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Two populations of smooth muscle cells in the guinea-pig gastric antrum

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Abstract

K^+ outward currents (I_K) expressed by guinea-pig antral smooth muscle cells were studied using the whole-cell voltage-clamp technique. In about 88% of cells depolarization steps applied from $V_h = -70$ mV activated a fast transient component ($I_{K(to)}$) with voltage-dependent characteristics, and a noninactivating component with slow activation kinetics ($I_{K(sl)}$). Both components were carried by K^+ ions. Apamin (10 nM to 1 μM) selectively depressed $I_{K(to)}$ in a concentration-dependent manner. $I_{K(sl)}$ was blocked by 1 mM tetraethylammonium or 0.1 μM charybdotoxin. 10 mM tetraethylammonium abolished both components of I_K. Nicardipine (1 μM) did not affect the voltage- and time-dependent characteristics of the net I_K, but reduced the current density of $I_{K(sl)}$ from 22.36 ± 1.38 $\mu A/cm^2$ to 13.06 ± 0.92 $\mu A/cm^2$ at $+40$ mV. In about 12% of the cells depolarization-evoked I_K could be separated as two pharmacologically distinguishable components: a glipizide-sensitive current (forming about 70% of the net I_K) and a charybdotoxin-sensitive current (30% of the net I_K). Nicardipine (1 μM) affected neither the amplitude nor the time-course of I_K of this cell population. The depletion of intracellular Ca^{2+} stores by thapsigargin (1 μM) or ryanodine (1 μM) led to a 50–200% increase of $I_{K(sl)}$ in the majority of cells and to an about 30% increase of the net I_K in 12% of cells. The data obtained suggest the existence of at least two populations of cells in guinea-pig antral smooth muscle. Twelve percent of cells seem to be responsible for the generation of slow wave potentials, while 88% of cells most probably respond passively to the electrotonically spread depolarization. © 1997 Elsevier Science B.V.

Keywords: Smooth muscle; K^+ channels; Ca^{2+} stores; Charybdotoxin; Apamin; Pacemaker cells

1. Introduction

The contraction of smooth muscle cells in the antral region of the stomach is initiated by electrical events, known as slow wave potentials (Papasova et al., 1968; Kuriyama et al., 1970). These consist of an upstroke depolarization and a plateau phase which persists for several seconds before repolarization occurs.

The precise sequence of events underlying the regular slow wave activity and the corresponding phasic contractions of the stomach antrum is still debatable. Physiologically, slow wave potentials originate in the corpus region. However, muscle strips removed from all areas aboral to the pacemaker possess the ability to generate slow waves. Most investigators have suggested that slow wave potentials are initiated within the longitudinal layer (Connor et al., 1977; Szurszewski, 1978), although others have suggested that they are a regenerative phenomenon, originating from multiple discrete sites, where pacemaker cell are located (Bauer et al., 1985a). The latter suggestion was recently supported by Post and Hume (1992), who recorded slow wave potentials from single smooth muscle cells, although very few cells were able to generate them. Thus, it was suggested that some of the antral cells which spontaneously generate slow waves serve as pacemakers (Bauer et al., 1985b; Noack et al., 1992), while the majority of cells respond passively to the electrotonically spread depolarization. Given that the separation of longitudinal and circular layers of the guinea-pig gastric antrum is impracticable (Boev, 1981), the only approach for isolation of pacemaker cells seems to be: (i) to cut off pieces from different regions of the antral smooth muscle wall, (ii) to isolate single cells from these pieces and (iii) to study numerous cells, to search for differences in the biophysical and pharmacological properties of the ion channel populations between cells, hoping that some of them may be pacemaker cells.

Briefly, Suzuki et al. (1993) and Petkov et al. (1994) have shown that apamin and charybdotoxin modulate the

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pattern of slow wave potentials and phasic contractions of the gastric antrum. Noack et al. (1992) have studied the ion currents of guinea-pig antral smooth muscle cells. They described two components of K^+ current (I_K). The so-called fast or transient component of I_K was triggered by the initial Ca^{2+} influx. The second, noninactivating I_K component was a Ca^{2+}-sensitive K^+ current that was activated by the second messenger Ca^{2+}.

