Two Components of Potassium Outward Current in Smooth Muscle Cells from the Circular Layer of Human Jejunum

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Abstract. Two components of the outward K^+ currents (I_K) of cells isolated from the circular layer of human jejunum were investigated using the conventional whole-cell voltage clamp method A fast transient I_K component could only be elicited by depolarization in cells dialysed with pipette solution of pCa < 7 4 This I_K component was strongly voltage dependent, and could be selectively abolished by 30 μmol/l quinidine Its amplitudes decreased in the absence of Ca entry, the decrease depending on the duration of cell exposure to media containing calcium-blockers, and disappeared after depletion of intracellular Ca^{2+} stores The steady-state component of I_K was sensitive to tetraethylammonium This component had comparable amplitudes at pCa = 8 4 or pCa = 7 4 of the pipette solution, and was present during a long lasting exposure of cells to solutions containing Ca^{2+} blocking drugs

Key words: Human jejunum — Potassium channels — Smooth muscle — Intracellular calcium

Introduction

Potassium currents of intestinal smooth muscle cells have been extensively investigated in laboratory animals by the whole-cell voltage clamp technique (Hu et al 1989, Gagov et al 1993, Duridanova and Boev 1995) However, there have been no studies on whole-cell currents from gastrointestinal smooth muscle in humans Recently, by using perforated patch-clamp technique, Farrugia et al (1993, 1995) studied human circular jejunum cells and described the presence of voltage sensitive

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K^+ and Ca^{2+} currents More specifically, these authors observed a non-inactivating I_K, a nicardipine-sensitive Ca^{2+} current, and a small Cl^- current (Farrugia et al 1995) It is to be noted that all currents revealed by the perforated patch clamp had slow kinetics In contrast, our pilot investigations of human circular jejunum performed by means of conventional whole-cell voltage clamp demonstrated that, in addition to the non-activating I_K, a fast transient component of I_K was also present Its kinetics was too fast to be detected by a perforated patch clamp study Furthermore, a fast whole-cell I_K component has been similarly recorded from intestinal smooth muscle cells in animals (Duridanova et al 1993) Hence, a precise description of the I_K currents in the human circular jejunum requires the whole-cell mode

The aim of the present work was to complement the results obtained by Farrugia and co-workers and to study precisely the role of Ca^{2+} ions in the regulation of K^+ conductivity in jejunal smooth muscle cells of humans under conventional whole-cell voltage clamp The Ca^{2+}-sensitivity of whole-cell K^+ current components with slow and fast kinetics was investigated

Materials and Methods

Human jejunal tissue was obtained as surgical waste tissue after official approval The cells were isolated as previously described for ventricular myocytes (Isenberg and Klockner 1982) Briefly, a circular layer was removed from sheets of intact muscle Tissue samples were cut into small pieces (2 mm × 2 mm) and placed in prewarmed solution for cell isolation (see below), containing 2 mg/ml collagenase type 1 (Sigma), 2 mg/ml trypsin inhibitor, and 2 mg/ml bovine serum albumin After about 50 min of incubation in this solution bubbled with O_2 at 37°C, pieces were carefully washed out by the enzyme and placed in a solution for cell storage (see below), where they were agitated mechanically until the solution became cloudy Single cells thus isolated were stored in this solution at 6°C for several hours All experiments were performed at 30 32°C

Temperated experimental solution (PSS), consisting of (mmol/l) 126 NaCl, 8 KCl, 1 2 $MgCl_2$, 20 glucose 20 taurine, 10 N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES), 5 Na-pyruvate, and 2 5 $CaCl_2$ (pH 7 4) was continuously perfused into the chamber at a rate of 0 7 ml min^{-1} and the drugs used were diluted in it The solution for cell isolation contained (in mmol/l) 58 NaCl, 68 $NaNO_3$, 5 KCl, 1 2 $MgCl_2$, 20 glucose, 20 taurine, 10 HEPES, 5 pyruvic acid (pH 7 3) The solution for cell storage consisted of (mmol/l) 108 NaCl, 7 KH_2PO_4, 5 KCl, 1 6 $MgCl_2$, 5 glucose, 20 HEPES 2 5 Na-pyruvate, 1 7 creatine, 2 oxalacetic acid, 1 5 Na_2ATP, 1 EGTA, and 1 mg/ml albumin (pH 7 25) The solutions used for intracellular dialysis consisted of (mmol/l) 105 (solution I) or 121 (solution II) KCl, 10 HEPES, 2 $MgCl_2$, 4 pyruvic acid, 5 succinic acid, 5 oxalacetic acid,

