Three Components of Potassium Outward Current in Cells Isolated from the Circular Layer of Guinea-pig Ileum

D. B. DURIDANOVA and K. K. BOEV

Institute of Biophysics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str, bl. 21, BU-1113 Sofia, Bulgaria

Abstract. Three different populations of voltage-activated K^+ channels were identified according to their voltage-dependences, inactivation kinetics and sensitivity toward selective K^+ channels antagonists in membranes of cells, isolated from the circular layer of guinea-pig ileum by the use of whole-cell voltage clamp method. Under holding potential $V_h = -50$ mV apamin (10^{-6} mol/l) inhibited $29 \pm 3\%$ of the whole-cell K^+ current (I_K) at 0 mV membrane potential. The apamin-blockable component of I_K disappeared after blockade of the inward Ca^{2+} current, while other I_K components remained unchanged. Glibenclamide (10^{-5} mol/l) inhibited $31 \pm 2.6\%$ of I_K at 0 mV membrane potential. Cromakalim ($5 \cdot 10^{-7}$ mol/l) increased I_K, and glibenclamide impeded this increase and reduced I_K amplitudes to the same extent as when applied alone. The difference between amplitudes of I_K, measured from $V_h = -80$ mV and the above ones, expressed from $V_h = -50$ mV, was defined as the potential-dependent part of I_K (K_{pd}). This component formed 36 to 47% of I_K in cells clamped at $V_h = -80$ mV, and was inhibited by charybdotoxin (10^{-6} mol/l). The physiological role of these components of I_K is discussed.

Key words: K^+ currents — Apamin — Charybdotoxin — Glibenclamide — Smooth muscle — Whole-cell voltage-clamp

Introduction

Potassium currents are essential for the membrane resting and action potentials (Wilde and Lee 1989). These currents play a main role in the determination of the state of excitability of gastro-intestinal smooth muscle (Barajas Lopez et al. 1989). In our preliminary studies we have shown the presence of two types of Ca^{2+} channels in membranes of smooth muscle cells, isolated from the circular layer of guinea-pig ileum: a high-threshold, dihydropyridine-sensitive L-type, and low-threshold, Ni^{2+}-blockable T-type potential-dependent Ca^{2+} channels (Duridanova et al. 1993b). It was assumed that the latter are responsible for the

initial depolarization phase of the slow-wave action potentials, while the former are the main pathway for refilling of intracellular calcium stores, and take part in maintaining the plateau phase. It is well known that K^+ channel blockers such as tetraethylammonium or 4-aminopyridine are able to induce regular phasic contractions in smooth muscle preparations, which do not possess spontaneous phasic activity (Suarez-Kurtz et al. 1991; Brading 1992; Allescher et al. 1992). This phenomenon has been explained by depolarization-evoked Ca^{2+} influx due to the blockade of voltage-operated K^+ channels, which results in contraction (see Huang et al. 1993). This assumption has to be confirmed by voltage-clamp studies of K^+ channel populations, present in membranes of the investigated cells.

To the best of our knowledge, outward potassium currents (I_K) in membranes of cells, isolated from the circular layer of guinea-pig ileum have not been described as yet. The aim of the present study is to present some data about the components of I_K, conducted through different K^+ channel populations in cells of the ileal circular layer, with respect to their potential dependence, time course and sensitivity to selective K^+ channel blocking substances.

Materials and Methods

Isolation procedure

Male guinea pigs, weighing 200–250 g, were killed by a headblow. After exsanguination, 10 cm of the ileum was excised, 5 cm proximally to the ileocecal sphincter and placed

Table 1. Composition of solutions (in mmol/l)

	Cell isolation	Bath solution	Pipette**
NaCl	120.0	115.0	
KCl	12.0	5.6	105.0
$MgCl_2$	1.2	1.2	2.0
Glucose	20.0	20.0	14.0
Taurine	20.0	20.0	
HEPES*	10.0	10.0	10.0
Pyruvate	5.0	5.0	
$CaCl_2$	0.0	2.5	1.0
EGTA***			11.0

* N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid. pH of bath solutions was adjusted to 7.35–7.4 with KOH