The aim of the present study was to investigate the depolarization-evoked K^+ current components in single smooth muscle cells isolated from different regions of the guinea-pig gastric antrum, on the assumption that a sufficient number of pacemaker cells would be suspended after enzymatic digestion of tissue pieces, in such a way as to find at least two populations of cells that differ in the voltage-dependent characteristics and calcium sensitivity of their K^+ currents.

2. Materials and methods

2.1. Whole-cell patch-clamp experiments

Male guinea pigs, weighing 300–400 g, were killed by a blow on the head. Muscle strips, isolated from the gastric antrum, were placed in physiological salt solution (solution A, Table 1) and cut into small pieces. The pieces were then transferred into solution B (Table 1), prewarmed at 37°C, to which 1 g/l collagenase (type 1A, Sigma), 0.5 g/l soybean trypsin inhibitor and 1.5 g/l bovine serum albumin were added. After 50–60 min of incubation at 37°C, the enzyme was carefully washed out from the pieces with 20 ml prewarmed solution B. Single smooth muscle cells were then obtained by gentle agitation of the pieces by two Pasteur pipettes with different tip openings in 1 ml fresh solution for cell isolation (see below) until the solution became cloudy. Cells were stored up to 12 h at 6°C in this solution. Solution C (Table 1) and the drugs, diluted in it, were perfused continuously through the bath chamber where the cells had adhered to the glass bottom.

Table 1
Composition of solutions (in mM)

	Solutions				
	A	B	C	I [b]	II [b]
NaCl	124.0	58.0	126.0		
$NaNO_3$		68.0			
$NaHCO_3$	15.5				
KCl	4.7	5.0	6.0	105.0	100.0
KH_2PO_4	1.2				
$MgCl_2$	1.2	1.2	1.2	2.0	2.0
Glucose	11.5	20.0	20.0		
Taurine		20.0	20.0		
HEPES [a]		10.0	10.0	5.0	5.0
Pyruvate		5.0	5.0	5.0	5.0
$CaCl_2$		0.0	2.5	0.3	1.0
EGTA [c]				4.0	11.0
$[Ca^{2+}]_i$ [d]				> 40.0	~ 4.0

[a] *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid. pH of bath solutions was adjusted with KOH to 7.35–7.4.
[b] Stock solution, used as intracellular medium. To 5 ml of it were added immediately before use (mM): 5.0 succinic acid; 5.0 oxalacetic acid; 3.0 sodium pyruvate; pH adjusted to 7.2 with KOH.
[c] Ethylene glycol-bis-(-aminoethyl ether) *N*,*N*′-tetraacetic acid.
[d] $[Ca^{2+}]_i$-calculated free Ca^{2+} concentration is given in nM.

2.2. Electrical recordings

The whole-cell mode of the patch-clamp technique was used (Hamill et al., 1981). The patch electrodes from borosilicate glass (Jencons Scientific), when filled with the internal solution, had resistances of about 2.5 MΩ. Membrane currents were recorded by an EPC-7 (List Electronics) amplifier. Series resistance was compensated at 70%, which is thought to provide sufficient removal of resistant artifacts (Hamill et al., 1981). Current signals were recorded and further analyzed on an AT 286 PC through a TL-1 DMA (AXOPATCH) interface, and Step Wave cell Tester, ver. 1.0, software (Shkodrov, 1995).

The values of the current densities, expressed as i_K ($\mu A/cm^2$) of the membrane surface, were estimated, assuming a specific membrane capacitance of 1 $\mu F/cm^2$ (Hamill et al., 1981), and were plotted versus the potential applied to obtain data for statistical analysis. In most of the experiments the holding potential (V_h) was −70 mV.