3 adenosine triphosphate sodium salt 11 (solution I) or 4 (solution II) EGTA, 1 (solution I) or 0 3 (solution II) $CaCl_2$, pH was adjusted to 7 2 with KOH giving a final K^+ concentration of 145 mmol/l

pCa of solution I was 8 4 (which is below the value of intracellular Ca^{2+} concentration – $[Ca^{2+}]_i$ – in the resting state) and pCa of solution II was 7 4 (which is close to the $[Ca^{2+}]_i$ at the time when spike depolarization starts – Vogalis et al 1992) The concentrations of Ca^{2+} and EGTA needed to obtain the desired final concentration of free Ca^{2+} in the pipette solutions were estimated according to the solution preparation guide of Schubert (1996) All substances used were obtained from Sigma (St Louis, MO, USA) except bovine serum albumin (fraction V Serva Heidelberg, Germany) and cyclopiazonic acid (RBI)

The whole-cell mode of the patch clamp technique was employed (Hamill et al 1981) The data were collected using a List Electronic amplifier via TL 1 DMA interface (Axon Instruments), and stored in a computer On-line registrations of ionic currents were performed by Square Wave cell Tester (Shkodrov 1995)

Results and Discussion

Data were obtained from 78 cells isolated from the circular layer of human jejunum of 23 surgical specimens The resting membrane potential measured in the current clamp mode of the whole cell configuration was -44 ± 6 mV ($n = 64$) Under holding potential (V_h) of -60 mV, all cells responded to depolarizing stimuli with uniform currents These currents consisted of an initial nicardipine-sensitive inward current overlapped by the outward currents at potentials positive to -20 mV (Fig 1*A*)

Outward currents were studied under a blockade of the inward Ca^{2+} current with 3 μmol/l nicardipine In most experiments, 50 μmol/l niflumic acid was also added to the bath to block chloride channels as they may be present in this tissue (Farrugia et al 1995) Under these conditions outward currents were carried exclusively by K^+, as verified by the differences in reversal potential of the tail currents at three different concentrations of K^+ added to the bath (not shown)

Whole-cell outward currents (I_K) were recorded from cells dialysed with low free Ca^{2+} containing solution (solution I with $[Ca^{2+}]_i = 5$ nmol/l) or with high free Ca^{2+}-containing solution (solution II with $[Ca^{2+}]_i = 40$ nmol/l)

In cells dialysed with pipette solution I I_K appeared after a delay of 10 15 ms, and developed slowly with times to peak between 50 and 100 ms I_K development and timing showed no voltage dependence (Fig 1*B*) In cells dialysed with high free Ca^{2+}-containing solution II, the same non-activating current was evoked, but in this case it was preceded and superimposed by a fast transient component (Fig 1*C*)

The non-activating steady state I_K, named $I_{K\,(slow)}$, had similar densities, voltage dependence, and kinetics in all cells dialysed with either solution I or solution II (Fig 1*D*) $I_{K\,(slow)}$ rectified outwardly and did not inactivate even at the end of

4s current pulses between −40 and +50 mV (Fig 1*B* and Fig 2*A*) It ran down very slowly, i e 1 h after the whole-cell configuration was obtained, its amplitudes reduction did not exceed 10% These results show that the net I_K in human jejunal smooth muscle cells recorded under our experimental conditions is similar to outward currents recorded by Farrugia et al (1993) in perforated patch configuration

