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An intermediate conductance K^+ channel in the cell membrane of mouse intestinal smooth muscle

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Abstract

Single channel currents were recorded from cell-attached and inside-out patches in smooth muscle cells of the mouse ileum in order to identify TEA-sensitive Ca^{2+}-dependent K^+ channels. Cells were bathed in high-K^+ (150 mM) solution with [Ca^{2+}] buffered to 80–150 nM with EGTA and patch pipettes were filled with low-K^+ (2.5 mM) physiological solution. Two distinct TEA-sensitive unitary outward current levels were identified at a holding potential (V_h) of 0 mV, corresponding to intermediate conductance (IK, ~40 pS) and large conductance (BK, >200 pS) K^+ channels. The open probability (P_o) of IK channels increased with depolarization, the voltage for half-maximal activation averaging +12 mV in 80 nM Ca^{2+}_{bath}. Raising the [Ca^{2+}] in the high-K^+ solution from 80 nM to 150 nM increased the P_o of IK channels at $V_h = 0$ mV from 0.078 to 0.21. Likewise, the open probability of BK channels at 0 mV was increased from 0.003 to 0.026. Unlike BK channels, IK channels inactivated with maintained depolarization with a voltage for half-maximal inactivation of −66 mV. IK channels were blocked by 2–5 mM external TEA and were sensitive to both charybdotoxin (100 nM) and apamin (500 nM). Our results suggest that IK channels contribute significantly to the Ca^{2+}-dependent K^+ conductance in visceral smooth muscle. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Ion channel; Visceral smooth muscle; Patch clamp; BK channel; Voltage-dependent potassium channel

1. Introduction

Patch-clamp studies on smooth muscle cells from various tissues have identified large-conductance (200–250 pS) Ca^{2+}- and voltage-activated K^+ (BK) channels as major contributors to the cellular membrane conductance to K^+ [1–5]. Because BK channels are typically blocked by externally applied 1–2 mM TEA and by charybdotoxin (20–100 nM) applied externally (see Ref. [5]), these pharmacological properties are often used to determine the contribution of BK channels to whole-cell currents and their participation in the electrical responses recorded from intact tissues [6]. Apart from BK channels however, smooth muscle cells may possess other Ca^{2+}-activated K^+ channels such as apamin-sensitive, but TEA (5 mM)-resistant small-conductance (<10 pS) (SK) channels, and TEA (2 mM)-sensitive intermediate-conductance (IK) channels [7–9]. In the present study, we investigated the voltage-dependence of IK channels in mouse ileal smooth muscle cells and their sensitivity to Ca^{2+} and to external charybdotoxin and apamin. Our results indicate that IK channels activate

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at more negative potentials than BK channels, are sensitive to both charybdotoxin and to apamin and can undergo steady-state inactivation. These properties, coupled with their sensitivity to TEA suggest that IK channels, as well a BK channels may be responsible for much of the TEA-sensitive electrical activity recorded from intestinal smooth muscles.

2. Materials and methods

Experiments were conducted on smooth muscle cells enzymatically dispersed from the muscularis of the mouse ileum as described previously [9]. Current recordings were obtained from cell-attached patches and from inside-out patches using an Axopatch 200 A amplifier (Axon, Foster City, CA) coupled to a PC computer running pClamp 6.03 (Axon), through an A/D converter (Axon). Recordings were low-pass filtered at 160–250 Hz (four-pole Bessel filter, Ithaca, NY) and digitized at 1 KHz. Pipettes were drawn from borosilicate glass (Clark) to have resistances of 5–10 MΩ when filled with physiological solution of the following composition (in mM): NaCl, 150; KCl, 2.5; $MgCl_2$ 2; HEPES, 10. pH was adjusted to 7.4 with 10 M NaOH. The recording chamber was perfused continuously with high-K^+ solution of the following composition (in mM): KCl, 150; $MgCl_2$, 1; $CaCl_2$, 1; HEPES, 10; EGTA, 2.0. The pH of this solution was adjusted to 7.2 with 10 M KOH yielding a $[Ca^{2+}]$ of ~150 nM (Eqcal, Biosoft, MO). Lower $[Ca^{2+}]$ was obtained by increasing [EGTA] while maintaining the pH. Drugs were applied in the pipette filling solution, or to the bathing solution. All recordings were obtained at room temperature (22–25°C). Recordings were analysed using Fetchan and Pstat (Axon). The open probability (P_o) of channels was determined by fitting sums of Gaussian functions to all-points histograms using a least-squares fitting procedure in Fetchan (Axon) (see Fig. 2B). P_o was determined by summing the proportion of each Gaussian component multiplied by its corresponding channel level, and dividing this total by the maximum number of channels that were open simultaneously in that patch at the most depolarized potential (+30 or +20 mV), which was taken as an indication of the total number of channels (N) in that patch. This assumption however is likely to underestimate the number of channels in the patch, especially if the open probability is low and no overlapping openings are observed.