2.3. Solutions

The solution used for cell isolation contained (mM): 85 KCl, 30 KH_2PO_4, 5 $MgCl_2$, 20 taurine, 5 Na_2-ATP, 5 Na-pyruvate, 5 creatine, 5 oxalacetate, 1 g/l bovine serum albumin (pH 7.2). The Ca^{2+} concentrations of the pipette solution (Table 1) were estimated according the solution preparation guide of Schubert (1996). All substances were products of Sigma, except for ryanodine and glipizide, which were products of Research Biochemicals International.

Most of the experiments were performed at a temperature of 31 ± 2°C.

3. Results

3.1. Resting potential and passive membrane characteristics

Antral smooth muscle cells kept a membrane potential of about −62 mV (−62 ± 6 mV, $n = 95$), measured under current-clamp mode. No significant differences were found in the values of the resting membrane potentials between cells. As the input impedance of the resting cells was about 1–2 GΩ (estimated by small hyperpolarizing pulses applied under current-clamp conditions), and the

seal resistance was usually 7–10 GΩ, the values obtained for the resting potential would be shifted by 10–15%.

The cell capacitance determined from the compensation necessary for removing the capacitive artifact was 59 ± 5 pF ($n = 102$), which was taken to be the value for the capacitive surface area, assuming 1 $\mu F/cm^2$ as the specific membrane capacitance. The validity of this approach was verified in the same cells by estimation of the visible cell area, and the difference between the two values was about 10%. No significant differences between cells were found in the visible and capacitive surface areas.

3.2. Properties of K^+ currents in 88% of the cells

About 88% of the cells responded to depolarizing stimuli with an initial fast activating and rapidly inactivating component of I_K–$I_{K(to)}$ (Fig. 1A), followed by a more sustained component, $I_{K(sl)}$, which did not inactivate even at the end of 4 s pulses (Fig. 1B).

3.2.1. Voltage dependence of $I_{K(to)}$

At voltages above zero mV the transient component ($I_{K(to)}$) peaked within 35 ms (Fig. 1) had an activation threshold around −50 mV and inactivated in a time- and voltage-dependent manner in 78 ms at +10 mV membrane potential (Fig. 1D). The steady state inactivation

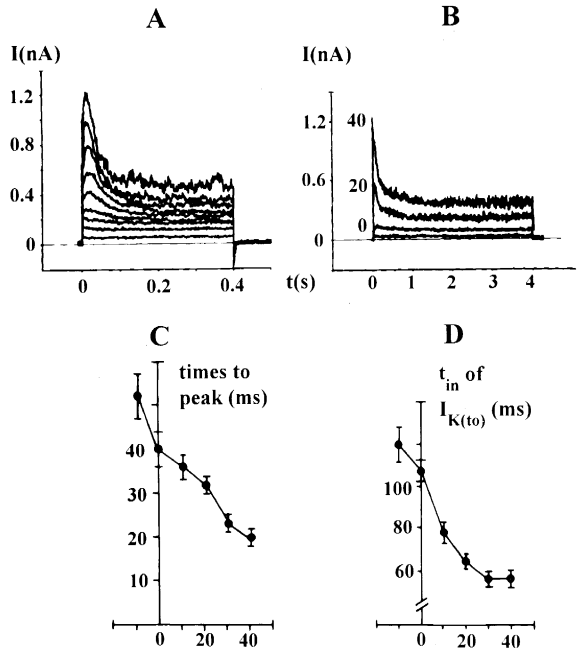

Fig. 1. Typical outward current waveforms, elicited by rectangular depolarization pulses lasting 400 ms (A) or 4000 ms (B) in the majority of antral smooth muscle cells of guinea pig. $V_h = -70$ mV. In (A) depolarization steps applied were: −50, −30, −20, −10, 0, 10, 20, 30 and 40 mV. Cells were bathed in external solution C for 10 min and 3 μM nicardipine was added to the bath right before obtaining the giga-seal. (C, D) Voltage dependence of the time to peak (C) and the inactivation time constants t_{in} (D) of $I_{K(to)}$. All currents were measured 15 min after the start of dialysis. Data are means ± SEM for 25 cells.

