Journal of Physiology (1993), **460**, *pp.* 135–152
With 11 *figures*
Printed in Great Britain

COMPARISON OF IONIC CURRENTS FROM INTERSTITIAL CELLS AND SMOOTH MUSCLE CELLS OF CANINE COLON

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(*Received 26 November 1991*)

SUMMARY

1. Voltage-dependent ionic currents of isolated interstitial cells were characterized using the whole-cell voltage clamp technique, and compared with currents recorded from circular muscle cells. Both cell types were isolated from the submucosal pacemaking region in the canine distal colon.

2. Upon depolarization, interstitial cells and smooth muscle cells generated transient inward, followed by slowly inactivating outward, currents.

3. After blocking inward current and much of the Ca^{2+}-dependent outward current, interstitial cells displayed voltage-dependent outward current that rapidly activated, reached a peak, and then inactivated. This current was resistant to 4-aminopyridine(4-AP; 1 mM). Smooth muscle cells expressed a similar current but it was reduced by about 40% at a test potential of +20 mV by 4-AP (1 mM).

4. The inactivation characteristics of the voltage-dependent outward currents of interstitial cells and smooth muscle cells were compared. The outward current of interstitial cells inactivated at more negative potentials; half-inactivation occurred at −53 mV, whereas half-inactivation occurred at −20 mV in smooth muscle cells.

5. Inward currents were not strikingly different in the two cell types when dialysing pipettes were used. When the perforated patch technique (using Amphotericin-B) was used, a negatively activating inward current was observed in interstitial cells that had a resolution threshold of −70 to −60 mV. This current peaked at −10 mV. Inward currents in smooth muscle cells were resolved at test potentials positive to −50 mV and peaked at 0 to +10 mV.

6. When interstitial cells were held at −40 mV, inward current could not be resolved with test depolarization negative to −30 mV. From this holding potential, peak amplitude was reduced by 85% with test depolarizations to −10 mV. Holding smooth muscle cells at −40 mV also reduced inward current, but the peak current in these cells was reduced by only 39% at 0 mV.

7. Ni^{2+} partially inhibited peak inward current in interstitial cells and abolished a 'hump' in the *I–V* curve that occurred at negative potentials. In dialysed cells where this 'hump' was not apparent, addition of nifedipine unmasked a 'hump'. The presence of both nifedipine and Ni^{2+} abolished inward current.

8. A portion of the inward current in smooth muscle cells was sustained and

persisted for the duration of test pulses. Very little sustained inward current was observed in interstitial cells.

9. The time course of inactivation of inward current in interstitial cells was fitted with two exponentials. The slow component of inactivation showed U-shaped dependence on test potential, suggesting 'current' dependency. 'Current' dependence was not apparent in the fast component. Characterizations of steady-state inactivation showed that the inward current inactivated at more negative potentials in interstitial cells (half-inactivation −45 mV) than in smooth muscle cells (half-inactivation −30 mV).

10. Inward current activated close to the resting potential in interstitial cells. This feature, along with the negatively inactivating outward current, may facilitate the spontaneous activity of these cells.

INTRODUCTION

Contractions of colonic muscles occur as a result of rhythmic electrical activity (Christensen, Caprilli & Lund, 1969; Sanders & Smith, 1986*b*; Barajas-Lopez & Huizinga, 1989). In the colon there are two electrical rhythms; a 6 cycle per minute rhythm known as *slow waves* and a 17 cycle per minute rhythm termed *myenteric potential oscillations* (MPOs) (for review see Sanders & Smith, 1989). The strength of contractions is regulated by the amplitude and duration of the spontaneous electrical events (Christensen *et al.* 1969; Sanders & Smith, 1986*b*; Barajas-Lopez & Huizinga, 1989), and the electrical activity is regulated by neurotransmitters, hormones, and paracrine substances (Huizinga, Chang, Diamant & El-Sharkawy, 1984; Sanders & Smith, 1986*a*; Ward, Dalziel, Thornbury, Westfall & Sanders, 1992). Although the amplitude and duration of slow waves and MPOs are regulated by these substances, the *occurrence* of electrical events is not blocked by the loss of regulatory mechanisms or the blockade of nerves (Sanders & Smith, 1986*a*). Thus, the electrical activity has been termed myogenic. This term, however, may not accurately describe the mechanism of pacemaker activity in colonic muscles, because: (i) although isolated circular muscle cells appear to express the ionic conductances necessary for slow waves, isolated cells do not spontaneously generate these events (Langton, Burke & Sanders, 1989*a*); (ii) resolvable inward currents are activated in isolated cells at potentials positive to −50 mV, whereas membrane potentials of cells in the slow wave pacemaker region *in situ* sit 20 to 30 mV negative to this level (Smith, Reed & Sanders, 1987; Langton *et al.* 1989*a*, Ward, Keller & Sanders, 1991; Ward & Sanders, 1992); and (iii) removal of thin strips of tissue along the myenteric and submucosal surfaces of the circular layer abolishes pacemaker activity in the bulk of the remaining circular muscle (Smith *et al.* 1987). These observations suggest that pacemaker activity may be generated in specific regions of the circular muscle layer and possibly by a cell type other than smooth muscle.

Others have suggested that pacemaker activity in the gastrointestinal (GI) tract is generated by cells referred to as interstitial cells (Thuneberg, 1982; Thuneberg, Johanson, Rumenssen & Anderson, 1983; Suzuki, Prosser & Dahms, 1986). This hypothesis has been supported by several anatomical observations, including: (i) interstitial cells populate regions identified as pacemaker regions (Berezin, Huizinga & Daniel, 1988; Ward & Sanders, 1990; Suzuki *et al.* 1986); (ii) interstitial cells are

coupled via gap junctions to smooth muscle cells; (iii) chemicals which are selectively taken up by interstitial cells *vs.* smooth muscle cells have been shown to abolish pacemaker activity (Thuneberg *et al.* 1983; Ward, Burke & Sanders, 1990). It has been very difficult to rigorously test the interstitial cell hypothesis with functional studies. This is because recordings with a single microelectrode from a network of electrically coupled cells provides no information about the source of the electrical activity (cf. Barajas-Lopez, Berezin, Daniel & Huizinga, 1989), and simultaneous recordings from coupled smooth muscle and interstitial cells, demonstrating that electrical events originate in interstitial cells, are unavailable. The first evidence that isolated interstitial cells are in fact excitable cells (i.e. express voltage-dependent ionic channels and produce regenerative electrical events upon depolarization) was recently obtained from isolated cells (Langton, Ward, Carl, Norell & Sanders, 1989*b*). These cells were also spontaneously active, generating slow wave-like depolarizations, when membrane potential was current-clamped in the range of physiological resting potentials. Although one would expect differences between the ionic currents of pacemaker and non-pacemaker cells, previous studies have failed to detect such differences (Langton *et al.* 1989*a*, *b*).