** To 5 ml of this solution, used as a stock, were added immediately before use (mmol/l): 4 pyruvic acid, sodium salt; 4 succinic acid; 4 oxalacetic acid; 1.5 adenosine triphosphate sodium salt; 0.001 cyclic adenosine monophosphate sodium salt; pH adjusted to 7.2–7.3 with KOH

*** Ethylene glycol-bis-(-aminoethyl ether) N,N' tetraacetic acid

in Krebs' solution at room temperature. Strips of 5 mm length were removed from the circular muscle layer of the terminal ileum and placed in separate Petri dishes, containing Ca^{2+}-free solution, for cell isolation (see Table 1). The muscle strips were cut into pieces, 2.5 mm in length, and incubated in the same Ca^{2+}-free solution, into which 0.7 g/l collagenase (type 1A, SIGMA Chemical Co.), 1.5 g/l soybean trypsin inhibitor and 2 g/l bovine serum albumin were added. The Krebs' solution was of the following composition (mmol/l): Na^+ 139.5; K^+ 5.9; Mg^{2+} 1.2; Cl^- 136.2; HCO_3^- 15.5; $H_2PO_4^-$ 1.2; glucose 11.5; pH 7.3–7.4; it was bubbled with 95% O_2 and 5% CO_2. The composition of solutions used for cell isolation, in experimental bath medium, and the pipette solution are given in Table 1. A more detailed description of the isolation procedure was published elsewhere (Gagov et al. 1993b). The obtained cell suspension was stored in cold (8 °C) "KB" medium for up to 24 hours in (see Isenberg and Klockner 1982). For the purpose of the study, only fully relaxed cells were used. The material was drawn from these stocks and placed into the experimental chamber with a volume of 150 μl. Cells which reduced their length by more than 35% or did not contract at all after the application of 10^{-7} mol/l acetylcholine (ACh) were discarded, as were those which did not relax after ACh-induced contraction.

All experiments were performed at 33 ± 2 °C.

Electrophysiological techniques

The patch-clamp technique in whole-cell configuration was employed (Hamill et al. 1981). Electrodes were made from borosilicate glass, 1.8 mm o.d. (Jencons Scientific Ltd.). The resistance of an electrode filled with intracellular solution (pipette solution, Table 1) was about 2 MΩ. The experimental chamber consisted of a 10 mm square dish with a glass coverslip at the bottom, to which the dispersed cells usually adhered after a 20 min settling time. All experiments were performed at 28–30 °C. Temperated solution (Table 1) was continuously perfused into the chamber at a rate of 0.7 ml/min. The experimental chamber was observed under a light microscope (magnification 1000×) connected to a CCD-camera (VHS) and displayed on a TV-screen (14″) using a VHS video-recorder.

A List EPC-7 amplifier was used. Giga-ohm seals (usually 2–5 GΩ) were established by the usual suction method (Hamill et al. 1981). The signals were digitized by the use of a Labmaster TL-1 interface (Axon Instruments Inc.). Data analyses were performed with an IBM/AT 16-bit microcomputer with pCLAMP software. Current traces were simultaneously displayed and photographed from an oscilloscope monitor (Hewlett Packard).

Drugs

All the drugs used were products of SIGMA Chemical Co. (St. Louis, MO), except bovine serum albumin (fraction V, SERVA, cat. No. 11922).

Results

General description

In cold "KB" solution (8 °C) most cells were elongated and fully relaxed. In 2.5 mmol/l external Ca^{2+} – $[Ca^{2+}]_o$ – (bath solution, Table 1) and at 33 °C they tended to contract.

