125 Hz. Exposure to nifedipine (10^{-6} M) decreased the transient current and abolished the sustained component, as described above. After a stable effect was observed, the cells were exposed to Ni^{2+} (0·040 mM). This abolished the remaining transient current, suggesting that all of the inward current is carried by Ca^{2+} ions (Fig. 6).

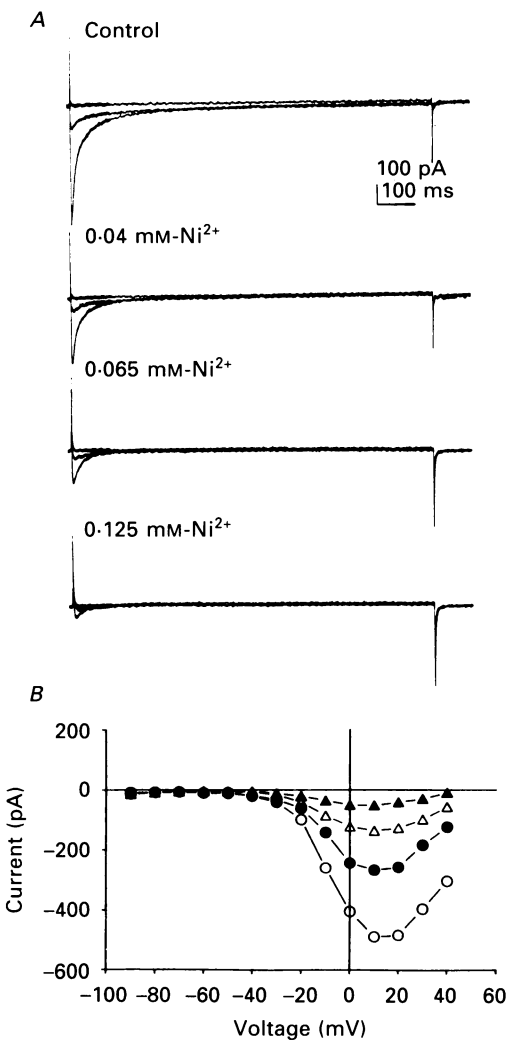

Fig. 5. Effect of nickel on inward current. Low concentrations of this divalent ion blocked most inward currents. *A*, data records show currents elicited by test potentials of −50, −20 and 0 mV under control conditions (top trace) and in the presence of concentrations of Ni^{2+} noted. Ni^{2+} reduced amplitude of both components of the inward current. At 0·125 mM there was little or no current available with pulses negative to −20 mV (maximum level reached by slow waves in intact muscles). Experiments performed at 35 °C. Panel *B* shows *I–V* curves obtained in the presence of several concentrations of Ni^{2+}. ○, control; ●, 0·04 mM-Ni^{2+}; △, 0·065 mM-Ni^{2+}; ▲, 0·125 mM-Ni^{2+}.

Since all of the inward current could be abolished by a combination of nifedipine and Ni^{2+}, we tested whether this combination of agents could abolish slow waves in intact muscles (see Ward & Sanders, 1992, for procedures). Cells near the submucosal muscle were impaled and typical slow wave activity was recorded. Application of