In the present study we have re-evaluated the inward and outward currents of interstitial cells and compared them with currents recorded from circular smooth muscle cells isolated from the same anatomical region and studied under identical conditions. Experiments were performed at more physiological temperature and many experiments used the perforated patch technique (Horn & Marty, 1988; Rae, Cooper, Gates & Watsky, 1991) to conserve intracellular regulatory mechanisms. A preliminary report of these findings has been presented (Lee, Thornbury & Sanders, 1991).

METHODS

Cell preparation and solutions

Mongrel dogs of either sex were anaesthetized with sodium pentobarbitone (45 mg/kg I.V.). A segment of distal colon, 4–8 cm from the rectum, was removed, and the dog was then killed by an overdose of pentobarbitone. The colonic segment was opened along the mesenteric border, and faecal material was removed by washing with Krebs–Ringer bicarbonate solution. The resulting sheet of colon was pinned out in a dissecting dish, muscle strips (1 mm × 10 mm) were cut parallel to the longitudinal fibres, and mucosal tissue was removed.

A thin strip of tissue along the submucosal surface of the circular muscle layer was carefully dissected from each strip of muscle and cut into small pieces (1–2 mm^3). The muscles were incubated in oxygenated, Ca^{2+}-free Hanks' solution of the following composition (mM): Na^+, 141; K^+, 5·8; Cl^-, 130·4; HCO_3^-, 15·5; HPO_4^{2-}, 0·34; $H_2PO_4^-$, 0·44; dextrose, 10; sucrose, 2·9. After equilibration with 97% O_2–3% CO_2, this solution had a pH of 7·4 at room temperature. The tissue pieces were transferred to dispersion medium containing (per ml of Hanks' Ca^{2+}-free solution) collagenase CLS II (196 U/ml, Worthington Biochemical, Freehold, NJ, USA), 1·5 mg bovine serum albumin (fatty acid free; Sigma, St Louis, MO, USA), 2 mg trypsin inhibitor (Sigma), 0·11 mg Na_2ATP (Sigma) and incubated at 37 °C for 30 min. The supernatant was removed, and the tissue pieces were resuspended in Ca^{2+}-free Hanks' solution. Gentle trituration detached single interstitial cells and smooth muscle cells (Langton *et al.* 1986*b*). 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. Interstitial cells and smooth muscle cells were identified under phase contrast optics by criteria previously discussed (Langton *et al.* 1989*b*). Cells were stored at 4 °C and used within 7 h.

Patch clamp experiments

For measurement of net currents, cells were perfused with an external solution buffered with HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) containing (mM): Na^+, 130;

K^+, 5·8; Ca^{2+}, 1·8; Mg^{2+}, 0·9; Cl^-, 135; HCO_3^-, 4·17; HPO_4^{2-}, 0·34; $H_2PO_4^-$, 0·44; SO_4^-, 0·4; dextrose, 10; sucrose, 2·9; HEPES, 10. Solution pH was adjusted to 7·4 with NaOH. The standard pipette solution contained (mM): K^+, 140; Na^+, 12; Mg^{2+}, 0·5; gluconate, 110; Cl^-, 21; ATP, 5·0; GTP, 1·0; phosphocreatine, 5; HEPES, 5; ethyleneglycol-bis(β-aminoethylether)-*N,N,N'N'*-tetra-acetic acid (EGTA), 0·1. The pH was adjusted to 7·2 with KOH, and the solution was filtered and stored frozen.

To isolate voltage-dependent (non-Ca^{2+}-dependent) outward currents, $NiCl_2$ (100 μM) and nifedipine (1 μM) were added to the bath solution, and EGTA (1 mM) was added to the pipette solution.

To isolate inward currents a perfusion buffer was used containing (mM): Na^+, 89·8; K^+, 5·8; TEA, 40; Cl^-, 135; HCO_3^-, 4·17; HPO_4^{2-}, 0·34; $H_2PO_4^-$, 0·44; Ca^{2+}, 2·5; Mg^{2+}, 0·9; SO_4^{2-}, 0·4; dextrose, 10; sucrose, 2·9; HEPES, 10. Solution pH was adjusted to 7·4 with NaOH. In these experiments the pipette solution contained (mM): Cs^+, 130; Na^+, 14; Mg^{2+}, 2; aspartate, 110; Cl^-, 22; ATP, 5·0; GTP, 1·0; phosphocreatine, 2·0; HEPES, 5; EGTA, 1·0. The pH was adjusted to 7·2 with CsOH, and the solution was filtered and stored frozen.

Pipette resistance was 1–3 MΩ. After obtaining gigaseals and after access to the cell interior was gained, 3 min were allowed for cell dialysis. Temperature was continuously monitored and regulated at 35 ± 1 °C with a bipolar temperature controller (Medical Systems Corp., Greenvale, NY, USA). Voltage clamp test pulse protocols were delivered, and membrane current responses were recorded by means of a patch clamp amplifier (Axon Instruments, Axopatch 1A) interfaced to a 12-bit analog-to-digital converter (Axon Instruments, TL-1) and an AT-style computer running pClamp software (Axon Instruments). Currents were low-pass filtered at 2 kHz. Currents displayed in figures were corrected for leakage.

Data were analysed with pClamp software, and hard copies were made with a Hewlet-Packard digital plotter and Sigmaplot software (Jandel Scientific). Averaged data are expressed as the mean ± S.E.M.; *n* represents the number of cells.

Perforated patch technique

In some experiments the perforated patch technique (Horn & Marty, 1988) was used with Amphotericin-B (Sigma) (Rae *et al.* 1991) to preserve intracellular regulatory systems and reduce 'run-down' of the inward current. A fresh stock solution of Amphotericin-B was made each day by dissolving the drug in dimethyl sulphoxide (DMSO; 0·6 mg/μl). The stock solution was diluted with pipette solution (4 μl stock solution/ml pipette solution) and mixed by vortexing and/or sonicating. Pipette tips were briefly dipped into pipette solution and then back-filled with pipette solution containing amphotericin-B. After formation of gigaseals, 5–10 min were allowed for amphotericin-B to diffuse into the cell membrane and form pores. During this period access resistance dropped to about 6–10 MΩ.