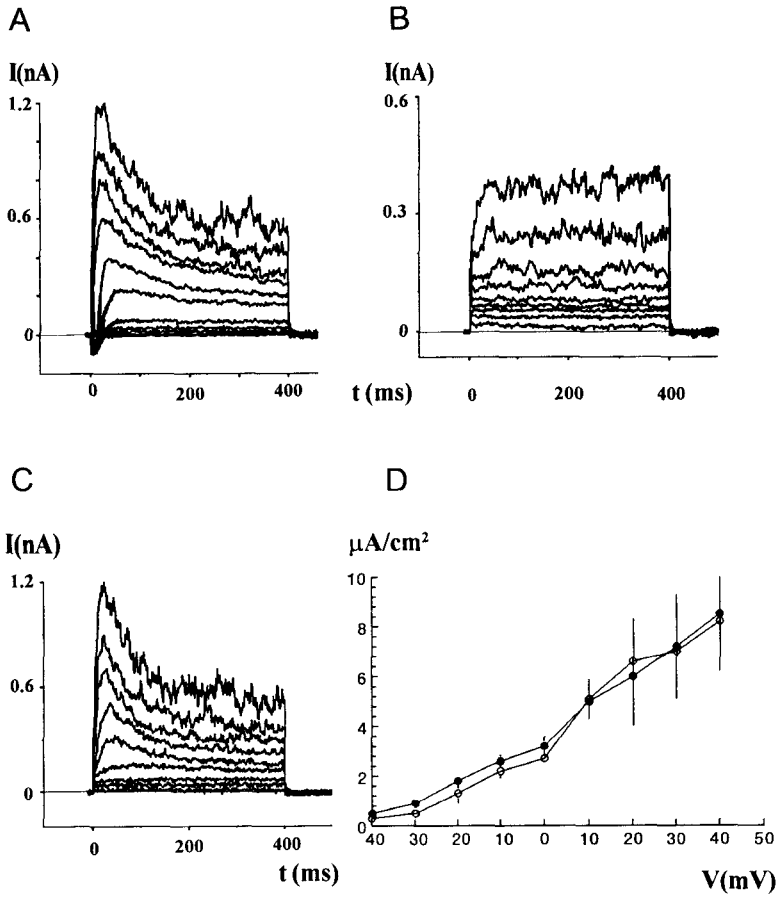

Figure 1. Typical outward current waveforms elicited by rectangular depolarization pulses of 400 ms duration from V_h = −60 mV in a jejunal smooth muscle cell in control experiments in the absence of nicardipine dialysed with high free Ca^{2+}-containing pipette solution (*A*) (*B*) and (*C*) represent the current waveforms evoked by the same protocol in cells dialysed with low free Ca^{2+}-containing (*B*) or high free Ca^{2+}-containing (*C*) pipette solution after the blockade of Ca^{2+} entry with 3 μmol/l nicardipine added right before obtaining the giga-seal The cells had a capacitance of 65 pF (*D*) Voltage dependence of the current densities of the late (measured at 400th ms after the pulse onset) I_K current, expressed in cells dialysed with solution I (open circles n = 8) or solution II (closed circles n = 9) V_h= −60 mV Data are means ± S E M