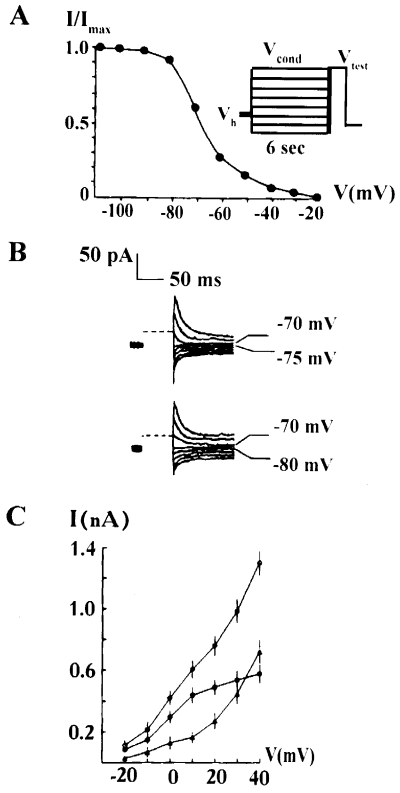

Fig. 2. (A) Steady-state inactivation properties of $I_{K(to)}$. Conditioning prepulses from −120 mV to +40 mV were applied for 300 ms, followed by a test pulse to +40 mV for 25 ms in the presence of 1 μM charybdotixin in the bath to block the noninactivating I_K. Data are fitted by the function: $I/I_{max} = 1/(1+\exp(V - V_{half})/k)$, where I_{max} is the amplitude of the current measured at the test pulse following the most negative conditioning prepulse; V_{half} the potential at which 50% of the current was inactivated; k the slope of the curve. Estimated V_{half} for $I_{K(to)}$ was −67 mV. (B) Tail current protocols performed to reveal the selectivity of $I_{K(to)}$ (upper traces) and $I_{K(sl)}$ (lower traces). Cells were depolarized to +40 mV for 50 ms (for $I_{K(to)}$) or 600 ms (for $I_{K(sl)}$) and then repolarized to various voltages between −110 and −50 mV. Bath solution contained 3 μM nicardipine. $V_h = -70$ mV, $T^o = 25$°C. The obtained values for the reversal potentials were −74 mV for $I_{K(to)}$ and −78 mV for $I_{K(sl)}$. Estimated reversal potential for K^+ was −76 mV. (C) Current-voltage relationships of $I_{K(to)}$ amplitudes expressed at $V_h = -70$ mV (open circles, $n = 9$), or $V_h = -30$ mV (triangles, $n = 7$) and the difference current, representing the potential-sensitive part of I_K (closed circles, $n = 8$). Data are means ± SEM.

curve of $I_{K(to)}$ showed that this component was fully available in potentials negative to −90 mV (down to −110 mV), and had a half-inactivation potential around −60 mV (Fig. 2A). The double-pulse protocols performed to determine the reversal potential of the tail currents proved that channels conducting $I_{K(to)}$ were highly selective for K^+ ions (Fig. 2B, upper traces). The comparison between the currents activated from $V_h = -70$ mV and those obtained from $V_h = -30$ mV demonstrated that $I_{K(to)}$ was totally abolished by the more positive membrane voltages (Fig. 2C). This confirmed the suggestion that $I_{K(to)}$ undergoes voltage-dependent inactivation.

3.2.2. Pharmacology of $I_{K(to)}$

$I_{K(to)}$ was selectively abolished by 1 μM apamin, which affected neither the amplitude nor the kinetics of the noninactivating component ($I_{K(sl)}$). Apamin (10 nM to 1 μM) reduced the amplitude of $I_{K(to)}$ in a concentration-dependent manner (Table 2), as 50% inhibition of $I_{K(to)}$ measured at +40 mV membrane potential was induced by 0.07 μM apamin. Tetraethylammonium in concentrations over 10 mM also blocked this component, but it blocked $I_{K(sl)}$ as well (see below). We did not observe significant changes in the amplitude and in the time-course of $I_{K(to)}$ when we decreased the Ca^{2+} concentration in the bath (to 0.1 mM Ca^{2+}), or when we added 1 μM nicardipine (or 1 μM methoxyverapamil) to block Ca^{2+} entry.