Drugs

4-Aminopyridine, EGTA, tetraethylammonium chloride, and nickel chloride were obtained from Sigma (St Louis, MO, USA), and nifedipine from Boehringer Mannheim (Indianapolis, IN, USA). Nifedipine was dissolved in 100% ethanol at 10^{-2} M and stored at −20 °C until use. The nifedipine stock solution was diluted with the perfusion buffer.

RESULTS

Comparison of net currents

Net currents recorded from interstitial cells were compared with smooth muscle cells using dialysing patch pipettes. Cells were held at −80 mV and stepped for 500 ms to test potentials ranging from −90 to +20 mV (Fig. 1*A*). Depolarizing test potentials positive to −40 mV elicited transient inward current followed by outward current that persisted for the duration of the test pulse in both types of cells. Averaged current–voltage (*I–V*) relationships for net currents recorded from interstitial cells are plotted in Fig. 1*B*. The currents elicited by interstitial cells and smooth muscle cells were not significantly different (data for smooth muscle not shown).

Comparison of voltage-dependent outward currents

Non-Ca^{2+}-dependent, voltage-dependent outward currents of interstitial cells and smooth muscle cells were also compared. In these experiments, inward currents were blocked by adding Ni^{2+} (100 μM) and nifedipine (1 μM) to the bath solution. We have

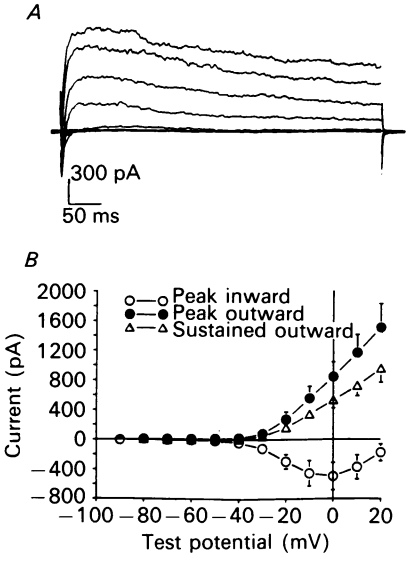

Fig. 1. Net currents recorded from interstitial cells. Cells were held at −80 mV and currents were recorded in response to 500 ms test depolarizations (−90 to +20 mV). *A*, depolarization induced a transient inward current followed by a sustained outward current. *B*, average *I–V* relationship summarizing responses of twelve cells.

previously shown that addition of Ni^{2+} and nifedipine quantitatively blocks the inward current in colonic myocytes (Ward & Sanders, 1992), and the effectiveness of these agents in blocking the inward currents of interstitial cells was shown in the present study. The EGTA concentration in the pipette solution was also raised (to 1 mM) to increase intracellular Ca^{2+} buffering. Under these conditions, very little or no Ca^{2+}-dependent K^+ current was elicited at test potentials negative to 0 mV (i.e. physiological range of potentials). Interstitial cells were held at −80 mV and stepped to potentials ranging from −100 to +20 mV for 500 ms. Depolarization positive to −40 mV caused rapid activation of an outward current which reached a maximum of 1073 ± 179 pA ($n = 7$) at +20 mV within $14 \pm 1{\cdot}2$ ms. The outward current inactivated to $61 \pm 6{\cdot}7$% of the peak within 500 ms (Fig. 2*A*). 4-Aminopyridine (4-AP; 1 mM in bath solution) did not significantly affect this current (Fig. 2*B*). Figure 2*C* summarizes the *I–V* relationship for the voltage-dependent outward current and the effects of 4-AP in seven interstitial cells.

Smooth muscle cells were studied under identical conditions with the same voltage clamp protocol. Depolarization of smooth muscle cells also elicited a rapidly activating outward current that partially inactivated during the time course of the test potentials (Fig. 3*A*). This current was similar to the voltage-dependent outward

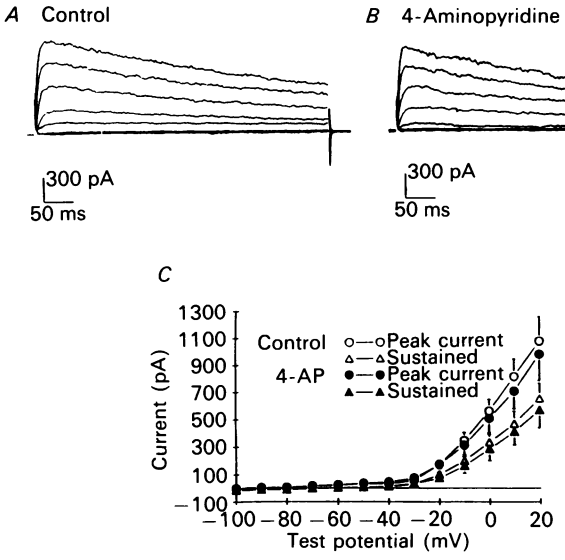

Fig. 2. Voltage-dependent outward current of interstitial cells. Inward current was blocked with nifedipine (1 μM) and Ni^{2+} (100 μM) in the bath solution. Cells were held at −80 mV and currents were recorded in response to 500 ms test potentials (−100 to +20 mV). *A*, a family of outward currents. Outward current activated rapidly and inactivated over time course of test potentials. *B*, the current was insensitive to 1 mM 4-aminopyridine (4-AP). *C*, average *I–V* relationships from seven interstitial cells before and after 4-AP application (means ± S.E.M.).

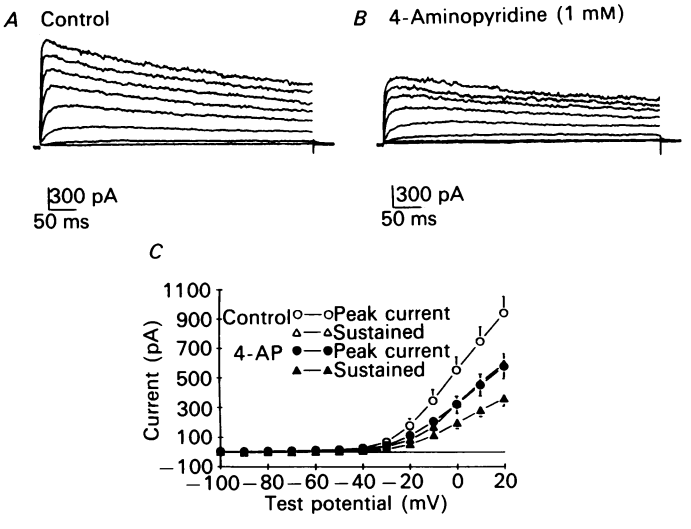

Fig. 3. Voltage-dependent outward current of circular smooth muscle cells. Cells were held at −80 mV and currents were recorded in response to 500 ms test potentials (−100 to +20 mV). *A*, outward currents of smooth muscle cells looked similar to those of interstitial cells (see Fig. 2). *B*, 4-AP reduced the outward current of smooth muscle cells by about 40%. *C*, average *I–V* relationship from five smooth muscle cells before and after 4-AP application (means ± S.E.M.).

current described in circular muscle cells of the proximal colon (Thornbury, Ward & Sanders, 1992). At first inspection, the outward currents in interstitial cells and smooth muscle cells appeared very similar. However, the outward current of smooth muscle cells was more sensitive to 4-AP. The peak current and the current remaining at the end of 500 ms were inhibited approximately 40% by the addition of 1 mM 4-AP (+20 mV test potential; Fig. 3*B*). Figure 3*C* summarizes the *I–V* relationship for the voltage-dependent outward current recorded from five smooth muscle cells.