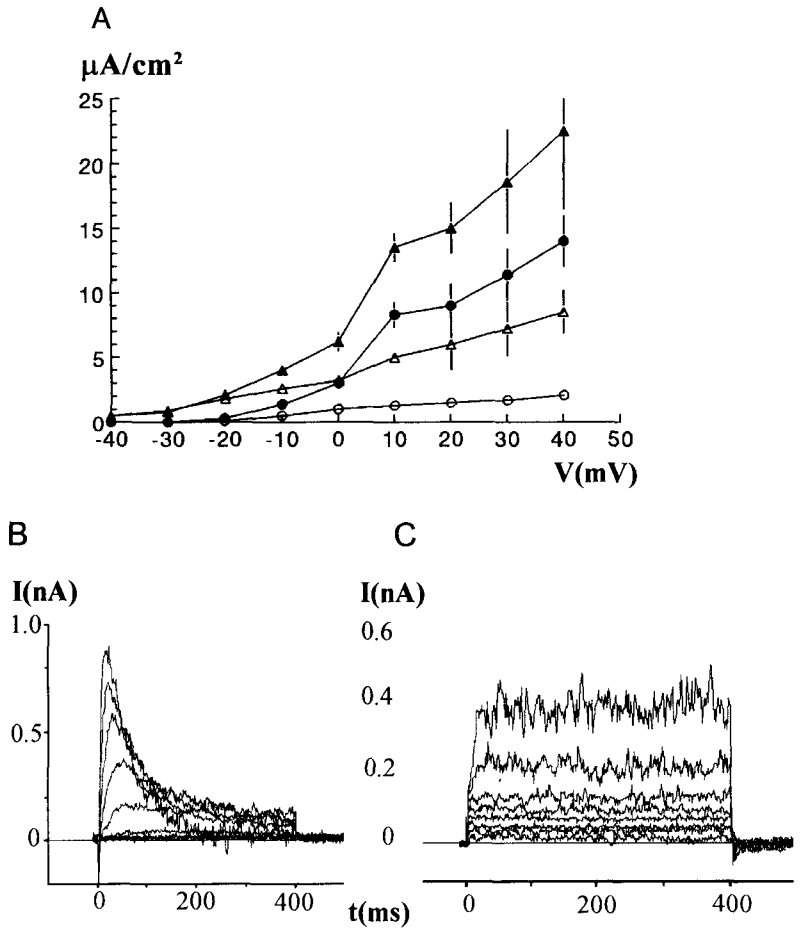

Figure 2. Voltage dependence of the current densities of the peak (closed triangles, $n = 6$) and late (measured 600 s after the pulse onset – open triangles, $n = 8$) I_K, expressed in cells, dialyzed by solution II and bathed in PSS. Closed circles ($n = 6$): I/V relationship for the net I_K after the blockade of the late component with 5 mmol/l TEA – a typical I_K waveform, expressed in cells in the presence of TEA is shown in (*B*). Addition of 30 μmol/l quinidine to the TEA-containing bath resulted in a total blockade of I_K, leaving only the leak conductance (open circles, $n = 7$). A typical I_K waveform obtained after the blockade of $I_{K(fast)}$ with quinidine is shown in (*C*). $V_h = -60$ mV. Data in (*A*) are means ± S.E.M

The transient I_K component – $I_{K(fast)}$ – was only activated in cells dialysed with high free Ca^{2+}-containing solution. $I_{K(fast)}$ formed the peak of the whole-cell I_K, and inactivated within 200 ms. Figure 2*A* shows the voltage dependence of

the densities of the peak I_K and $I_{K(slow)}$. Figure 2A, B illustrates that addition of 5 mmol/l tetraethylammonium (TEA) to the bath abolished $I_{K(slow)}$ while 30 μmol/l quinidine selectively suppressed $I_{K(fast)}$ (Fig. 2C). In the presence of both drugs, a leakage current was detected only (Fig. 2A). It is thus demonstrated that the two kinetically different components of I_K could be blocked selectively by TEA and quinidine. Apamin (1 to 10 μmol/l), charybdotoxin (0.1 to 10 μmol/l), and 4-aminopyridine (0.1–3 mmol/l) had no effect on the net I_K (not shown).

In order to specify the sources of Ca^{2+} needed for the activation of $I_{K(fast)}$ and $I_{K(slow)}$ we studied the changes in the amplitudes of both I_K components and the time course of $I_{K(fast)}$ under Ca^{2+}-entry blockade. Figure 3A presents the dependence of $I_{K(fast)}$ and $I_{K(slow)}$ amplitudes on the duration of Ca^{2+} entry blockade. In the first series of experiments the Ca^{2+} entry was interrupted after the cells were bathed in PSS for 20 min. In the second series of experiments Ca^{2+} entry was prevented throughout the experiment. In both series the amplitudes of the net I_K decreased after Ca^{2+} entry blockage as compared to the controls (Fig. 3). The decrease in amplitude was more pronounced at negative membrane potentials which suggested that both components of I_K were activated by Ca^{2+} in the physiologically relevant range of depolarizations. Moreover, in the absence of Ca^{2+} entry throughout the study the amplitudes of $I_{K(fast)}$ also decreased significantly at positive membrane voltages (as can be deduced from Fig. 3A) which was accompanied by an increase of times to peak (Fig. 3B) and acceleration of $I_{K(fast)}$ inactivation (Fig. 3C). At the same time the amplitudes and the time course of $I_{K(slow)}$ remained practically unchanged.