3.2.3. Voltage dependence of $I_{K(sl)}$

The noninactivating component $I_{K(sl)}$ was studied under blockade of $I_{K(to)}$ by apamin (1 μM). $I_{K(sl)}$ was selective for K^+ ions (Fig. 2B, lower traces) and had an activation threshold of about −35 mV (Fig. 3A). Its activation kinetics did not show voltage dependence (not shown).

3.2.4. Pharmacology of $I_{K(sl)}$

The amplitude of $I_{K(sl)}$ depended on Ca^{2+} entry. Thus, in the presence of 1 μM nicardipine or 1 μM methoxyverapamil the amplitude of $I_{K(sl)}$ decreased about twice at membrane voltages higher than zero as compared to control (Fig. 3A). Three min after addition of 10 μM $LaCl_3$ to the nicardipine-containing bath solution $I_{K(sl)}$ disappeared (Fig. 3B), suggesting that at least some Ca^{2+} enters the cell via nicardipine-insensitive pathways. Tetraethylammonium (1 mM) or charybdotoxin (0.1 μM) reversibly blocked $I_{K(sl)}$ (Fig. 3B), which suggested that $I_{K(sl)}$ was conducted through Ca^{2+}-activated K^+ channels with a large conductance.

Table 2
Concentration-dependent inhibition of $I_{K(to)}$ by apamin

Voltage (V)	Current density of $I_{K(to)}$ (μA/cm²) concentration of apamin (M)			
	without apamin [a]	10^{-8} [b]	10^{-7} [c]	10^{-6} [d]
+40	8.2±0.9	5.9±0.6	2.7±0.3	0.17±0.0
+30	7.8±0.7	5.6±0.5	2.5±0.3	0.08±0.0
+20	6.4±0.7	4.6±0.5	2.1±0.2	0.01±0.0
+10	4.7±0.5	3.4±0.4	1.6±0.2	0.0
0	2.7±0.3	1.9±0.2	0.9±0.1	0.0
−10	1.3±0.1	0.9±0.1	0.4±0.0	0.0
−20	0.6±0.0	0.4±0.0	0.2±0.0	0.0
−30	0.4±0.0	0.2±0.0	0.1±0.0	0.0
−40	0.2±0.0	0.1±0.0	0.0	0.0
−50	0.1±0.0	0.0	0.0	0.0

[a] Data are means±SEM for 18 cells.
[b] Data are means±SEM for 12 cells.
[c] Data are means±SEM for 16 cells.
[d] Data are means±SEM for 9 cells.