Comparison of steady-state inactivation of outward current

The steady-state inactivation characteristics of the outward current of both cell types was studied using a two-pulse protocol. Studies described in the previous section showed that a steady-state level of inactivation was not achieved during 500 ms depolarization. Therefore, conditioning pulses, 5 s in duration, were applied to potentials ranging from −110 to 0 mV. Then the cells were returned to the holding potential (−80 mV) for 10 ms before stepping to a test potential of 0 mV. The peak current activated at each test potential was normalized against the maximum current generated during test potentials. Maximum current was generated in most cells with conditioning potentials of −110 to −100 mV. The maximum current values were averaged and plotted as a function of conditioning potential. The curve was fitted to a Boltzman equation by a non-linear least-squares method (Langton *et al.* 1989*a*):

$$I = (C_1 - C_2)/[1 + \exp((V - V_h)/V_s)] + C_2,$$

where C_1 and C_2 are the maximum and minimum in the normalized current, V_h is the conditioning potential for half-inactivation and V_s is the slope factor.

Figure 4*A* and *B* shows a comparison of the inactivation characteristics of the voltage-dependent outward current in interstitial cells and smooth muscle cells. The outward current in interstitial cells inactivated over a more negative potential range; half-inactivation occurred at −53 mV ($n = 6$). In smooth muscle cells half-inactivation occurred at −20 mV ($n = 5$). The slope factor of interstitial cells ($18 \pm 0{\cdot}8$) was significantly different from that of smooth muscle cells ($12 \pm 1{\cdot}3$; $P < 0{\cdot}05$).

Comparison of inward currents

In studies of inward currents, outward currents were blocked by addition of TEA (40 mM) to the bathing solution and by replacing K^+ in the pipette solution with Cs^+. Two patch recording techniques were used to describe the inward current: conventional whole-cell recording conditions in which cells were dialysed with the pipette solution through a ruptured patch, and the perforated patch technique in which monovalent cations from the pipette exchange with cellular cations (see Rae *et al.* 1991).

Cells were held at −100 mV and stepped to potentials ranging from −100 to +30 or +40 mV. Depolarization elicited inward currents that were characterized by rapid activation followed by inactivation to a small sustained inward current. In dialysed cells, repetitive single steps from −100 to 0 mV resulted in constant run-down of the inward current. In contrast, inward currents recorded with the perforated technique showed little (or relatively slow) run-down (Fig. 5*A*). The

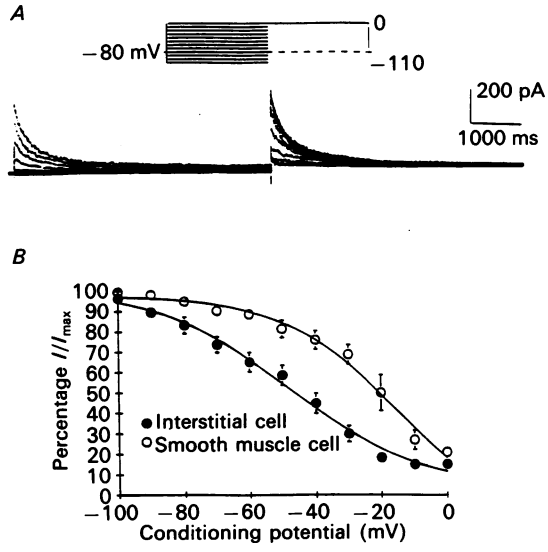

Fig. 4. Comparison of the voltage dependence of inactivation of outward currents of interstitial cells and smooth muscle cells. *A*, example of two pulse protocols used (inset) and typical current responses in an interstitial cell. *B*, plots of normalized maximum currents generated during test potentials as a function of conditioning potential. The current from interstitial cells inactivated in a more negative potential range (half-inactivation = −53 mV). The current from smooth muscle cells inactivated in a more positive potential range (half-inactivation = −20 mV). Data are means ± S.E.M. from six interstitial cells and five smooth muscle cells.

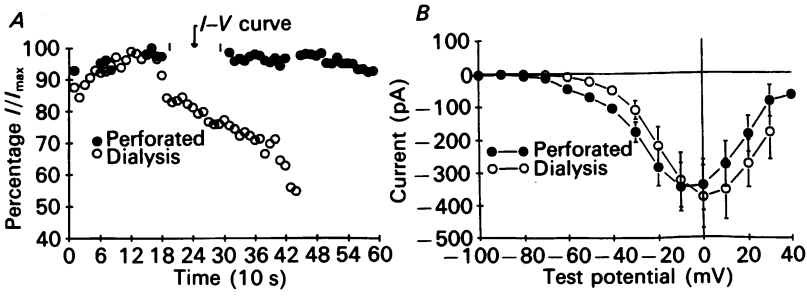

Fig. 5. Comparison of inward currents in dialysed interstitial cells and cells studied with the perforated patch technique (see text for details). *A*, 'run-down' of inward current as a function of time. Cells were stepped from a holding potential of −100 to 0 mV every 15 s. Peak current responses are plotted. The current from perforated patch cells (●) showed little 'run-down' as compared with dialysed cells (○). *I–V* curve was produced during the gap in the plot of the perforated patch cell. *B*, in interstitial cells, more current was resolved at negative test potentials using the perforated patch technique (●) than in dialysed cells (○). Data are means ± S.E.M. from five dialysed cells and four perforated patch cells.

characteristics of the inward currents recorded from interstitial cells under the two recording conditions were different. Average *I–V* curves (Fig. 5*B*) show that with the perforated patch technique inward currents were resolved at more negative potentials and the amplitude of the current recorded by test potentials in the range of −70 to −30 mV were greater than in dialysed cells.