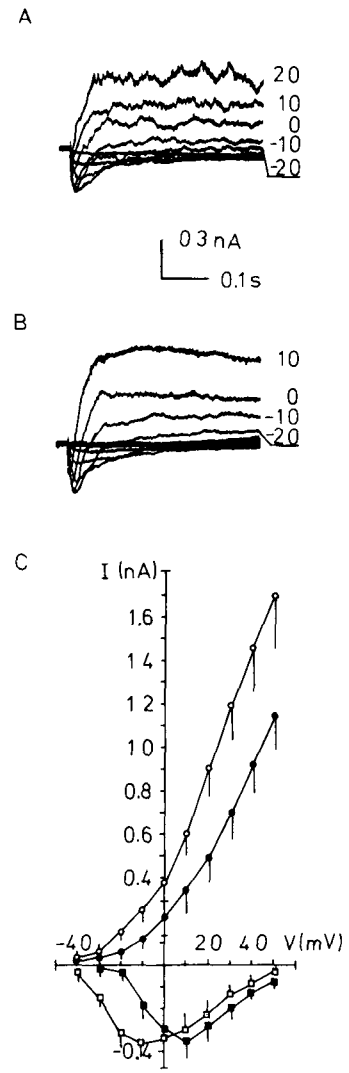

Figure 1. Characteristic responses of a cell held at $V_h = -50$ mV (A) and at $V_h = -80$ mV (B). Depolarization steps were applied to the potentials marked with digits. Cell with a capacitance of 71 pF and an impedance of 1.6 GΩ. (C) I/V curves of peak inward (squares) and outward (circles) currents, elicited by depolarization steps of 600 ms duration from $V_h = -50$ mV (closed symbols) and $V_h = -80$ mV (open symbols). The curves were constructed from net currents measured from the same current waveform, obtained during every test pulse regardless to inward and outward current overlaps. Data are means for 9 cells. S.E.M. half-bars are presented for the clarity of the drawing.

Under these experimental conditions a value of -49 ± 5.6 mV ($n = 67$) was found for the resting potential.

Total membrane currents

Figure 1A, B shows the characteristic whole-cell current responses of a cell, isolated from the circular layer of the guinea-pig ileum, bathed in Ca^{2+}-containing solution. In voltage-clamp mode depolarizing steps produced the usual waveforms of an initial inward current, followed by a more sustained outward current with a considerable overlap. The current-voltage (I/V) curves for the whole-cell inward

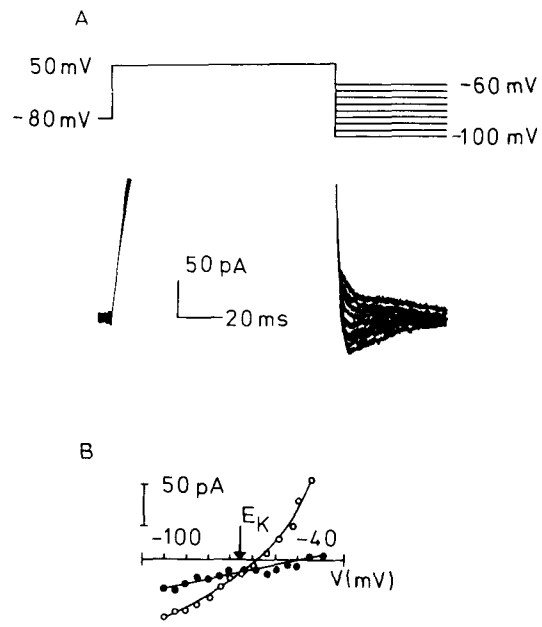

Figure 2. Tail current analysis of net I_K expressed in cells from the circular layer under $V_h = -80$ mV in the presence of 10^{-6} mol/l nicardipine and 0.3 mmol/l $NiCl_2$ to block I_{Ca}. Test pulse to +50 mV for 100 ms activated an outward current which deactivated upon returning to various after-pulse potentials between −100 mV to −60 mV in 5 mV increments (*A*). Tail current size with the leak current and the leak current, measured 1 s after the end of the test pulse, were plotted against after-pulse potential for the same experiment. Points were normalized with respect to the current at the end of the test pulse and zero holding current occurred at the resting membrane potential of the cell. The straight line was fitted and the intersection point gave the reversal potential (*B*).

and outward currents are shown in Figure 1*C*. The activation time constants of the net outward current, elicited by depolarization steps from holding potential $V_h = -80$ mV in the presence of 10^{-6} mol/l nicardipine and 0.3 mmol/l $NiCl_2$ to block I_{Ca}, were 20.3 ± 6 ms at −10 mV, 16.6 ± 2 ms at 0 mV, 17.5 ± 2 ms at +10 mV, 18.9 ± 3 ms at +20 mV, 29.5 ± 3 ms at +30 mV and 22.2 ± 3 ms at +40 mV ($n = 15$ cells).