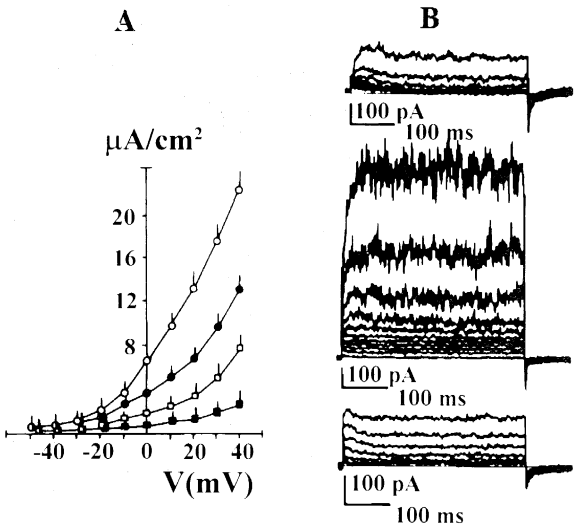

Fig. 3. (A) Voltage dependence of the densities of the peak I_K, measured in solution C (open circles, $n=6$), or after the blockade of $I_{K(to)}$ with 1 μM apamin (closed circles, $n=5$). With apamin the amplitudes of $I_{K(sl)}$ were reduced by 50% with 1 μM nicardipine or 1 μM methoxyverapamil (open squares, $n=7$), and the addition of 10 μM $LaCl_3$ to apamin- and nicardipine-containing solution left only the leakage current (closed squares, $n=5$). $V_h=-70$ mV. Data are means±SEM. (B) Representative whole-cell I_K waveforms, expressed in a cell bathed with apamin- (1 μM) and tetraethylammonium- (1 mM) containing solution C (upper traces), and 5 min after replacement of this solution with apamin-containing (1 μM) solution C without tetraethylammonium (middle traces). Subsequent addition of 0.1 μM charybdotoxin to the bath effectively abolished $I_{K(sl)}$ (lower traces). Rectangular pulses in 10 mV increments lasting 400 ms were applied from $V_h=-70$ mV. Leak and inward currents were subtracted. Cell with capacitance 61 pF and impedance 1.6 GΩ.

Other K^+ channel blockers were also investigated for effects on both $I_{K(to)}$ and $I_{K(sl)}$. Glibenclamide (10 nM to 10 μM) or glipizide (10 nM to 10 μM), used as selective blockers of the ATP-sensitive K^+ channels (Nelson, 1993), as well as 4-aminopyridine (0.1 to 5 mM) failed to affect the amplitude of both components of I_K. Iberiotoxin (0.1 μM), like charybdotoxin, blocked $I_{K(sl)}$, while quinidine (10 μM and above) decreased by about 15% the amplitude of $I_{K(to)}$ in 23% of the cells ($n=42$) (not shown).

3.3. Properties of K^+ currents in 12% of the cells

In 12% of cells depolarizing pulses positive to −60 mV evoked a noninactivating I_K. This current consisted of two pharmacologically distinguishable components: a tetraethylammonium- (1 mM) or charybdotoxin- (0.1 μM) sensitive one, which formed about 30% of the net I_K, and a glipizide-sensitive (0.2 μM) one, which conducted about 70% of the net I_K (Fig. 4A). Both components were highly selective for K^+ ions (Fig. 4B), had similar voltage dependences and activated simultaneously. However, the charybdotoxin-sensitive component was abolished by addition of