Inward currents of interstitial cells were compared with inward currents of smooth muscle cells using the perforated patch technique. Inward current was resolved at more negative potentials in interstitial cells than in smooth muscle cells (Fig. 6*A*). When the peak current was plotted as a function of test potentials, the *I–V*

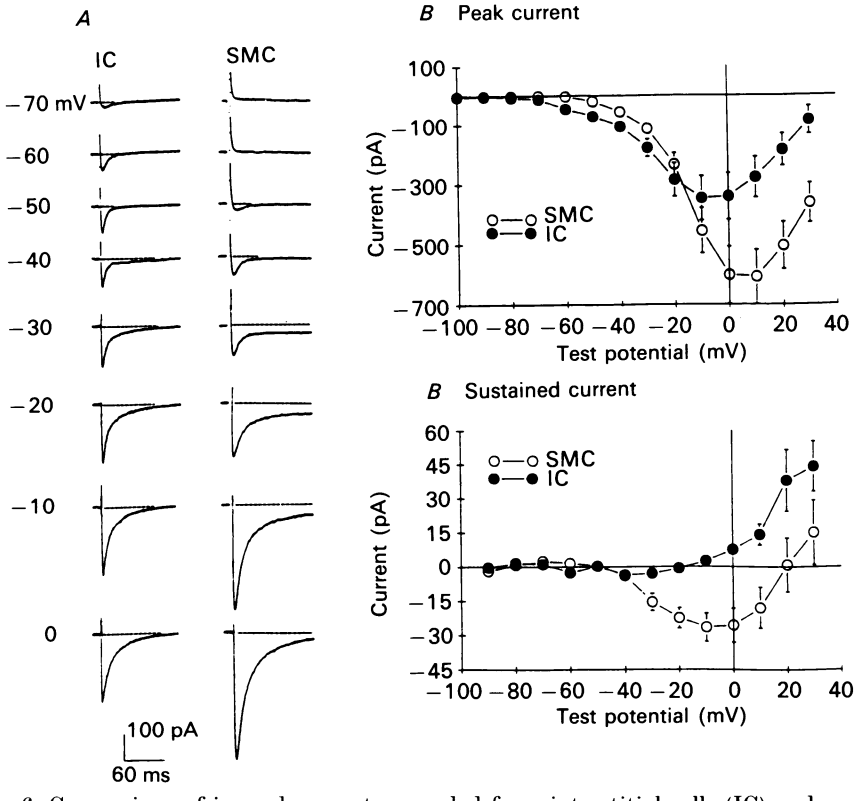

Fig. 6. Comparison of inward currents recorded from interstitial cells (IC) and smooth muscle cells (SMC). *A*, current records elicited by test potentials noted at left of traces. Inward current was resolved at a test potential of −70 mV in this cell. At potentials negative to −20 mV the current generated by interstitial cells was greater than in smooth muscle cells. Inward current was resolved in smooth muscle cells at −50 mV. *B*, plot of *I–V* relationship of peak inward current. Current reached maximum at −10 mV in interstitial cells, current peaked at between 0 and +10 mV in smooth muscle cells. *C*, plot of *I–V* relationship of current at end of 500 ms test pulse (sustained current). Little current remained in interstitial cells, while smooth muscle cells displayed sustained current. Data in *B* and *C* are means ± S.E.M. from four interstitial cells and six smooth muscle cells.

relationships of the interstitial cells showed a distinctive 'hump' at negative potentials (cf. Fig. 9*A*). The magnitude of the inward current that activated at negative potentials varied from cell to cell, but the averaged *I–V* curves from interstitial cells showed that inward current could be elicited at more negative potentials than in smooth muscle cells (Fig. 6*B*). In smooth muscles the *I–V* curves were smooth and bell-shaped as previously reported (Fig. 6*B*; see also Langton *et al.* 1989*a*; Ward & Sanders, 1992). Inward current of interstitial cells peaked at −10 mV, whereas inward current of smooth muscle cells peaked at 0 to +10 mV.

Although the magnitude of the inward current of interstitial cells was greater over the range of −70 to −20 mV, smooth muscle cells generated more current positive to −20 mV (Fig. 6*A* and *B*).

The sustained component of inward current was also compared. In interstitial cells, there was little or no sustained inward current component (see Fig. 6*A*). Figure 6*C* summarizes the *I–V* relationships for the sustained current at the end of a 500 ms test depolarization. At potentials positive to −30 mV, significant sustained inward current was observed in smooth muscle cells ($n = 6$, $P < 0{\cdot}05$), but in interstitial cells the sustained component was not significantly greater than 0. For example, at −20 mV, the current remaining at the end of 500 ms averaged $-1 \pm 2{\cdot}2$ pA ($n = 4$), as compared with $-22 \pm 4{\cdot}6$ pA in smooth muscle cells ($n = 6$). In these studies the sustained component of inward current was less than observed previously in these cells (Langton *et al.* 1989*b*). This discrepancy could be due to the differences in experimental conditions between studies (i.e. 20–24 °C and 20 mM EGTA in Langton *et al.* 1989*b* *vs.* 35–36 °C and perforated patch conditions used in the present study). In the current study, the magnitude of the sustained component of inward current was not significantly different in dialysed cells (1 mM EGTA) and in cells using the perforated patch technique.

Separation of two types of Ca^{2+} current by varying holding potentials

The hump in the *I–V* relationship in most of the interstitial cells is typical of cells that express low-threshold Ca^{2+} currents (Mitra & Morad, 1986; Fox, Nowycky & Tsien, 1987; Schroeder, Fishbach & McCleskey, 1990). Voltage clamp protocols used previously to separate different components of Ca^{2+} current (Bean, 1985; Friedman, Suarez-Kurtz, Kaczorowski, Katz & Reuben, 1986; Fox *et al.* 1987) were employed to attempt to separate low- and high-threshold currents in interstitial cells. The cells were held at −100 mV and stepped to potentials ranging from −100 to +40 mV. Then cells were held at −40 mV and depolarized to potentials from −100 to +30 mV (Fig. 7*A* and *B*). The current elicited from a holding potential of −100 mV was larger than currents elicited from −40 mV. For example, decreasing the holding potential from −100 to −40 mV reduced the peak amplitude by 85% (at −10 mV). The current elicited from −40 mV was only −62 pA. The difference current between two holding potentials peaked at −10 mV with an amplitude of −295 pA and reversed between +30 and +40 mV. Inward currents of smooth muscle cells were studied with the same protocol (Fig. 8*A* and *B*). In smooth muscle cells, changing the holding potential from −100 to −40 mV reduced the peak amplitude by 39% (at 0 mV). The current elicited from −40 mV in smooth muscle cells was −392 pA at the peak, which was 6-fold larger than that of interstitial cells. The difference current between two holding potentials peaked at −10 mV and reversed between +30 and +40 mV, similar to that of interstitial cells. The peak amplitude of the difference current was −214 pA which was slightly smaller than that of interstitial cells.