The tail current analyses of the net outward current were performed in the presence of Ca^{2+}-channel blocking drugs (10^{-6} mol/l nicardipine and 0.3 mmol/l $NiCl_2$) to study the nature of ions which carry the outward currents (Fig. 2*A*). The value of the reversal potential (V_{rev}) of the net outward current was found to be -76 ± 3 mV (Fig. 2*B*), which is close to the value of V_{rev} for K^+ ions, estimated according to the Nernst equation (−78.4 mV).

Ca^{2+}- and apamin-blockable component

To eliminate the contamination of I_{Ca} in the net current waveforms, nicardipine (10^{-6} mol/l) was used to block L-type calcium channels, and 0.3 mmol/l $NiCl_2$ was added to the bath solution to block T-type calcium channels under V_h = −80 mV (see Duridanova et al. 1993b). Preliminary studies have shown that $NiCl_2$ (up to 3 mmol/l under isoosmotic conditions) was without effect on K^+ current characteristics (V_h = −50 mV, data not shown; see also Huizinga 1991). In cells clamped at V_h = −80 mV the effect of I_{Ca} blockade on amplitudes and time course of the net I_K was investigated. In control solution the whole cell I_K amplitudes remained practically unchanged (2–4% decrease of maximum and late, measured at 4000 ms, I_K amplitudes recorded at 45 min as compared to the amplitudes, measured at 7 min after obtaining the whole cell configuration). The same percentages of decrease in I_K amplitudes were found at 45 min of the experiments in the presence of 10^{-6} mmol/l nicardipine and/or 0.3 mmol/l $NiCl_2$ in the bath.

The effect of calcium entry blockers was measured at 5 min after the drug applications into the bath, and further measurements were made every 5 min up to 40 min from the beginning of the experiment. At 5 min, the amplitudes of depolarization-evoked I_K measured at 350 ms from the pulse onset decreased by 17 to 22% if compared to the control ones (Fig. 3*A*, open squares), and no further decrease could be observed up to 40 min. During this time the peak current amplitudes remained practically unchanged.

Apamin (10^{-6} mol/l) was added to the bath as a probe of the presence of Ca^{2+}-activated K^+ channels with small conductance (SK^+). It inhibited 18 ± 2% of the net I_K at 0 mV and 21 ± 1.6% at +40 mV membrane potential (if V_h = −80 mV, Fig. 3*A*), or 15 to 29% of it (if V_h = −50 mV, not shown), measured at 350 ms from the pulse onset. The amplitudes and the time course of apamin-blockable I_K, as well as those of the other I_K components described below, were determined by subtracting the current, elicited by depolarization pulses in the presence of K^+ channel blocking drug, from the waveforms of I_K measured before the drug application. In this way, the apamin-blockable I_K was found to have a peak amplitude of 345 ± 57 pA at +40 mV membrane potential (Fig. 3*A*, closed circles; see also Fig. 3*C*) which was reached between 250 and 350 ms from the beginning of the pulse (n = 14 cells); see Fig. 3*B*. The I/V curves of the apamin-blockable I_K component, expressed in 18 cells clamped at V_h = −80 mV or V_h = −50 mV, are presented in Figs. 3*C* and 3*D*. This I_K component had an activation threshold of about −40 mV membrane potential (−43 ± 3 mV; n = 10).

In the presence of nicardipine ($10^{-6} - 5 \cdot 10^{-5}$ mol/l), used to block I_{Ca} (under V_h = −50 mV), apamin ($10^{-6} - 10^{-5}$ mol/l) failed to affect I_K (Fig. 3*D*). On the other hand, in cells clamped at V_h = −80 mV, nicardipine ($10^{-6} - 5 \cdot 10^{-5}$ mol/l) did