Therefore the derived parameter should be regarded as an 'apparent' P_o. Where N is not known, as for BK channels, NP_o is given. Single channel current amplitude was measured either from all-points histograms or by eye. Channel open times were measured using the 50% threshold criterion. Because of the low cutoff frequencies used to filter records, openings < 5 ms in duration were rejected. This was because a cutoff frequency of 200 Hz introduces a time constant (τ) of ~1 ms to a step input signal. Therefore only events of durations $> 5\tau$ could be measured with certainty. Mean open times were determined by fitting exponential functions to dwell time histograms using pStat (Axon). Boltzmann fits to the data were performed using Sigmaplot 4.0 (Jandel). Averaged data are expressed as mean ± S.E.M. where n equals the number of patches (cells). Unpaired t-tests were used to determine statistically significant differences ($P < 0.05$) between means.

3. Results

Prior to seal formation, cells were perfused with high-K^+ solution in order to null their resting potentials. Contraction was precluded by adjusting the $[Ca^{2+}]$ in the bathing solution ($[Ca^{2+}]_{bath}$) to 80–150 nM. Under these conditions depolarization of cell-attached patches from a negative holding potential (V_h) of −80 mV triggered outward single channel currents. As shown for a typical patch in Fig. 1A, the magnitude of the unitary current (i) was dependent on the driving force on K^+. The current–voltage relationship for i averaged from 10 patches was well-fitted by the Goldman–Hodgkin–Katz equation for an asymmetric K^+ gradient (Fig. 1B, filled squares). The value for permeability obtained from this fit predicted a unitary conductance of between 40–50 pS at very positive potentials (> 200 mV). This value agreed well with the conductance determined from unitary currents recorded from one cell-attached patch under a quasi-symmetric K^+ gradient (44 pS; Fig. 1B, pen squares) ($[K^+]_{pipette} = 150$ mM). At test potentials positive of 0 mV, a larger unitary current was also recorded with less frequency (see