Pharmacological properties of inward current in interstitial cells

Low-threshold Ca^{2+} currents have been reported to be blocked by low concentrations of Ni^{2+} and resistant to block by dihydropyridines, while 'L'-type, high-threshold currents are sensitive to dihydropyridine but more resistant to Ni^{2+}

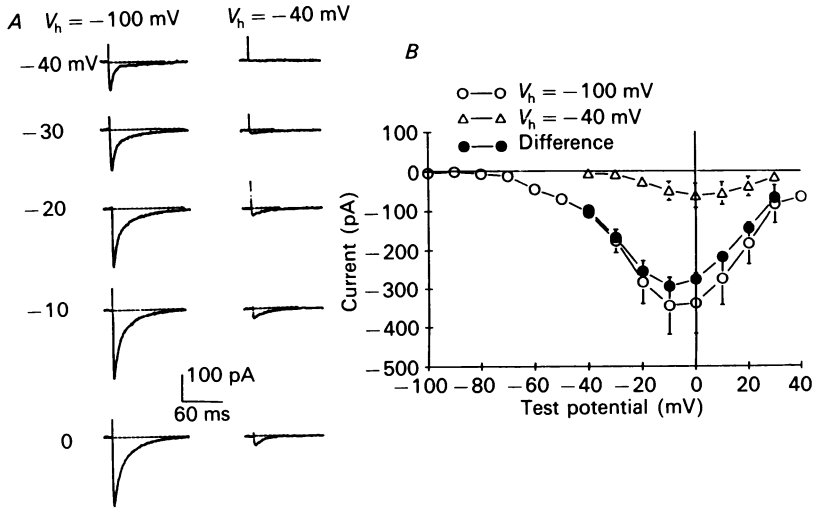

Fig. 7. Inward currents generated in interstitial cells from different holding potentials. *A*, holding potentials (V_h) of −100 mV (left traces) and −40 mV (right traces) were used, and cells were depolarized to various levels (test potentials noted at left of traces). Inward current was largely inactivated at −40 mV. *B*, average currents elicited from −100 and −40 mV and plotted as a function of the test potential. Difference current peaked at −10 mV with an average amplitude of 295 pA. Data are means ± S.E.M. from four cells.

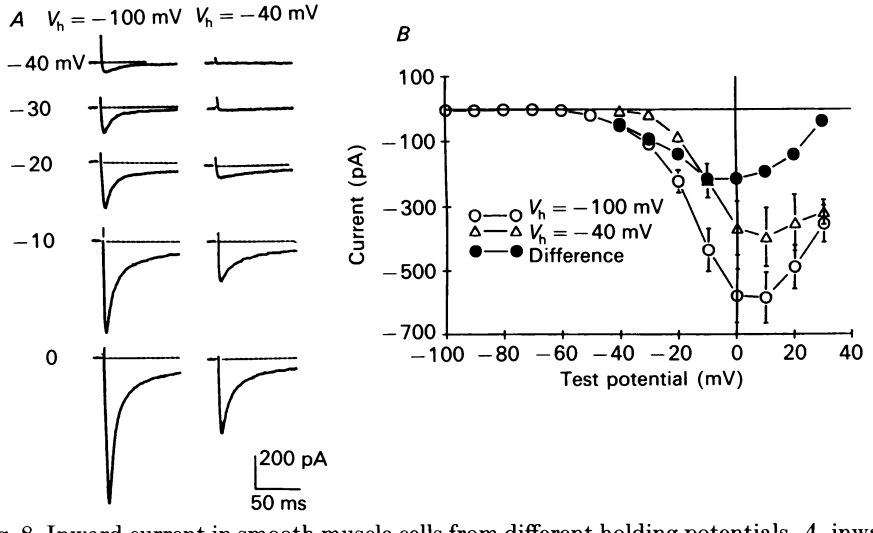

Fig. 8. Inward current in smooth muscle cells from different holding potentials. *A*, inward current was less inactivated in smooth muscle cells by holding at −40 mV; 61% of the peak inward current remained when holding potential (V_h) was changed from −100 to −40 mV. *B*, average currents elicited from −100 and −40 mV and plotted as a function of test potential. Difference current peaked at −10 mV with an average amplitude of 214 pA. Data are means ± S.E.M. from six cells.

(Friedman *et al.* 1986; Loirand, Pacaud, Mironneau & Mironneau, 1986; Fox *et al.* 1987; Hagiwara, Irisawa & Kameyama, 1988). In interstitial cells studied with the perforated patch technique, 40 μM Ni^{2+} partially inhibited inward current. The effect

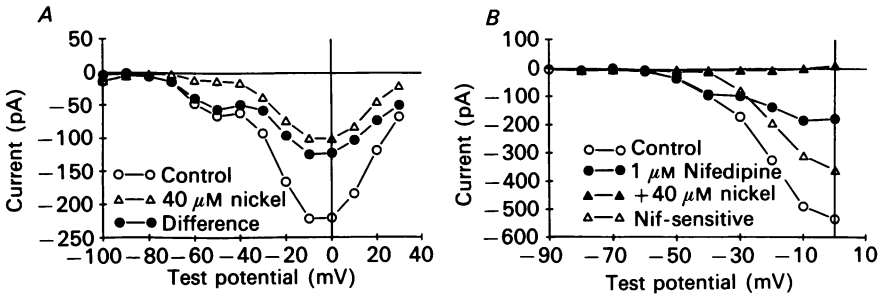

Fig. 9. Pharmacological properties of inward current of an interstitial cell. *A*, nickel (40 μM) partially inhibited inward current and abolished most of the 'hump' in the *I–V* relationship. *B*, in a dialysed cell where a 'hump' in the *I–V* was not apparent, addition of nifedipine (1 μM) revealed a 'hump'. Nifedipine-sensitive current (△) was subtracted from control. Addition of nickel (40 μM) in the presence of nifedipine abolished all inward current.