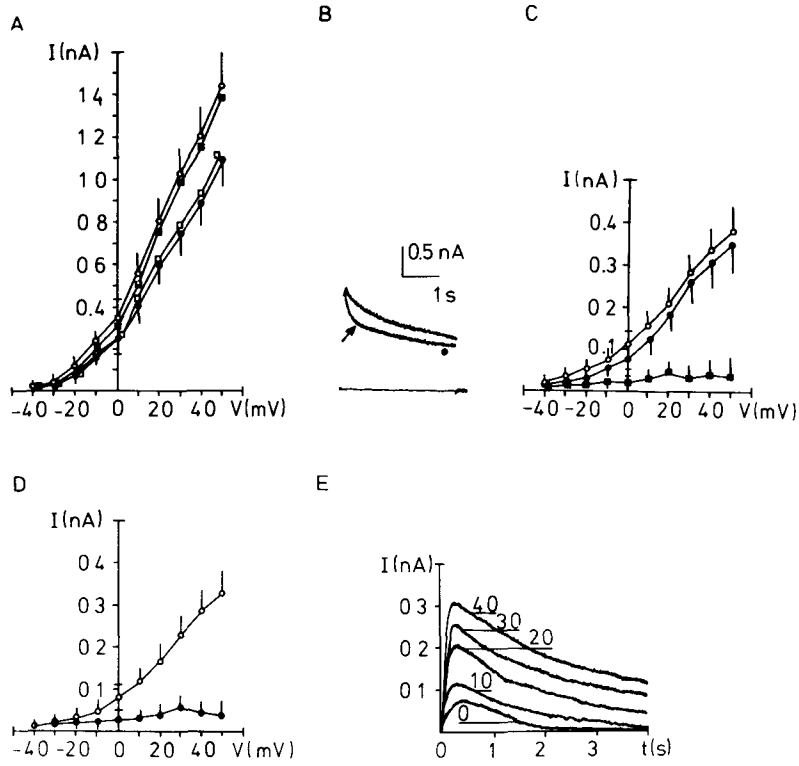

Figure 3. (*A*) I/V characteristic of I_K, inhibited by Ca^{2+} blocking drugs: 10^{-6} mol/l nicardipine (closed squares), its combination with 0.3 mmol/l $NiCl_2$ (open squares) or 10^{-6} mol/l apamin (closed circles), as compared to the control values (open circles). Currents were measured at 8 min after the drug application to the bath solution, at 350 ms from the onset of rectangular pulses of 4000 ms duration, applied from $V_h = -80$ mV. Data are means ± S.E.M. for total of 14 cells. (*B*) Effect of 10^{-6} mol/l apamin (the trace marked with the circle) on the net I_K waveform, elicited by depolarization pulse to +40 mV from $V_h = -80$ mV, with a duration of 4 s. The moment at which current amplitudes were measured is marked with the arrow. Cell with a capacitance of 73 pF and an impedance of 1.4 GΩ. (*C*) I/V-characteristics of the apamin-blockable I_K (open circles) in cells clamped at $V_h = -80$ mV, and effects of 10^{-6} mol/l nicardipine (closed circles) or its combination with 0.3 mmol/l $NiCl_2$ (squares) on this component, measured at 8 min after their application to the bath. Data are means ± S.E.M. for 7 cells. (*D*) I/V characteristics of the apamin-blockable I_K (open circles) and its absence at 8 min after the addition of 10^{-6} mol/l nicardipine into the bath. $V_h = -50$ mV. Data are means ± S.E.M. for 5 cells. (*E*) Current traces of apamin-blockable I_K, obtained by subtracting the current measured at 8 min after the drug application to the bath from the net I_K waveforms. The voltages applied are indicated. $V_h = -50$ mV. Cell with a capacitance of 68 pF and an impedance of 1.3 GΩ.

not change the depolarization-evoked I_K amplitudes or time course, and apamin was still effective (see Fig. 3*C*). If both nicardipine (10^{-6} mol/l) and $NiCl_2$ (0.3 mmol/l) have been added to the bath, the amplitudes of I_K decreased within 3 min by 17 to 22% depending on the voltage applied (Fig. 3*A*, open squares), and apamin even at $5 \cdot 10^{-5}$ mol/l could not express its K^+ channel blocking properties (Fig. 3*C*, squares). From Fig. 3*A* it can be seen that the I/V curve for the apamin-blockable I_K showed a close resemblance to that of the I_{Ca}-sensitive component.