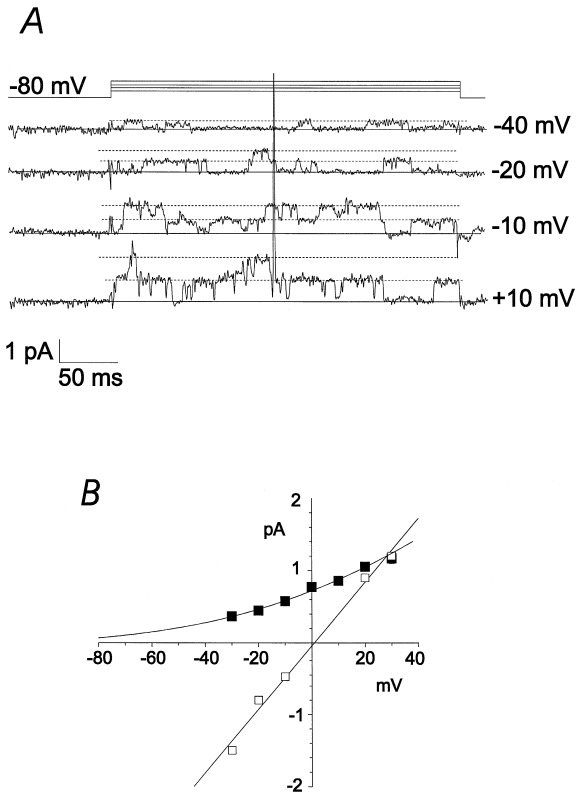

Fig. 1. Voltage-dependence and conductance of IK channels in smooth muscle cells of the mouse ileum. (A) Cell-attached patch recording from a cell bathed in 120 nM Ca^{2+}. Channel openings are upward. The patch was held at −80 mV and the patch potential was stepped positive, every 10 s, to the potentials indicated. Capacitance current artifacts have been digitally subtracted using 'null' traces. Depolarization activated mainly IK channels. A single BK channel was opened at +10 mV. (B) Current–voltage relationship of single channel IK currents, recorded under asymmetric (filled squares) and symmetric (empty squares) K^+ gradients. Data points obtained under the asymmetric K^+ gradient are averages from 10 patches. The points obtained under the symmetric K^+ gradient represent data from one patch. The filled squares were fitted with the GHK equation and the empty squares were fitted by linear regression yielding a maximal slope conductance of 48 pS and 44 pS, respectively.

lower panel in Fig. 1A) which averaged 5.6 pA at 0 mV (e.g., see Fig. 4A). Based on the magnitude of the unitary currents that were recorded under an asymmetric K^+ gradient, the two types of unitary current were attributed to IK and to BK channels, respectively.

As illustrated in Fig. 1A, the frequency of opening of IK channels was clearly dependent on the test potential. IK channels were activated, after a variable latency, with increasing depolarization, usually in the absence of BK channels. The increase in P_o of IK channels was reflected in the all-points histogram plots of the data recorded during the depolarizing steps and the distributions were well fitted by Gaussian functions (Fig. 2A). The open probability (P_o) of IK channels derived from such histograms clearly displayed a sigmoidal dependence on the test depolar-

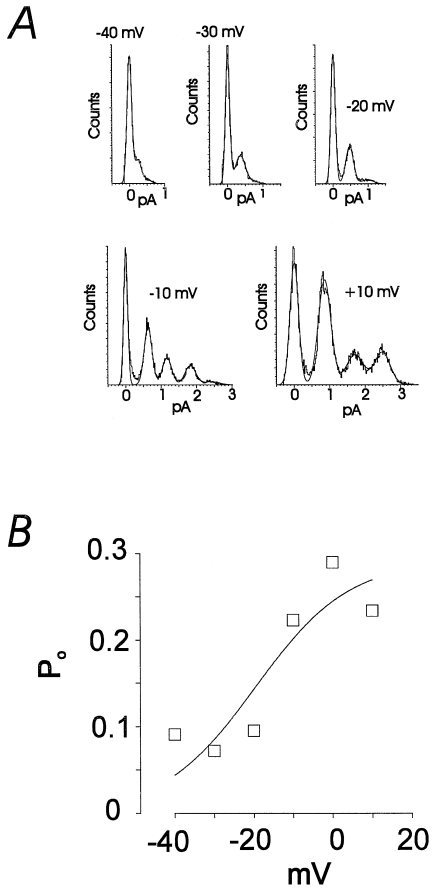

Fig. 2. Determination of open probability of IK channels. (A) All-point histogram plots constructed from channel activity triggered by 30 consecutive step depolarizations to the potentials indicated, from a holding potential of −80 mV (data are from the cell depicted in Fig. 1A). The histograms are overlaid with fitted Gaussian functions from which P_o was estimated. (B) P_o plotted as a function of voltage for the patch depicted in panel (A). The data points are fitted with a Boltzmann function of the form: $P_o = P_{o\,MAX}/(1+\exp(-(V_h - V_{act})/k))$, where V_{act} is the voltage at which P_o is half-maximal and k is the slope. V_{act} was −19 mV and the slope was +11.9 mV.

ization (Fig. 2B). The data points from this patch when fitted with a Boltzmann function yielded a voltage of half-maximal activation (V_{act}) of −19 mV and a slope of +11.9 mV.