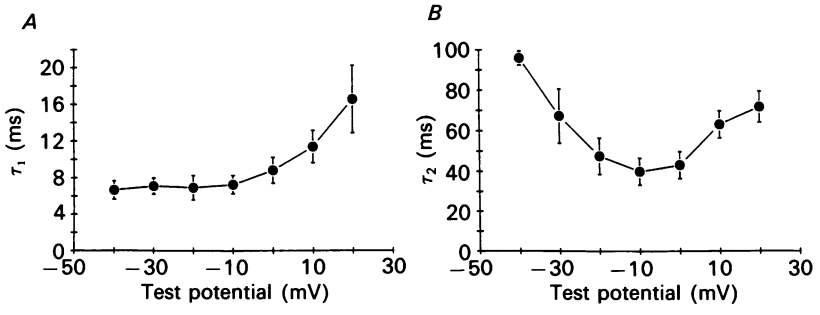

Fig. 10. Inactivation time constants of inward currents of interstitial cells plotted as functions of test potential. Inactivation time course was fitted with two exponentials. *A*, time constant for fast component of inactivation (τ_1) was not very dependent on membrane potentials at test potentials negative to −10 mV. *B*, time constant for slow component of inactivation (τ_2) was characterized as U-shaped dependence on membrane potential.

of Ni^{2+} was proportionally bigger at negative potentials (Fig. 9*A*). In dialysed cells where a 'hump' was not apparent in the *I–V* curves, addition of nifedipine (1 μM) unmasked a 'hump' (Fig. 9*B*). Presence of both nifedipine and Ni^{2+} abolished all inward current. This suggests that inward current was carried by Ca^{2+} and was as a mixture of low- and high-threshold currents.

Inactivation characteristics of inward currents in interstitial cells

The time course of inactivation of Ca^{2+} currents in interstitial cells was fitted with two exponentials. Figure 10 shows average time constants for the fast and slow components of inactivation plotted as a function of test potential. The time constant for the slow component showed a U-shaped dependence on membrane potential (Fig. 10*B*). The dependency of the fast component on membrane potential was not obvious at negative potentials, while at potentials positive to −10 mV, inactivation rate slowed as the membrane depolarized (Fig. 10*A*).

Steady-state inactivation was characterized with the following protocol: cells were stepped to conditioning potentials ranging from −110 to 0 mV for 1 s. After stepping back to the holding potential (−90 mV) for 10 ms, the cells were stepped to a test potential of 0 mV. The peak current activated at each test potential was normalized

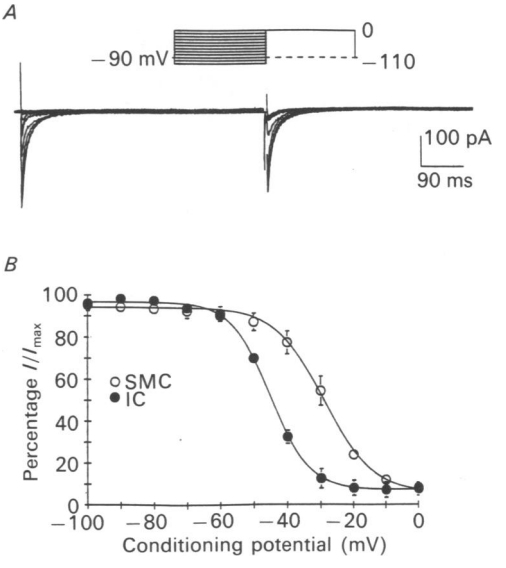

Fig. 11. Comparison of steady-state inactivation of inward currents of interstitial cells (IC) and smooth muscle cells (SMC). *A*, example of two pulse protocols used (inset) and typical current responses in an interstitial cell. *B*, plots of normalized currents as a function of conditioning potential. Current of interstitial cells inactivated at more negative potentials; half-inactivation = −45 mV. Current of smooth muscle cells inactivated at more positive potentials; half-inactivation = −30 mV.

against the maximum current generated during test potentials. Maximum current was generated in most cells with conditioning potentials of −110 to −100 mV. The maximum current values were averaged and plotted as a function of the conditioning potential. The curve was fitted to a Boltzman equation by a non-linear least squares method as used in outward current.

Figure 11*A* and *B* shows a comparison of the steady-state inactivation curves obtained from interstitial cells and smooth muscle cells. Inward current from interstitial cells inactivated over a more negative range of potentials than inward current of smooth muscle cells. The current available at −40 and −30 mV in interstitial cells averaged 33±3% and 13±5% of maximum, respectively. Half-inactivation occurred at −45 mV ($n = 3$). At the same potentials, 78±6% and 55±7% of the maximal current was available in smooth muscle cells. Half-inactivation in smooth muscle cells occurred at −30 mV ($n = 5$). The slope factor for interstitial cells (6±0·7) was not significantly different from that of smooth muscle cells (9±1·5; $P > 0{\cdot}05$).

DISCUSSION

This study describes some of the phenotypic differences between interstitial cells and smooth muscle cells. Interstitial cells express at least two ionic currents that differ from those expressed by circular smooth muscle cells from the pacemaker region along the submucosal surface in the colon (Smith *et al.* 1987). The two currents found in interstitial cells but not in smooth muscle cells were: (i) a voltage-dependent outward current that was relatively resistant to 4-AP and inactivated over a negative range of potentials, and (ii) a voltage-dependent Ca^{2+} current that activated and inactivated near the negative resting potentials of cells in the pacemaker region. The voltage-dependent outward current in interstitial cells was likely to be carried by K^+, since it was blocked by intracellular Cs^+ and extracellular TEA, and the inward current appeared to be carried by Ca^{2+}, since it was blocked by Ni^{2+} and nifedipine. These currents, especially the negatively activating inward current, may contribute to the generation of spontaneous activity in interstitial cells and the propagation of excitable events in the network of interstitial cells (Berezin *et al.* 1988; Ward & Sanders, 1990).

Several mammalian cells, including neurones (cf. Carbone & Lux, 1984; Nowycky, Fox & Tsien, 1985), cardiac muscle cells (cf. Bean, 1985; Nilius, Hess, Lansman & Tsien, 1985; Mitra & Morad, 1986; Hagiwara *et al.* 1988), smooth muscle cells (cf. Bean, Sturek, Puga & Hermsmeyer, 1986; Benham, Hess & Tsien, 1987; Aaronson, Bolton, Lang & Mackenzie, 1988; Ganitkevich & Isenberg, 1990; Vivaudou, Singer & Walsh, 1991), have been reported to express low- and high-threshold Ca^{2+} currents. These currents have been separated on the basis of differing voltage dependencies (Bean *et al.* 1986; Fox *et al.* 1987), different sensitivities to organic and inorganic Ca^{2+} channel blockers (Nilius *et al.* 1985; Bean *et al.* 1986; Fox *et al.* 1987), and inactivation kinetics (Klöckner & Isenberg, 1985). Other excitable cells that appear to serve as pacemakers also express low- and high-threshold Ca^{2+} currents. For example, sino-atrial cells express a Ca^{2+} current (a 'T-like' current) that is resolvable positive to -50 mV, inactivates at negative potentials, and may contribute to the pacemaker current generated by these cells (Hagiwara *et al.* 1988). Similar currents exist in cardiac Purkinje cells (Tseng & Boyden, 1989; Bossu, Fagni & Feltz, 1989) and thalamo-cortical cells (Soltesz, Lightowler, Leresche, Jassik-Gerschenfeld, Pollard & Crunelli, 1991), and may contribute to the spontaneous electrical rhythmicity of these cells. In interstitial cells, which have been suggested as pacemaker cells in GI smooth muscles (Thuneberg *et al.* 1983; Suzuki *et al.* 1986; Langton *et al.* 1989*b*), there appears to be a significant component of this fast, transient type of inward current. The current in interstitial cells peaked at -10 mV, which is similar to the current in sino-atrial cells (Hagiwara *et al.* 1988).