The inactivation time courses of the apamin-blockable I_K (Fig. 3*E*) and of I_K, which disappeared after I_{Ca} blockade, could be best fitted by single exponentials with very similar inactivation time constants: 2218 ± 235 ms at 0 mV, 2153 ± 103 ms at +10 mV, 1766 ± 99 ms at +20 mV, 1889 ± 131 ms at +30 mV and 1559 ± 120 ms at +40 mV for the apamin-blockable component; and 2102 ± 118 ms at 0 mV, 2001 ± 143 ms at +10 mV, 1865 ± 101 ms at +20 mV, 1793 ± 92 ms at +30 mV and 1491 ± 152 ms at +40 mV for (nicardipine + $NiCl_2$)-blockable component ($V_h = -80$ mV; $n = 15$). These constants did not change significantly upon changing the holding potential between −80 and −50 mV (not shown).

It was suggested that the activation of apamin-blockable SK^+ requires voltage-gated calcium entry.

Glibenclamide-blockable component

The cells isolated from the circular layer of the guinea-pig ileum expressed a markable glibenclamide-blockable component of I_K although there was 1.5 mmol/l ATP in the pipette solution. Glibenclamide was used as a specific probe for the presence of ATP-blockable K^+ channels and the amplitudes of this component have been estimated by subtraction, and measured after I_{Ca} blockade. This component was found to peak at about 120 ms of the test stimulus (Fig. 4*B*). Glibenclamide (10^{-5} mol/l) inhibited 31 ± 2.6% of whole cell I_K at 0 mV membrane potential (from $V_h = -50$ mV; Fig. 4*A*, closed squares), and the amplitudes of the glibenclamide-blockable component did not depend on V_h (not shown). Cromakalim ($5 \cdot 10^{-7}$ mol/l), usually described as an activator of ATP-sensitive K^+ channels (for review see Davis et al. 1991; Takano and Noma 1993), increased the whole cell I_K by about 45% at 0 mV, and lowered the net I_K amplitudes by 20% at +40 mV membrane potential (Fig. 4*A*, closed circles). Glibenclamide (10^{-5} mol/l) impeded the effect of cromakalim and reduced I_K amplitudes to the same extent as when applied alone (Fig. 4*A*, open squares). These findings were taken as an evidence that there is a population of ATP-sensitive K^+ channels in cells investigated, a part of which remained conductible even in the presence of milimolar concentrations of ATP.

The glibenclamide-blockable component had an activation threshold of about −45 mV (-44 ± 4 mV; $n = 9$), which was independent of V_h. The inactivation time course of this current (see Fig. 4*B*) was well fitted by a single exponential with time constants 975 ± 53 ms at 0 mV, 820 ± 43 ms at +10 mV, 760 ± 25 ms

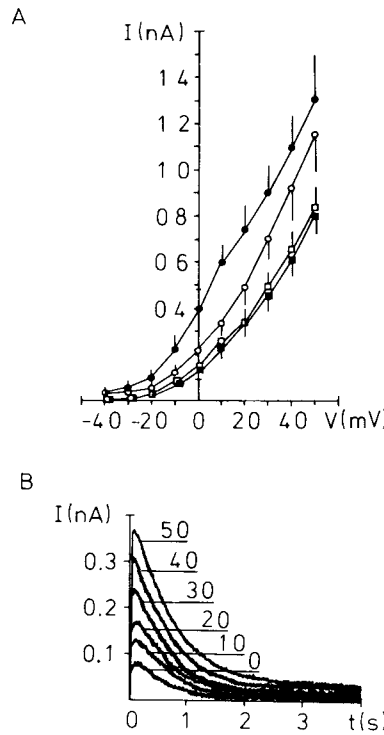

Figure 4. (*A*) I/V-relationship of the peak I_K at 7 min after the addition of 10^{-5} mol/l glibenclamide (closed squares) or $5 \cdot 10^{-7}$ mol/l cromakalim (closed circles) into the bath solution as compared to its amplitudes in the absence of any drugs (open circles). Open squares represent the blocking ability of glibenclamide (10^{-5} mol/l) on I_K, activated by $5 \cdot 10^{-7}$ mol/l cromakalim. Data are means ± S.E.M. for 6 cells. $V_h = -50$ mV. (*B*) Glibenclamide-blockable component of I_K, obtained by subtracting from the whole-cell current waveforms. $V_h = -80$ mV. Cell with a capacitance of 72 pF and an impedance of 1.4 GΩ. Currents were measured in the presence of 10^{-6} mol/l nicardipine (*A*) or 10^{-6} mol/l nicardipine and 0.3 mmol/l $NiCl_2$ (*B*).

at +20 mV, 722 ± 39 ms at +30 mV and 655 ± 50 ms at +40 mV ($n = 8$ cells; $V_h = -80$ mV).