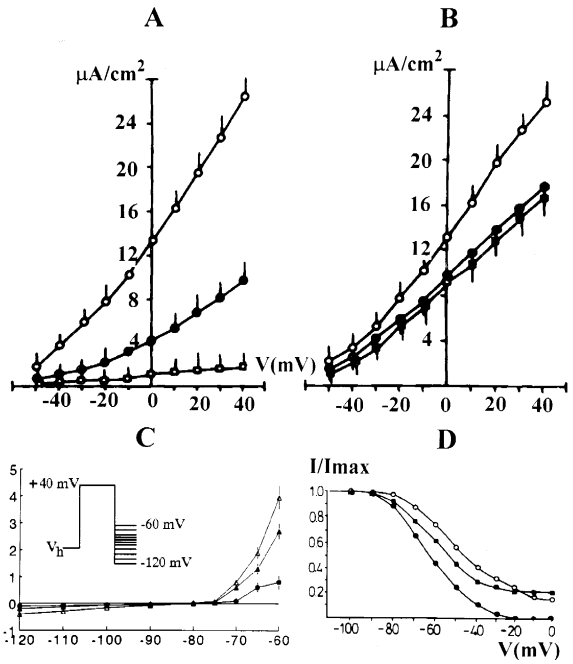

Fig. 4. (A) Voltage dependence of the I_K densities in the minority of guinea-pig antral smooth muscle cells, as measured in solution C (open circles, $n=6$) and after consecutive addition of 0.5 μM glipizide (closed circles, $n=5$) and 0.1 μM charybdotoxin (open squares, $n=6$). $V_h=-70$ mV. (B) Selective inhibition of charybdotoxin-blockable component of I_K by $LaCl_3$. Voltage dependence of the I_K density was obtained in solution C (○, $n=6$) and after bath application of 10 μM $LaCl_3$ (●, $n=7$). In the presence of $LaCl_3$ 0.5 μM charybdotoxin (□, $n=5$) failed to affect I_K. $V_h=-70$ mV. Data are means ± SEM. (C) I/V characteristics of the tail currents, obtained after a conditioning potential to +40 mV, followed by various repolarizing stimuli between −120 and −50 mV, as shown in the inset. Data are presented as means ± SEM for 14 cells after subtraction of the linear leakage current. The selectivity of the glipizide-sensitive component was studied in the presence of 1 μM charybdotoxin, and that of the charybdotoxin-sensitive current — in the presence of 10 μM glipizide. Bath solution contained 3 μM nicardipine and 50 μM niflumic acid (Cl^- channel blocker). $V_h=-70$ mV, $T^o=32$°C. The obtained values for the reversal potentials were -80 ± 2 mV for the glipizide-sensitive current and -81 ± 3 mV for the charybdotoxin-sensitive current. The estimated reversal potential was −82.1 mV. (D) Steady state inactivation properties of the glipizide-sensitive I_K obtained in the presence of 1 μM charybdotoxin (○, $n=7$; $V_{half}=-43$ mV) and of the charybdotoxin-blockable I_K obtained in the presence of 10 μM glipizide in control experiments (●, $n=5$, $V_{half}=-60.5$ mV) or in the presence of 1 μM thapsigargin in the bath (□, $n=6$, $V_{half}=-51$ mV).

10 μM $LaCl_3$ to a nicardipine-containing (1 μM) bath medium (Fig. 4C), while the glipizide-sensitive component remained unaffected.

Apamin (10 nM to 10 μM) did not influence the I_K components in this population of cells. 4-aminopyridine (over 5 mM) decreased the amplitude of the glipizide-sensitive component by 12 ± 3% in 3 out of 11 cells ($p>0.1$).

The steady state inactivation curves for glipizide- and charybdotoxin-sensitive components of I_K were obtained by using a conventional double-pulse protocol. This protocol consisted of a conditioning prepulse between −120 mV and +40 mV, which lasted 6 s, followed by test stimulus to +40 mV for 500 ms. The study was performed in the presence of 0.5 μM charybdotoxin (for the glipizide-sensitive component) or 1 μM glipizide (for the charybdotoxin-sensitive component). The data obtained showed that about 20% of the glipizide-sensitive component was still available at 0 mV membrane potential. Under the same experimental conditions (> 40 nM free Ca^{2+} into the pipette solution), the charybdotoxin-sensitive K^+ channels were 50% available at −60 mV membrane potential and were fully inactivated by a 4 s conditioning prepotential to −20 mV (Fig. 4D, $n=12$).

3.4. Modulation of I_K by intracellularly stored Ca^{2+}

In 88% of cells the depletion of intracellular Ca^{2+} stores by thapsigargin (0.1–1 μM), an inhibitor of Ca^{2+}-ATPases of the sarcoplasmic reticulum, or ryanodine (0.5 μM), an activator of Ca^{2+}-induced Ca^{2+} release channels, increased by 50–200% the amplitude of $I_{K(sl)}$ at potentials positive to +10 mV (Fig. 5A), and accelerated the activation of $I_{K(sl)}$ (not shown).

In 12% of cells thapsigargin (0.1 μM) or ryanodine (0.5 μM) caused a 30% ± 2% ($n=10$) increase of the net I_K amplitude (Fig. 5B). The thapsigargin-induced elevation of subplasmalemmal Ca^{2+} shifted the steady state inactivation curve of the charybdotoxin-sensitive I_K to more positive potentials: steady state half-inactivation appeared at −49 mV, and about 23% of this component persisted at voltages up to +20 mV (Fig. 4D, squares). This effect of the Ca^{2+}-depleting agents was manifested even when voltage-sensitive Ca^{2+} entry was interrupted with 1 μM nicardipine at the start of the experiment.