The P_o of IK channels was generally higher when patches were depolarized to 0 mV from a negative holding potential than during sustained depolarization at 0 mV. This tendency for IK channels to inactivate was studied in six cell-attached patches from cells bathed in 120–150 nM Ca^{2+}. In one such patch, inactivation of IK channels was apparent during the step depolarization as a lower incidence of openings at the end of short (320 ms) depolarizing steps (Fig. 3A(i)) and as a slow decay in the average current constructed from 30 consecutive traces (Fig. 3A(ii)). In the other patches, however, this time-dependent inactivation was less obvious and inactivation in these patches was expressed as a marked decrease in P_o for IK channels at 0 mV, as V_h was made more positive. The averaged data when fitted with a Boltzmann function (Fig. 3B) yielded a voltage of half-maximal inactivation of −66 mV and a slope of −9 mV. IK channels however did not fully inactivate with maintained depolarization (minutes) and continued to gate at positive potentials with a finite probability (0.01) (Fig. 3B).

The P_o of IK channels during sustained depolarization was also voltage-dependent (Fig. 4A). The averaged data from six patches representing P_o at various holding potentials recorded from cells bathed in 80 nM Ca^{2+} is plotted in Fig. 4B (filled diamonds) and has been fitted with a Boltzmann function with a V_{act} of −4 mV and a slope of +12 mV. When the data points representing open probability that was not normalized for the number of channels in the patch (N) (i.e., NP_o) were fitted individually, V_{act} averaged +12.3 ± 8 mV and the slope factor averaged +11.7 ± 2 mV.

In four patches from cells that were bathed in 150 nM Ca^{2+}, P_o of IK channels was enhanced at all potentials (Fig. 4B, empty diamonds) and the P_o–V relationship appeared shifted to more negative potentials. A Boltzmann function fitted to the pooled data yielded a V_{act} of −37 mV and a slope of +22 mV.

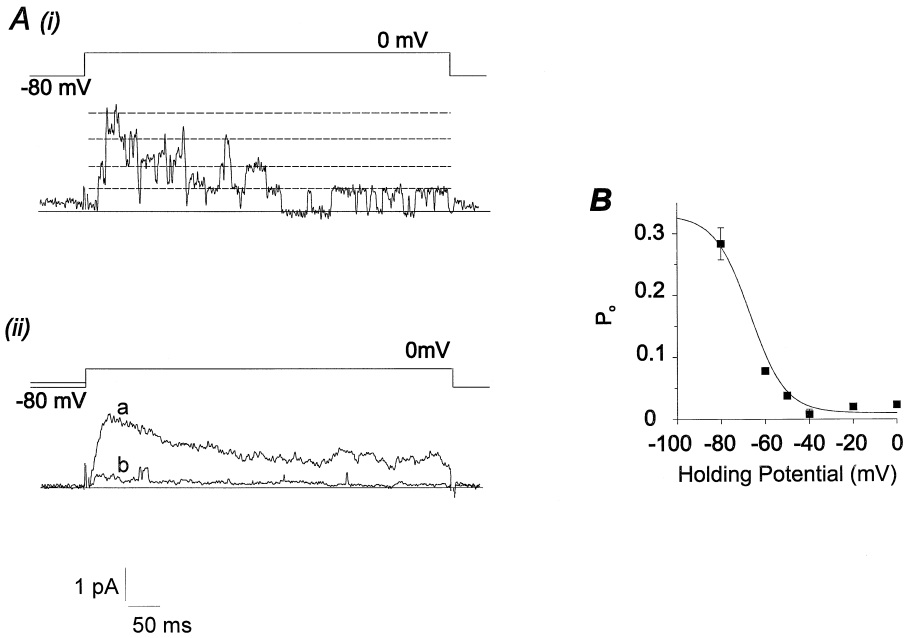

Fig. 3. Inactivation of IK channels in a cell-attached patch. (A)(i), IK channel openings (four channels) recorded from a cell-attached patch in response to step depolarization from −80 mV to 0 mV showing fewer openings at the end of the pulse. Capacitance current artifacts have been digitally subtracted using 'null' traces. (A)(ii), Mean currents averaged from 30 traces triggered by depolarization to 0 mV from a holding potential of −80 mV (a) and −60 mV (b). Solid line indicates zero current level. (B) Pooled data obtained from four patches on cells bathed in 120–150 nM Ca^{2+} showing P_o at 0 mV plotted as a function of holding potential. Data points are fitted with a modified Boltzmann equation with a non-inactivating component to obtain a voltage of half-maximal inactivation of −66 mV and a slope of −8 mV and a non-inactivating P_o of 0.01.