Charybdotoxin-blockable component

The I/V-curves of I_K, obtained from $V_h = -80$ mV showed a considerable increase in the net I_K amplitudes if compared to the current which could be elicited from $V_h = -50$ mV (Fig. 1*B*). The difference between I_K amplitudes measured at the given membrane potential from $V_h = -80$ mV and $V_h = -50$ mV was defined as potential-dependent I_K, or K_{pd} (see Fig. 5*B*). As can be seen from Fig. 5*A* this current, named $[I_{K(-80)} - I_{K(-50)}]$, coincided with the charybdotoxin-blockable part of I_K, activated from $V_h = -80$ mV. The toxin expressed maximum effectiveness at 10^{-6} mol/l. Further increase in toxin concentration did not intensify the blockade. On the other hand, I_K activated by depolarization steps from $V_h = -50$ mV had lower amplitudes as compared to those measured at the given membrane potential from $V_h = -80$ mV (see Fig. 1), and the blocking activity of charybdotoxin on this I_K was weak (it inhibited 9 to 15% of the net I_K) even at concentration of 10^{-5} mol/l (not shown). Thus, K_{pd} coincided with the charybdotoxin-sensitive component of I_K, as well as with the active component of the residual current, i.e.

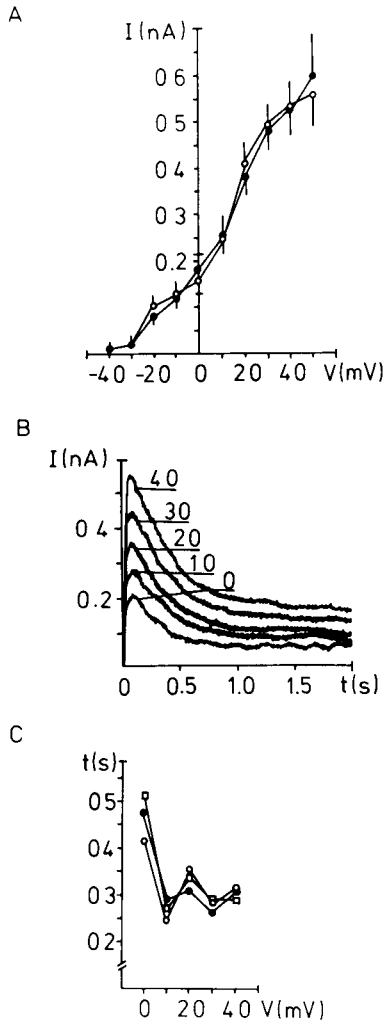

Figure 5. (*A*) I/V curves of the peak K_{pd} defined as the difference $[I_{K(-80)} - I_{K(-50)}]$ (open circles), compared to that of the peak charybdotoxin-blockable I_K, elicited from V_h = −80 mV in the presence of 10^{-6} mol/l charybdotoxin (closed circles). Depolarizing pulses with a duration of 4 s were applied in 10 mV increments. Data are means ± S.E.M. for 6 cells. (*B*) Current traces of K_{pd}, obtained by subtracting the net I_K, elicited by depolarization from V_h = −50 mV from that activated from V_h = −80 mV to the potentials indicated. Cell with a capacitance of 71 pF and an impedance of 1.4 GΩ. (*C*) Voltage dependence of inactivation time constants of K_{pd} (open circles), charybdotoxin-blockable I_K (closed circles), and (apamin + glibenclamide)-insensitive I_K (squares). V_h = −80 mV. Data are means for 5 cells. S.E.M. bars have been omitted for the sake of clarity of the drawing. All experiments were performed in the presence of 10^{-6} mol/l nicardipine and 0.3 mmol/l $NiCl_2$ in bath solution.