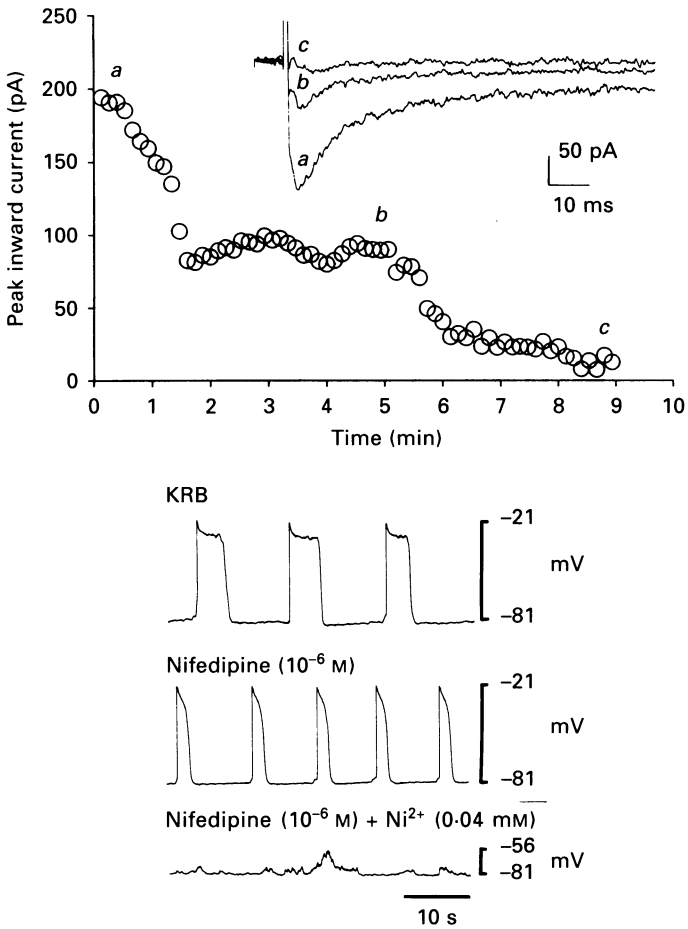

Fig. 6. Combined effects of nifedipine and Ni^{2+}. Figure shows amplitude of peak inward current elicited by repetitive depolarizations to −30 mV. At *a* nifedipine (10^{-6} M) was added This caused about a 50 % reduction in the peak inward current. At *b* Ni^{2+} (0·04 mM) added in addition to the nifedipine. This essentially abolished the remaining inward current. Raw data at points *a–c* shown in inset in upper panel. Bottom traces show effects of nifedipine and Ni^{2+} on slow waves recorded from an intact strip of muscle from the canine proximal colon. First trace shows typical slow wave activity from a cell near the submucosal border of the circular muscle layer (see Ward & Sanders, 1992, for details of preparation and recording). Nifedipine (10^{-6} M) reduced the amplitude and duration of the plateau phase of the slow waves (middle trace). Addition of Ni^{2+} (0·04 mM) along with nifedipine blocked slow wave activity (bottom trace).

nifedipine (10^{-6} M) reduced the amplitude and duration of the plateau phase and increased the frequency of slow waves (as described in Ward & Sanders, 1992; see also Fig. 6). Further application of Ni^{2+} (0·04 mM) abolished slow wave activity (Fig. 6).

Activation and inactivation characteristics of Ca^{2+} current at 35 °C

Voltage-dependent activation was determined for six cells (test potentials from −90 to +60 mV) from the peak inward current corrected for the driving force at each test potential (Klockner & Isenberg, 1985). Driving force was determined as the

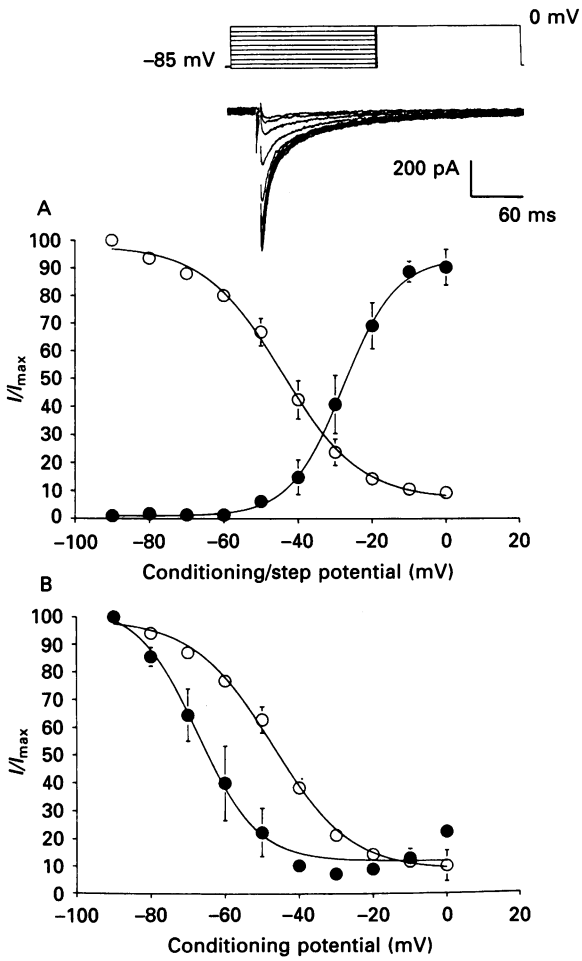

Fig. 7. Voltage-dependent activation and inactivation of inward current. Panel *A* shows plots of averaged data describing activation (●; $n = 6$) and inactivation (○; $n = 4$). Points are fitted with Boltzmann functions. See details in text explaining how data were obtained. Inactivation protocol and example of current responses to test depolarizations to 0 mV in one cell shown in inset. All experiments performed at 35 °C. Panel *B* shows effects of nifedipine on voltage-dependent inactivation in three cells. Inactivation curves generated under control conditions (○) and then repeated in presence of 10^{-6} M-nifedipine (●). Nifedipine caused a pronounced negative shift in inactivation relationship.

difference between test potential and the observed reversal potential. Peak conductance was normalized to 100 and plotted against test potential. This yielded a sigmoidal relationship which was fitted with a Boltzmann function as previously described (Langton *et al.* 1989). Maximal conductance may have been somewhat

underestimated by the technique used because at the time when peak conductance was reached some inactivation may have already occurred. Figure 7*A* shows an activation curve fitted to averaged data. Half-activation occurred at −28 mV and the steepness of the activation curve was −7·2.