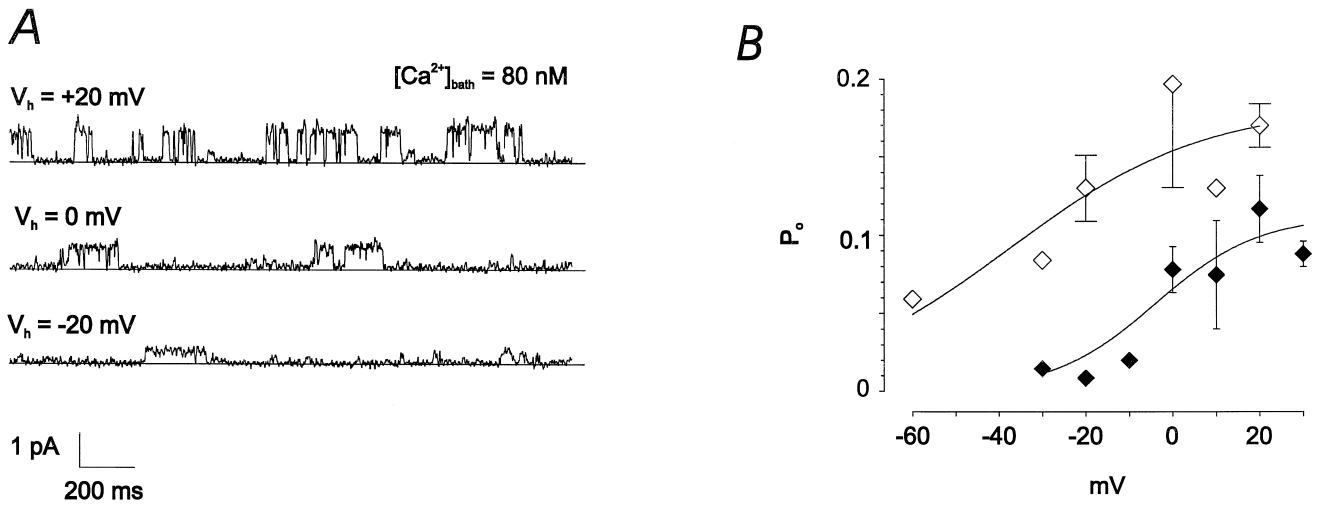

Fig. 4. Effect of $[Ca^{2+}]$ on steady-state activation of IK channels. (A) Typical IK channel activity recorded from a cell-attached patch in a mouse ileal smooth muscle cell at three different holding potentials (V_h). The cell was bathed in high-K^+ solution containing 80 nM Ca^{2+}. (B) Voltage-dependence of the open probability (P_o) of IK channels recorded from six cells bathed in 80 nM Ca^{2+} (empty diamonds) and from four cells bathed in 150 nM Ca^{2+} (filled diamonds). The averaged data points have been fitted to a Boltzmann functions. V_{act} obtained from fits to the pooled data was -4 mV and -37 mV for the cells in 80 nM and 150 nM Ca^{2+}_{bath}, respectively. The corresponding slopes were 12 and 22, respectively.

Although we cannot be certain that the increase in $[Ca^{2+}]_{bath}$ is reflected in a commensurate increase in cytoplasmic $[Ca^{2+}]$, the fact that the open probability of BK channels (expressed as NP_o) was also increased by switching from 80 nM to 150 nM Ca^{2+}-containing high-K^+ solution, from 0.003 ± 0.006 ($n = 10$) to 0.026 ± 0.007 ($n = 10$), respectively, suggests that cytoplasmic $[Ca^{2+}]$ is indeed elevated. Moreover the data plotted in Fig. 4B suggest that the P_o of IK channels is both voltage- and Ca^{2+}-dependent. The higher P_o of IK channels compared to BK channels under these conditions is partly attributable