A significant feature of the low-threshold inward current of interstitial cells was that resolvable current was observed at test potentials as negative as -70 mV. This is close to the resting potentials of cells in the pacemaker region in the colon (Smith *et al.* 1987; Ward *et al.* 1991), suggesting that the threshold for regenerative events may lie at negative potentials in these cells. An average of 50 pA of current was observed in interstitial cells with a test depolarization to -60 mV. This may not be sufficient current to depolarize the smooth muscle syncytium that is coupled via gap

junctions to the interstitial cells (Berezin *et al.* 1988; Ward & Sanders, 1990), but interstitial cells are also highly coupled to each other, forming a network. The negatively activating inward current would facilitate propagation of excitability through this network, and it is possible that the *network of interstitial cells* produces enough current to pace the smooth muscle syncytium. As the smooth muscle cells depolarize, voltage-dependent inward current is activated (Langton *et al.* 1989*a*; Ward & Sanders, 1992), and a threshold for regenerative slow waves is reached 10 to 20 mV positive to the resting potential. Thus the pacemaker current provided by interstitial cells may depolarize smooth muscle cells which may, in turn, regenerate the activity.

The low-threshold current of interstitial cells was transient, having little or no sustained component. In smooth muscles, the expression of a higher-threshold, more sustained current would appear to be favourable because the primary function of these cells is to generate force during phasic contractions. In fact, it appears that the sustained component of the inward current provides most of the Ca^{2+} influx during the plateau phase of slow waves (see Vogalis, Publicover, Hume & Sanders, 1991; Sanders, 1992), and this appears to regulate the force of contractions in the colon and in other regions of the GI tract (cf. Ozaki, Stevens, Blondfield, Publicover & Sanders, 1991).

Inactivation of inward currents in interstitial cells were fitted with two exponentials. The slow component of inactivation was characterized by a U-shaped dependence on membrane potential. This dependence was similar in shape to the *I–V* relationship, reaching a peak at approximately the same potential where Ca^{2+} current was maximal. This 'current' dependence suggests that this component of inactivation may be related to the influx of Ca^{2+}. In contrast, the fast component of inactivation may not be significantly dependent on Ca^{2+} influx at potentials negative to -10 mV. This relative lack of dependence on Ca^{2+} current is consistent with descriptions of 'T'-type Ca^{2+} currents in other cell types (Bossu & Feltz, 1986; Fox *et al.* 1987). At potentials positive to -10 mV, it would be difficult to distinguish 'T'-type from 'L'-type current, and this may explain why the fast component only showed current dependence positive to this level.

An interesting observation in this study was the differences in currents recorded from dialysed and non-dialysed interstitial cells. In the latter, the contents of the pipette, with the exception of monovalent ions, do not dialyse the cell (Horn & Marty, 1988; Rae *et al.* 1991). In these cells the rate of run-down of the Ca^{2+} current was reduced, and the low-threshold current was more prominent at negative potentials. These data suggest that low- and high-threshold Ca^{2+} currents require regulation by cytoplasmic factors. It may be necessary to study the low-threshold current with the perforated patch technique unless the necessary cytoplasmic factors can be determined and reconstituted in the filling solution of dialysing pipettes.

We reported previously that interstitial cells express Ca^{2+}-dependent K^+ channels, and these channels appear to contribute to macroscopic current (Langton *et al.* 1989*b*). In the present study we attempted to reduce contamination from Ca^{2+}-dependent currents by blocking Ca^{2+} entry and by using relatively high Ca^{2+} buffering in the pipette solution. Although these manoeuvres are unlikely to block Ca^{2+}-activated K^+ channels (they could still be activated by depolarization), it is

likely that reducing cytosolic Ca^{2+} shifted the activation of these channels to more positive potentials. We were primarily interested in voltage-dependent K^+ currents in the physiological range of potentials (i.e. negative to −20 mV), and current records generated in this range of test potentials did not appear contaminated with large conductance channels (i.e. records had very low amplitude noise). Under these conditions we resolved a rapidly activating and inactivating voltage-dependent outward current in interstitial cells that appeared to be carried by K^+, because it was blocked by Cs^+ in the pipette and TEA (40 mM) in the external bathing solution. This current was resistant to block by 4-AP (1 mM) and was half-inactivated at −53 mV. The voltage-dependent outward current of circular muscle cells had different properties: it was half-blocked by 1 mM 4-AP and inactivated at more positive potentials (half-inactivation at −20 mV). Previous work has suggested that the voltage-dependent outward current in smooth muscle cells balances with the sustained inward current to allow the plateau phase of slow waves (cf. Sanders, 1992). At present, the specific role of the voltage-dependent outward current in the spontaneous activity of interstitial cells is unclear, but the fact that it inactivates at relatively negative potentials may mean that less outward current is available to oppose depolarization. For example, at −60 mV, 36% of the voltage-dependent outward current was inactivated. This may facilitate, or at least not interfere with, spontaneous regenerative activity.

In summary, this study has documented several differences in the ionic currents expressed by interstitial cells and smooth muscle cells from the pacemaker region of the colon. It would appear that the ionic conductances of interstitial cells may facilitate spontaneous activity and might allow these cells to serve as pacemakers. Smooth muscle cells are also excitable, but may need 10 to 20 mV of depolarization before slow waves can be generated. Further studies will be needed to determine how the ionic currents of interstitial cells are regulated and whether manoeuvres known to have chronotrophic effects in intact muscles will affect the ionic conductances of interstitial cells in a consistent manner.

This work was supported by a Program Project Grant from National Institute of Diabetes and Digestive and Kidney Diseases (NIDDKD) (P01 DK 41315). The authors would like to thank Dr Keith Thornbury for valuable contributions and discussions during the outward current studies and Drs Jamie Campbell, Adam Rich and Sean Ward for comments about the manuscript.

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