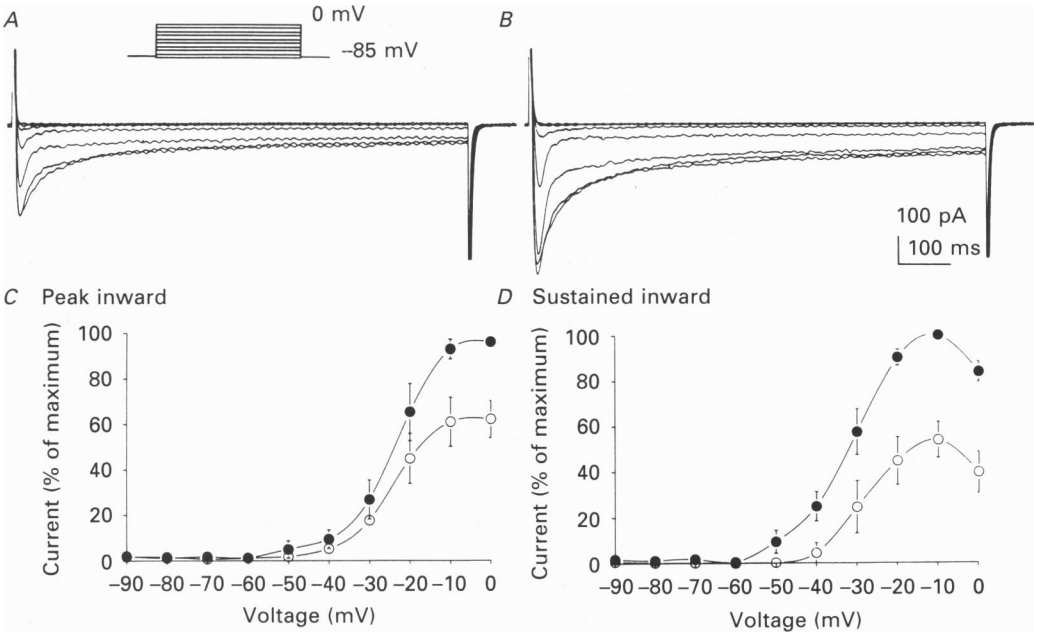

Fig. 8. Effects of Bay K 8644 on inward currents. Example of families of currents generated in one cell under control conditions (*A*) and in presence of Bay K 8644 (10^{-6} M; *B*). Note increase in the amplitude of the transient and sustained phases of current. Panels *C* and *D* show effects of Bay K 8644 on normalized currents. ○, control; ●, 10^{-6} M-Bay K 8644. These plots show that the increase in the transient phase (*C*) was smaller than the increase in the sustained phase (*D*). Data are means ± standard error of the mean ($n = 4$). Note increase in sustained current in the range of −50 to −30 mV which is the range of the plateau phase of slow waves in intact muscle.

Steady-state inactivation was studied in four cells using a two-pulse protocol consisting of 500 ms conditioning pulses ranging from −90 to 0 mV, a 5 ms hyperpolarization to −90 mV, and a test potential to 0 mV. Peak currents were normalized against the maximum current response and plotted as a function of conditioning potential. The resulting inactivation curves were averaged and fitted with a Boltzmann function as previously described (Langton *et al.* 1989). Half-maximal inactivation occurred at −44 mV, and the steepness factor was 10·5 mV. These values are similar to previously reported values for inactivation of Ca^{2+} current in colonic myocytes characterized at 23 °C (i.e. holding potential, V_h, = −43 mV and steepness factor = 8·3 mV; see Langton *et al.* 1989). An example of one of these experiments and a summary inactivation curve is shown in Fig. 7*A*.

We also tested the effects of nifedipine on voltage-dependent inactivation in three cells. In these experiments, inactivation protocols, as described above, were

performed before and in the presence of nifedipine (10^{-6} M). Nifedipine shifted the inactivation curves to more negative potentials (average shift in $V_h = -20 \pm 2{\cdot}8$ mV; see Fig. 7*B*).