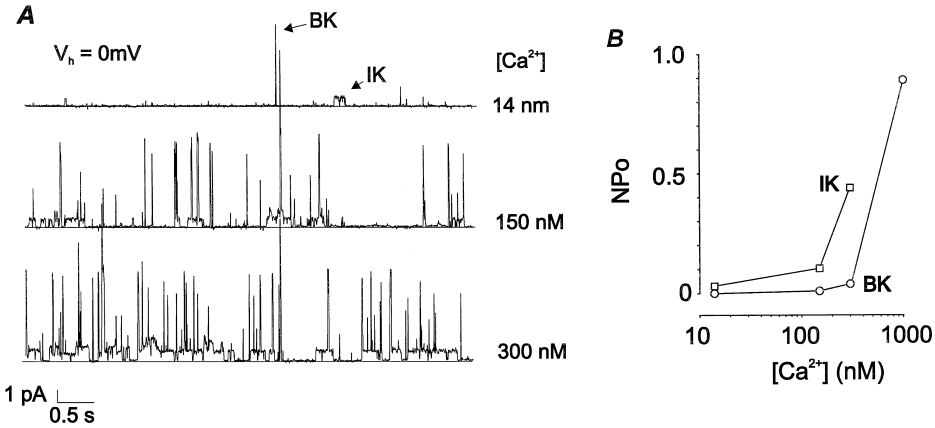

Fig. 5. Ca^{2+}-dependence of IK channels in an inside-out patch. (A) Openings of both IK and BK channels were increased by raising $[Ca^{2+}]$ in the high-K^+ bathing solution at a holding potential of 0 mV. (B) The open probability (NP_o) of IK (squares) and BK channels (circles) plotted as a function of $[Ca^{2+}]$. NP_o of IK channels increased steeply at $[Ca^{2+}]$ between 150 and 300 nM.

to the significantly ($P < 0.05$) longer mean open time (τ_{open}) of IK channels which at 0 mV and in the presence of 150 nM $[Ca^{2+}]_{bath}$ averaged 83 ± 17 ms ($n = 9$) and 9.1 ± 1.6 ms ($n = 10$), respectively (e.g., see Fig. 5A).

In order to assess dependence of IK channels on cytoplasmic $[Ca^{2+}]$ directly, we attempted to record IK channel activity from inside-out patches. This patch configuration however led to loss of IK channel activity within 5–10 min, suggesting that IK channels are regulated by cytoplasmic factors. In one patch however we were able to demonstrate the stimulatory effect of increasing $[Ca^{2+}]_{bath}$ on IK channel activity (Fig. 5A). As shown in Fig. 5B, the open probability (NP_o) of IK channels increased steeply at $[Ca^{2+}] >$ 150 nM at a maintained depolarization (0 mV) while the increase in the NP_o of BK channels was more pronounced at ~ 1 μM Ca^{2+} at which the NP_o of IK channels could not be measured because currents could not be clearly distinguished from the multiple BK channel openings. These recordings, however, suggest that IK channels may be activated by lower levels of cytoplasmic Ca^{2+} than BK channels.

To test whether IK channels are inhibited by blockers known to inhibit BK channels, a saturating concentration of charybdotoxin (100 nM) was added to the external (pipette) solution facing cell-attached patches in cells bathed in 150 nM Ca^{2+}. Under these conditions, openings of BK channels were absent at $V_h = 0$ mV (cf. Fig. 6A and B) and the P_o of IK channels was decreased to 0.05 ± 0.007 ($n = 5$) from 0.21 ± 0.06 ($n = 6$) in control patches. Although this difference did not reach significance ($P > 0.05$), the mean open time (τ_{open}) of IK channels was significantly shorter ($P < 0.05$) in patches exposed to charybdotoxin (25 ± 5 ms, $n = 5$) than in control patches (83 ± 17 ms, $n = 9$). Under similar conditions, the effect of apamin (500 nM) was tested on IK channels. When apamin was included in the pipette solution, P_o was decreased to 0.05 ± 0.015 ($n = 8$) and τ_{open} of IK channels was significantly reduced ($P < 0.05$) to 13 ± 2 ms ($n = 7$). Tetraethylammonium (TEA, 2 mM), a known blocker of BK channels also inhibited IK channel openings and when applied to the pipette solution induced short openings of IK channels the duration of which could not be measured (Fig. 6C). At 5 mM, TEA completely blocked IK channel openings (data not shown).