These findings suggest that with all other conditions being the same, $I_{K(slow)}$ is mainly regulated by voltage sensitive Ca^{2+} entry while $I_{K(fast)}$ is maintained by a gradual leak of Ca^{2+} from intracellular Ca^{2+} stores. This suggestion is further supported by the fact that intracellularly applied ruthenium red (a conventional blocker of Ca^{2+}-induced Ca^{2+} release channels – Ma 1993) or the depletion of intracellular Ca^{2+} stores with 20 min lasting incubation of cells with 1 μmol/l cyclopiazonic acid (inhibitor of Ca^{2+} ATPase of the sarcoplasmic reticulum – Raeymaekers and Wuytack 1993) totally abolished $I_{K(fast)}$ (Fig. 4A) and did not affect the amplitudes and time course of $I_{K(slow)}$ (Fig. 4B). Thus at intracellular Ca^{2+} concentrations close to the level of the resting state (i.e., right before the initial depolarization phase of the spike potential) human jejunal cells are able to generate both $I_{K(fast)}$ and $I_{K(slow)}$.

The present data demonstrate that human jejunal smooth muscle cells respond to depolarizing stimuli with two major Ca^{2+}-sensitive components of I_K: a non-inactivating TEA-sensitive current activated by the Ca^{2+} entry and a fast transient quinidine-sensitive component activated by the release of Ca^{2+} from intracellular Ca^{2+} stores.

$I_{K(slow)}$ behaves like most of the non-inactivating I_K components described so