Effects of Bay K 8644 on inward current

We found that Bay K 8644 substantially increased the amplitude and duration of slow waves in intact muscles (Ward & Sanders, 1992). The effect on slow wave duration was far more pronounced than the increase in the upstroke component, suggesting a differential enhancement of the currents contributing to the plateau *vs.* the upstroke. Therefore, we tested the effects of Bay K 8644 on four cells to determine its effects on the transient and sustained components of inward current.

Bay K 8644 increased the amplitude of the transient and sustained components. Figure 8 shows an example of the effects of Bay K 8644 in one cell and normalized *I–V* curves for the peak and sustained components before and in the presence of Bay K 8644. These data showed a significant enhancement in the inward current generated by test potentials in the physiological range. For example, peak inward current increased from $-49 \pm 17{\cdot}8$ to $-113 \pm 27{\cdot}2$ pA, from -184 ± 86 to -377 ± 112 pA, and from -503 ± 267 to -781 ± 255 pA with test potentials of -40, -30 and -20 mV, respectively. The sustained component of current increased from -3 ± 3 to -21 ± 5 pA, from -22 ± 9 to -50 ± 8 pA, and from -42 ± 10 to -83 ± 12 pA at the same potentials. A comparison of the normalized effects of Bay K 8644 demonstrated that the increase in the magnitude of the sustained component was greater than the increase in the transient component with test potentials in the range of -50 to -20 mV (Fig. 8*C* and *D*). This may explain why the effects of Bay K 8644 are more pronounced on the plateau phase of slow waves than on the upstroke phase (see Fig. 5 of Ward & Sanders, 1992).

DISCUSSION

Based on this and previous studies, a picture has emerged of the ionic mechanism of slow waves in gastrointestinal smooth muscles (Langton *et al.* 1989; Cole & Sanders, 1989; Carl & Sanders, 1989; Sanders *et al.* 1990; Carl, McHale, Publicover & Sanders, 1990). Our data suggest that inward current occurs in two phases, both of which are important in the generation of slow waves. (i) The first phase is transient, partially resistant to block by dihydropyridines, and contributes to the upstroke depolarization. Although this phase is small in absolute magnitude in the presence of dihydropyridines, it appears to be sufficient to generate the upstroke depolarization (Ward & Sanders, 1992). This phase of the inward current was overlooked previously in studies performed at room temperature (Langton *et al.* 1989). (ii) The second phase of the inward current is sustained and is blocked by organic Ca^{2+} channel blocking drugs. The most important, physiological task of the sustained phase of the inward current is to provide Ca^{2+} influx during the plateau phase of slow waves (Vogalis, Publicover, Hume & Sanders, 1991), and this component appears to be the main signal for, and regulator of, excitation–contraction coupling in several regions of the gastrointestinal tract (Morgan & Szurszewski, 1980; Ozaki, Stevens, Blondfield, Publicover & Sanders, 1991).

The two phases of Ca^{2+} current could result from the expression of two types of Ca^{2+} channels with differing sensitivities to dihydropyridines. Two types of Ca^{2+} channels have been described in cardiac cells (Nilius, Hess, Lansman & Tsien, 1985; Mitra & Morad, 1986; Hagiwara, Irisawa & Kameyama, 1988; see also review by Bean, 1989) and in some smooth muscle cells (Bean *et al.* 1986). One class of channels, referred to as low-threshold or 'T' channels, has a small unitary conductance and activates at relatively negative potentials. The other class ('L-type') has a greater single-channel conductance and activates at more positive levels. The pharmacology of the two classes of channels is different. L-type channels are sensitive to dihyropyridines, but T-type channels are nearly unaffected by these compounds below 3 μM (Nilius *et al.* 1985; Hagiwara *et al.* 1988). There is also different sensitivity to inorganic Ca^{2+} channel blockers (Hagiwara *et al.* 1988), which is perhaps due to a difference in the molecular structure of the permeation pore. In the present study we found that Ni^{2+} (40 μM), which has been described as a relatively specific blocker of T-current at low concentrations (Hagiwara *et al.* 1988), blocked the nifedipine-resistant phase of current, and this concentration significantly reduced the rate-of-rise of slow wave upstrokes (see Fig. 4 in Ward & Sanders, 1992). But it should also be noted that Ni^{2+} also reduced the sustained component of inward current with similar potency.