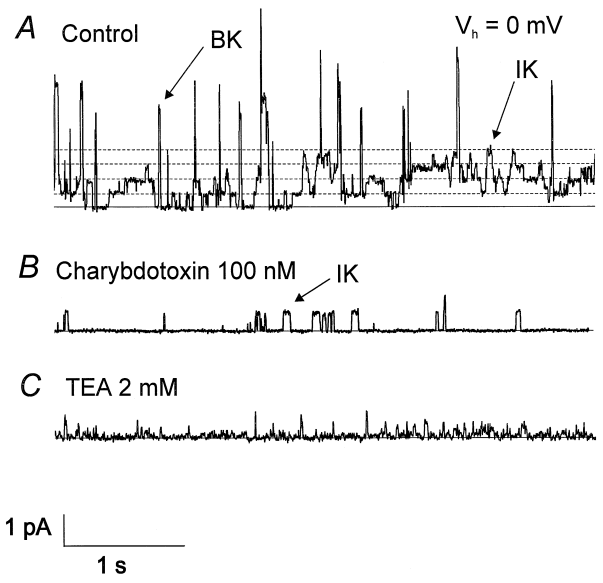

Fig. 6. Inhibition of IK channels by charybdotoxin and TEA. (A) Typical activity of IK channels and BK channels recorded from a cell-attached patch in a cell bathed in 150 nM Ca^{2+} at a holding potential of 0 mV. Solid line below the trace indicates the zero current level and dashed lines indicate IK open channel levels. (B) Channel activity recorded from a cell-attached patch from a different cell under similar conditions as in panel (A) with charybdotoxin (100 nM) added to the pipette solution. Note the absence of BK channels and the reduced number of openings of IK channels. (C) Cell-attached patch recording from another cell with TEA 2 mM added to the pipette solution showing abolition of BK channel currents and reduced amplitude and open times of IK channels.

4. Discussion

Large-conductance Ca^{2+}-activated K^+ (BK) channels are expressed ubiquitously in smooth muscle [1–5]. Their primary role is to repolarize cells following Ca^{2+} influx and cell depolarization. These channels are very sensitive to block by external TEA and usually 1–2 mM TEA is sufficient to effectively block current flow [4,10,11]. Ca^{2+}-activated K^+ (IK) channels of intermediate conductance (20–50 pS) which are as sensitive to external TEA as BK channels have been previously reported in rat cerebral arteries [7] and in rat renal arteries [8] and IK chan-

nels with lower sensitivities to TEA have also been reported in rabbit portal vein [1].

In the present study, we have demonstrated that IK channels in the mouse ileum are activated by both voltage and Ca^{2+}. The slope of the voltage-dependence of activation ($\sim$ 12 mV) of P_o is in the range reported for BK channels [11] suggesting that the voltage-sensing region of IK channels may be structurally similar to that of BK channels. The mechanism by which Ca^{2+} enhances P_o of IK channels at present is unclear. Because IK channels run down with patch excision the effect of $[Ca^{2+}]$ on the voltage-dependence of activation could not be determined. Such rundown of IK channels has also been reported in GH3 cells upon patch excision [12]. However, we found that when cells were exposed to a slightly higher $[Ca^{2+}]$ in the bathing solution, the maximal P_o of IK channels was increased and their activation by voltage was shifted to more negative potentials. Although the cytoplasmic $[Ca^{2+}]$ is not accurately known under these conditions, the fact that the open probability of BK channels was also higher in patches from cells bathed in the higher (150 nM) $[Ca^{2+}]$ suggests that cytoplasmic Ca^{2+} was elevated. Cytoplasmic Ca^{2+} may enhance the P_o of IK channels by increasing their sensitivity to voltage, in a manner similar to the effect of Ca^{2+} on BK channels [11]. However, we cannot rule out the possibility that an increase in cytoplasmic $[Ca^{2+}]$ may also make more IK channels available for activation without necessarily shifting their voltage-dependence. This possibility is suggested by the increase in peak P_o of IK channels in the presence of 150 nM Ca^{2+}_{bath}.