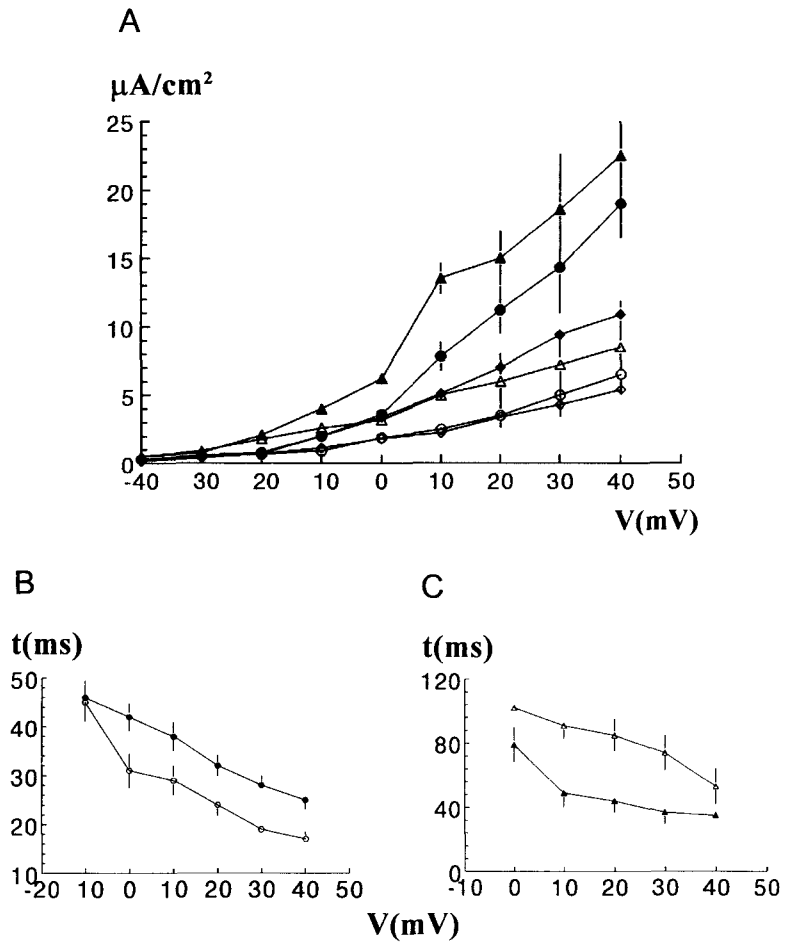

Figure 3 (*A*) Voltage dependence of the current densities of the peak (closed symbols) and late (measured 600 s after the pulse onset, open symbols) I_K expressed in cells dialyzed by solution II and bathed in PSS (triangles, $n = 13$) in PSS to which 1 μmol/l nicardipine was added after 20 min bathing (circles, $n = 12$) or in nicardipine containing PSS throughout the study (squares, $n = 14$). $V_h = -60$ mV. Data are means ± S.E.M. (*B*, *C*) Voltage dependencies of the times to peak (*B*) and inactivation time constants (C) of $I_{K(fast)}$ as obtained from cells bathed in PSS (open symbols, $n = 16$) or in nicardipine-containing PSS throughout the study (closed symbols, $n = 12$). Data are means ± S.E.M.

far in smooth muscle cells of different origin (Ohya et al. 1987; Noack et al. 1992). Given that these types of currents are activated by Ca^{2+} ions which enter the cell during depolarization, their participation in the repolarizing phase of the spikes is widely recognized (Ohya et al. 1987; Thornbury et al. 1992a).

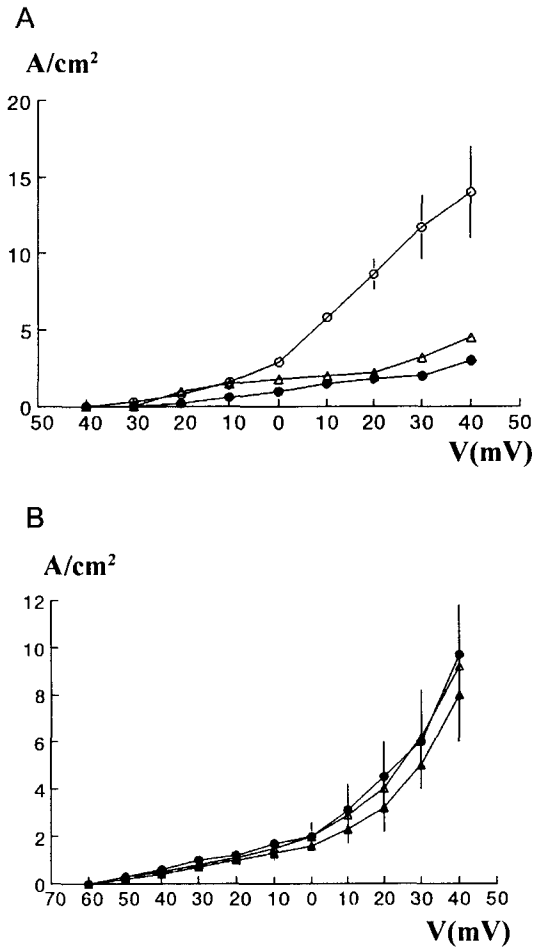

Figure 4 Voltage dependence of the current densities of $I_{K(fast)}$ (*A*) and $I_{K(slow)}$ (*B*) measured in cells dialysed with high free Ca^{2+} containing pipette solution in control experiments (open circles $n = 12$) and after the blockade of intracellular Ca^{2+} activated Ca^{2+} release with 1 μmol/l ruthenium red (closed circles $n = 9$) or 20 min after bath application of 1 μmol/l cyclopiazonic acid (triangles $n = 10$). PSS contained 3 μmol/l nicardipine and 50 μmol/l niflumic acid added after giga seal formation.

According to its kinetics and voltage dependence $I_{K(fast)}$ resembles the fast transient I_K components described in various gastrointestinal smooth muscles (Ohya et al. 1987, Thornbury et al. 1992b, Duridanova et al. 1993). However according to its unique sensitivity to quinidine and requirements for intracellular Ca^{2+} release, this component differs from all other transient I_K components

known to exist in smooth muscle cells Moreover, $I_{K(fast)}$ reported here reached its peak amplitudes 10 ms after the stimulus onset and could effectively counteract the depolarization induced Ca^{2+} entry This observation explains the lack of spontaneous spike activity in the circular layer of the jejunum (Hara et al 1986)

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