At present we are doubtful that a 'T-like' current is expressed in colonic myocytes. One of the common signatures of a T current in a mixed current–voltage relationship is a shoulder in the curve at negative potentials (i.e. -50 to -30 mV; cf. Schroeder, Fischbach & McCleskey, 1990). The *I–V* curve for colonic cells is monophasic and bell-shaped, and a lower threshold current is not easily identified in the presence of nifedipine. Clearly more extensive experiments, including single channel studies, will be needed to determine whether the two components of Ca^{2+} current in colonic cells are due to the activity of two or more specific classes of ion channels.

The two components of inward current could result from a single class of Ca^{2+} channels. The resistance of a portion of the inward current and the upstroke component of slow waves to dihydropyridines could result from the voltage dependence of the block of Ca^{2+} channels by dihydropyridines. Nelson & Worley (1989) have shown that dihydropyridine block of single channels of arterial smooth muscle is voltage dependent and suggested this could be explained by a model in which these compounds bind to the inactivated state of the channels with higher affinity than to the resting state. This is similar to the model originally proposed for dihydropyridine block of Ca^{2+} current in cardiac myocytes (Bean, 1984). Nelson & Worley (1989) found that a greater percentage of channels were blocked by nisoldipine when more positive holding potentials were used. At more positive holding potentials, more channels would be in the inactivated state and therefore in a high affinity state for binding nisoldipine. This property of dihydropyridine block was manifest as a negative shift in the apparent voltage dependence of inactivation. In the present study we found that the block of inward current by nifedipine was voltage dependent, and the magnitude of the block increased with depolarization (see Figs 3 and 4). Based on the findings of Nelson & Worley (1989), there may have been a low level of nifedipine binding at the negative holding potentials used in our experiments (-85 mV). Upon depolarization, binding would increase as inactivation

proceeded. Therefore, the nifedipine block might increase as a function of depolarization and time. This mechanism may also explain the relative resistance of the upstroke depolarization of slow waves to nifedipine in intact muscles (Ward & Sanders, 1992). At the negative resting potentials of colonic muscle cells (about −80 mV), the affinity of dihydropyridines for Ca^{2+} channels may be low. Even at a high concentration of nifedipine (such as 10^{-6} M) the availability of unblocked channels may be sufficient to support the upstroke depolarization. With time the block would increase, and therefore most of the sustained component of inward current (and therefore the plateau component of slow waves) may be blocked. Similar reasoning may explain why Bay K 8644 has a proportionally greater effect on the sustained phase of the inward current and the plateau phase of slow waves than it does on the transient phase of the inward current and the upstroke depolarization.

We examined the inward current of colonic myocytes in a previous study (Langton *et al.* 1989) and found this current, which was identified as a Ca^{2+} current, to be blocked by nifedipine (10^{-6} M). In the present study we found that physiological temperatures greatly enhanced the amplitude of the inward current, especially the peak of the transient phase. The enhanced transient current was reduced, but not blocked, by nifedipine. As discussed above, we have no reason to suggest that this was due to an additional population of channels that were not active at room temperature. An increase in the activation rate at 35 °C might explain the increase in the magnitude of the transient phase and the relatively greater resistance to dihydropyridine. Faster activation might increase resistance to dihydropyridine block since many channels may be able to open and reopen for a longer period before dihydropyridine binding occurs. Thus the emergence of a dihydropyridine-resistant current at 35 °C could be due to a disparity between the increase in activation rate and the rate of dihydropyridine binding.

This study, and the preceding one (Ward & Sanders, 1992), provide the concept that the transient and sustained phases of Ca^{2+} current are involved in the regulation of gastrointestinal motility. The first phase is responsible for timing in the electrically rhythmic regions of the gastrointestinal tract. This phase initiates slow waves and provides the depolarization necessary to reach a potential where the second, sustained phase is balanced by outward current (i.e. creating the plateau phase of the slow wave; see Sanders *et al.* 1990). While important for timing, the dihydropyridine-resistant, first phase is not very important in terms of contraction. Most physiological agonists have very little effect on the first phase of inward current (i.e. few agonists affect the upstroke depolarization; see Szurszewski, 1987). However, some agonists have an important chronotropic effect which could conceivably be due to a negative shift in the voltage dependence of activation. The second phase of the inward current appears to be crucial for excitation–contraction coupling. This phase of the inward current is blocked by dihydropyridines and other organic Ca^{2+} channel blockers. Our data suggest that the two phases of inward current could be generated by the complex activation and inactivation properties of a single class of Ca^{2+} channels. This concept of two phases of Ca^{2+} current, each with a distinct role in motility, may be useful in developing therapeutic agents directed at either chronotropic or inotropic regulation of gastrointestinal motility.

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