Unlike BK channels, IK channels in the mouse ileum tended to inactivate with depolarization. Inactivation however was not complete, and a fraction of IK channels remained active during maintained depolarization. The mechanism underlying inactivation of IK channels was not investigated in the present study. The fact that IK channels inactivated while $[Ca^{2+}]_{bath}$ was constant and recording pipettes were filled with nominally Ca^{2+}-free solution suggests that inactivation does not simply reflect a decrease in the Ca^{2+}-mediated activation of IK channels, as would be expected if there was Ca^{2+} influx through inactivating L-type Ca^{2+} channels in the patch. In rat chromaffin cells, cytoplasmic Ca^{2+} has been reported to inactivate a large-conductance Ca^{2+}-activated K^+ channel through an unidentified mechanism [13]. Evidence for Ca^{2+}-dependent inactivation is also evident in recordings of IK channel activity in GH3 cells stimulated by Ca^{2+}-influx [12]. Further experiments are needed to investigate the effect of cytoplasmic $[Ca^{2+}]$ on the availability and activation of IK channels. One approach may be to use an ionophore to selectively permeabilize cells to Ca^{2+} without disturbing the intracellular regulatory pathways.

In the present study we have shown that in addition to being blocked by external TEA (2–5 mM), IK channels in mouse ileal smooth muscle cells are also sensitive to external charybdotoxin, albeit at a concentration 10-fold higher than that which blocks BK channels, and also to apamin. The sensitivity to both charybdotoxin and apamin was manifest as a decrease in the mean open time. IK channels in rat renal arterioles are also sensitive to charybdotoxin and to apamin and also to TEA (0.1 mM) [8]. However in GH3 cells, IK channels with a conductance similar to IK channels in mouse ileal smooth muscle are relatively insensitive to external TEA (K_d = 17 mM) [12] and although their sensitivity to charybdotoxin was not tested, those channels were inhibited by apamin [12]. In cells of rabbit proximal tubules, IK channels were blocked by scorpion venom [14]. At present it is not clear whether IK channels in the various cell types represent a distinct family of ion channels. Recently a Ca^{2+}-activated K^+ channel which has been classified as either an intermediate- (hIK4) [15] or small-conductance K^+ (hSK4) [16] channel was cloned from human pancreas [15] and human placenta [16]. This channel is resistant to block by TEA and apamin, and has a much higher sensitivity to charybdotoxin (K_i = 2.5 nM) [17] than IK channels in mouse ileum. Moreover hIK4 has no appreciable voltage-dependence and shows inward current rectification [18], unlike the IK channel in mouse ileum. Our present study indicates that IK channels in the mouse ileum have similar, but not identical, pharmacological properties and Ca^{2+} sensitivity to BK channels. This suggests that the depolarization of visceral smooth muscle by TEA [17] and by charybdotoxin [18] may in fact be mediated in part by block of IK channels. The molecular identity of the IK channel in mouse ileum remains to be determined.

Despite having a smaller conductance, the higher P_o of IK channels compared to BK channels at

physiological potentials suggests that IK channels may be important in generating the Ca^{2+}-dependent K^+ conductance near the resting potential of ileal smooth muscle. Although IK channels may be substantially inactivated at the resting potential, a significant fraction of IK channels may be continuously active to generate a 'window' of outward current at potentials between -60 and -40 mV. The role of BK channels, which require a cytoplasmic $[Ca^{2+}] > 1$ μM for them to open at potentials below -40 mV [11], may be confined to generating outward current only at very depolarized potentials or during periods of rapid Ca^{2+} influx, for example during the rising phase of Ca^{2+}-dependent action potentials. IK channels on the other hand may be activated by modest increases in cytoplasmic $[Ca^{2+}]$ and channel openings may persist longer.

It is not yet clear if IK channels are expressed as ubiquitously in smooth muscle as BK channels. In smooth muscle cells of the mouse ileum, openings of IK channels were recorded from all cell-attached patches suggesting that IK channels are densely distributed on the cell membranes of these cells. Assuming a cell surface area of 5000 mm^2 and the area of a typical patch of 1–2 mm^2, extrapolation of the average patch current density in Fig. 3B to the total cell surface area would predict a typical peak whole-cell current of about 5 nA. Such a current can indeed be recorded from cells using perforated patch recordings or when cells are dialyzed with $[Ca^{2+}]$ of 150 nM [9]. Under conventional whole-cell dialyzing conditions however, this large current component is largely absent, consistent with the observed rundown in IK channels in excised patches. These data suggest that IK channels are regulated by intracellular factors and constitute a major component of Ca^{2+}-activated K^+ conductance in mouse ileal smooth muscle cells.

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