the depolarization-evoked I_K component, which remained after the blockade of both apamin- and glibenclamide-sensitive K^+ channel populations. The inactivation time constants of (apamin + glibenclamide)-insensitive and $[I_{K(-80)} - I_{K(-50)}]$ – components of I_K were undiscernible (Fig. 5*C*). Their inactivation time courses were best fitted with single exponentials with following inactivation time-constants: 420 ± 65 ms at 0 mV, 234 ± 38 s at +10 mV, 343 ± 74 ms at +20 mV, 282 ± 38 ms at +30 mV, 305 ± 81 ms at +40 mV, for $[I_{K(-80)} - I_{K(-50)}]$; and 482 ± 84 ms at 0 mV, 288 ± 62 ms at +10 mV, 304 ± 66 ms at +20 mV, 258 ± 43 ms at +30 mV, 299 ± 56 ms at +40 mV, for charybdotoxin-blockable I_K (n = 14 cells). The

inactivation time course of the (apamin + glibenclamide)–insensitive component is shown in the same Figure for comparison. The corresponding inactivation time constants were: 518 ± 39 ms at 0 mV, 270 ± 51 ms at +10 mV, 341 ± 31 ms at +20 mV, 287 ± 66 ms at +30 mV, and 280 ± 42 ms at +40 mV ($n = 6$).

The voltage dependences, inactivation time constants and pharmacological sensitivity characteristics of K^+ channels populations, expressed in cells from the circular layer of guinea-pig ileum are summarized in Table 2.

Table 2. Summary of characteristics of K^+ channel populations

Channel type	Blocked by	Time constant inactivation at +40 mV (ms) from $V_h = -80$ mV	Voltage threshold of activation (mV)
Ca^{2+}-sensitive K^+ channels with small conductance	apamin 10^{-6} mol/l	1491 ± 152 (15)	-43 ± 3 (10)
Ca^{2+}-sensitive K^+ channels with large conductance	charybdotoxin, 10^{-6} mol/l	299 ± 56 (14)	-70 ± 2 (13)
ATP-blockable	glibenclamide 10^{-5} mol/l	655 ± 50 (8)	-44 ± 4 (9)

Discussion

The sensitivity of SK^+, apamin-blockable channels, toward Ca^{2+}, which enters the cells during depolarization, has been previously described in a number of cells derived from different tissues (McManus 1991; Leinders and Vijverberg 1992; Sah 1992; Artalejo et al. 1993). We have also shown that the apamin-blockable I_K component, measured in cells from the longitudinal layer of the same organ, expressed marked sensitivity toward I_{Ca} as well (Gagov et al. 1993a). Here we show that even Ca-entry through T-type calcium channels (about 150 pA current at −30 mV per cell; see Duridanova et al. 1993b; Gagov et al. 1993b) is still sufficient to maintain the activity of K^+ channels with small conductance. This suggests that SK^+ may participate in keeping the resting membrane potential counteracting the membrane depolarization. Due to its slow activation and inactivation kinetics the apamin-blockable I_K component may act as a terminator of the duration of plateau-action potentials: a mechanism, which has already been proposed (Suzuki et al. 1993; Kitamura et al. 1993).

In a previous paper (Gagov et al. 1993a) we reported the existence of three pharmacologically distinguishable components of I_K in cells, isolated from the longitudinal layer of the same area of guinea-pig intestines. It is interesting to note that in cells from both layers a measurable glibenclamide-blockable component of I_K could be detected, with a voltage threshold of activation of about −45 mV. The inactivation kinetics of this component in longitudinal layer cells was faster than that of the apamin- or charybdotoxin-blockable ones (Duridanova et al. 1993a; Gagov et al. 1993a). In cells derived from the circular layer the glibenclamide-sensitive component of I_K inactivated more slowly.

K_{pd} seems to be the fastest inactivating component in these cells, and it may be speculated that it plays a role similar to that of the glibenclamide-blockable component in cells from the longitudinal layer. The observed voltage dependence of K_{pd} suggests its participation in impeding the depolarization due to the Ca^{2+} influx through T-type Ca^{2+}-channels (Duridanova et al. 1993b). The presence of charybdotoxin-blockable low voltage-activated K^+ channel populations in cells investigated may explain the absence of spontaneous electrical activity of ileal circular smooth muscle preparations (Kuriyama et al. 1966; Bywater and Taylor 1982; Hara et al. 1985). Thus, K_{pd} could effectively counteract depolarization caused by Ca^{2+} entry through T-type Ca^{2+} channels, the more so as both these currents appear simultaneously, at membrane potentials close to the resting one (Hara et al. 1985).

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