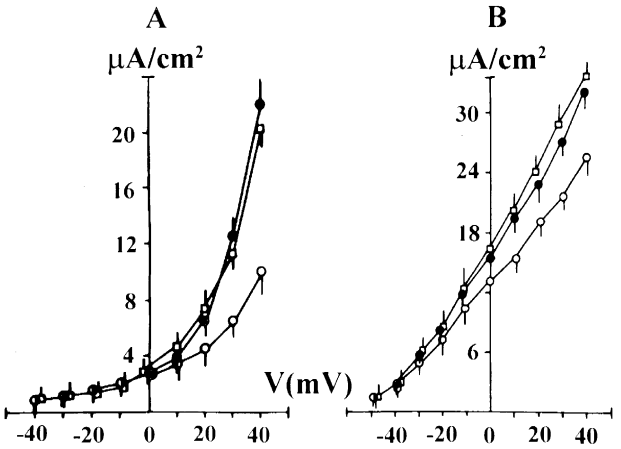

Fig. 5. (A) Voltage dependence of the density of the $I_{K(sl)}$, measured in 88% of the guinea-pig antral smooth muscle cells (A) and in the rest 12% of cells (B), 400 ms after the onset of 500 ms lasting pulses in solution C (○, $n=11$) and 5 min after bath application of 1 μM thapsigargin (●, $n=12$) or 0.5 μM ryanodine (□, $n=11$). $V_h=-70$ mV. Data are means ± SEM.

4. Discussion

The present data suggest the existence of at least two populations of smooth muscle cells in the guinea-pig gastric antrum, which differ in the voltage dependence, kinetics and pharmacological profile of their I_K components.

4.1. Role of $I_{K(to)}$

The majority of cells expressed an apamin-sensitive transient K^+ current, $I_{K(to)}$, which was depressed when V_h was more positive than −50 mV. This finding is in line with the data of Suzuki et al. (1993) and Petkov et al. (1994), who found that apamin (10 nM) increased the amplitude of the antral slow wave potential, and favors the view of Noack et al. (1992) that $I_{K(to)}$ determines the level of the upstroke depolarization. According to this, we suggest that cells expressing $I_{K(to)}$ cannot be pacemaker cells.

4.2. Role of the glipizide-sensitive component

The most appropriate candidate for pacemaker cells in antral smooth muscle are the cells which lack the 'fast' I_K component. The main part of the K^+ outflow in such cells is conducted through the glipizide-blockable, i.e. ATP-sensitive K^+, channels (Standen, 1992). It is well established that the ability of antral smooth muscle to generate slow waves depends on the temperature (Q_{10} between 3.3 and 3.5; Boev and Papasova, 1969; El-Sharkawy and Daniel, 1975). This means that slow wave potentials in the guinea-pig antrum are regulated by a metabolic pathway (Ward et al., 1991). Our data show that a substantial glipizide-sensitive I_K could be conducted during the plateau phase. ATP insufficiency will increase the opening of ATP-blockable channels, which will result in repolarization of the cell membrane and suppressed excitability upon conventional stimuli. This assumption is in agreement with the finding that full size action potentials could not be elicited between slow waves even by directly applied supramaximal depolarization (Boev et al., 1970). Therefore, the accumulation of ATP between contractions may trigger the next depolarization due to the ATP-dependent inhibition of glipizide-sensitive K^+ conductivity.

4.3. Role of the charybdotoxin-sensitive component

The depletion of intracellular Ca^{2+} stores selectively increased the amplitude of charybdotoxin-sensitive I_K. Most probably, charybdotoxin-sensitive I_K activates during the plateau phase to counteract the depolarization, and participates in the termination of the plateau phase when substantial amounts of Ca^{2+} have already been accumulated in the subplasmalemmal space (Sanders, 1992).

The depletion of intracellular Ca^{2+} stores in 'pacemaker' cells caused only a slight increase in I_K amplitude at positive membrane voltages, while at −40 mV this increase exceeded 75%, suggesting that the rise in the subplasmalemmal Ca^{2+} concentration in 'pacemaker' cells may serve as a negative feedback control to reduce the frequency of the spike potentials.

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