

Ca²⁺-activated K⁺ Currents of Pancreatic Duct Cells in Guinea-pig

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There are numerous studies on transepithelial transports in duct cells including Cl⁻ and/or HCO₃⁻. However, studies on transepithelial K⁺ transport of normal duct cells in exocrine glands are scarce. In the present study, we examined the characteristics of K⁺ currents in single duct cells isolated from guinea pig pancreas, using a whole-cell patch clamp technique. Both Cl⁻ and K⁺ conductance were found with KCl rich pipette solutions. When the bath solution was changed to low Cl⁻, reversal potentials shifted to the negative side, -75±4 mV, suggesting that this current is dominantly selective to K⁺. We then characterized this outward rectifying K⁺ current and examined its Ca²⁺ dependency. The K⁺ currents were activated by intracellular Ca²⁺. 100 nM or 500 nM Ca²⁺ in pipette significantly (P<0.05) increased outward currents (currents were normalized, 76.8±7.9 pA, n=4 or 107.9±35.5 pA, n=6) at +100 mV membrane potential, compared to those with 0 nM Ca²⁺ in pipette (27.8±3.7 pA, n=6). We next examined whether this K⁺ current, recorded with 100 nM Ca²⁺ in pipette, was inhibited by various inhibitors, including Ba²⁺, TEA and iberiotoxin. The currents were inhibited by 40.4±% (n=3), 87.0±% (n=5) and 82.5±% (n=9) by 1 mM Ba²⁺, 5 mM TEA and 100 nM iberiotoxin, respectively. Particularly, an almost complete inhibition of the current by 100 nM iberiotoxin further confirmed that this current was activated by intracellular Ca²⁺. The K⁺ current may play a role in secretory process, since recycling of K⁺ is critical for the initiation and sustaining of Cl⁻ or HCO₃⁻ secretion in these cells.

Key Words: Duct cells, Guinea pig, Pancreas, K⁺ currents, Ca²⁺

INTRODUCTION

Ducts play a critical role in whole secretory process as well as acini. First, the ducts provide a frame work for acinar cells, and they also function as a transport pathway for digestive enzyme and fluid secreted from acinar cells. In addition the ducts play a role of ion exchanges and water secretion. For example, in salivary glands, Na⁺, Cl⁻ and HCO₃⁻ are reabsorbed into the duct cells, while K⁺ is secreted into the lumen from the duct cells (Dinudom et al, 1995; Komwatana et al, 1996; Cook et al, 2002).

In pancreas, digestive enzymes are secreted from acini and bicarbonate-rich isotonic fluid is supplied from the duct cells. This fluid has several functions. The most important function is the flushing of digestive enzymes secreted by acini toward gut and neutralization of acidic chime which enters the duodenum from the stomach. In cystic fibrosis (CF), one of the fatal hereditary diseases among Caucasians, pancreatic ductal secretions of bicarbonate and fluid are reduced, leading to an increase of the concentration and precipitation of enzymes within duct lumen, followed by duct blockage and destruction of gland (Becq et al, 1993; Winpenny et al, 1995a). Therefore, defects in

ductal function may underlie the pathology that occurs in cystic fibrosis, pancreatitis and perhaps hypofunction of salivary glands.

During the last decade, there has been many studies on transepithelial Cl⁻ transport, including various ionic channels in duct cells; for example, the cystic fibrosis transmembrane conductance regulator (CFTR), Ca²⁺ activated Cl⁻ channel, and volume activated Cl⁻ channel (Gray et al, 1990a; Becq et al, 1992; Smith et al, 1995; Verdon et al, 1995; Nguyen et al, 1997; Winpenny et al, 1998). CFTR is a chloride channel regulated by cAMP and acts in parallel with chloride-bicarbonate exchangers to facilitate bicarbonate secretion across the apical plasma membrane of the duct cells (Gray et al, 1993; Winpenny et al, 1995). In CF (cystic fibrosis) cells, reduced bicarbonate secretion results from defective cAMP-activated Cl⁻ channels, and these defects are partially compensated with an increased sensitivity of CF cells to purinergic stimulation and alternative activation of Ca²⁺-activated Cl⁻ channels (Zsembery et al, 2000). Angiotensin II increases intracellular Ca²⁺ concentration and may elicit activation of Ca²⁺-mediated chloride channels (Fink et al, 2002).

However, all the Cl⁻ transporters in the duct cell membrane can work only when the intracellular electrical

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ABBREVIATIONS: BSA, bovine serum albumin; DMEM, Dulbecco's modified eagle medium; SBTI, soy bean trypsin inhibitor; FCS, fetal calf serum; TEA, tetra ethyl ammonium.

negativity is maintained by recycling of K^+ . Therefore, the transepithelial K^+ transport is important for the ductal Cl^- or bicarbonate secretion. Although K^+ currents in CFPAC-1 cells (Galletta et al, 1994) and in pancreatic ductal epithelial cell (PDEC) in dog (Nguyen et al, 1998) were reported, the K^+ current has not rigorously been characterized. In the present experiment, we studied the characteristics of the K^+ currents from single duct cells, using a whole-cell patch clamp technique.

METHODS

Isolation and culture of pancreatic ducts

Minced pancreatic tissues were treated by collagenase, and were then incubated for 30 min at 37°C in enzyme solution containing collagenase, hyaluronidase and BSA. After mechanical separation by pipetting, tissues were incubated again for another 30 min in new enzyme solution. After wash-out of tissues with normal DMEM, the tissues were then kept in refrigerator for 1 hr in storage solution containing SBTI and BSA. Small intra or inter lobular pancreatic ducts were microdissected from the digested tissue fragments under the stereomicroscope and maintained in culture for 24 hrs. Isolated ducts were cultured overnight on the cyclopore in culture medium containing FCS, glutamine, dexamethasone, and velosuline. After overnight culture, the cultured ducts were incubated in 50 U/ml elastase for 1 hr. Finally, the ducts were teased apart in calcium and magnesium free Ringer solution to obtain single cells. The anatomic diagram of pancreatic acini and duct is shown in Fig. 1.

Patch clamp recording

Cells in the experimental chamber (volume=400 μ l) on the inverted microscope were superfused with a bath solution at a rate of 2 ml/min. For the patch clamp experiment, pipettes were manufactured from haematocrit capillaries (Oxford Labware, St Louis, MO, USA), and the pipette solution contained: 145 mM KCl, 2 mM $MgCl_2$, 1.16 mM $CaCl_2$, 1.34 mM EGTA, 5 mM HEPES, pH was adjusted 7.3 with KOH, and a tip resistance of 3–5 M Ω . Whole cell currents were monitored, using an Axopatch 200

amplifier (Axon Instruments, CA, USA). Command potentials were generated by pClamp software (Version 6; Axon Instruments), and the resultant currents were recorded on a DAT recorder (DTR1204, Bio-logic, France) or computer hard disk. The experiments were performed at room temperature (22–25°C). Iberitoxin was purchased from Alomon (Israel).

RESULTS

We obtained single duct cells by enzyme digestion of the isolated ducts which had been cultured for 24 hrs after microdissection. This maneuver excluded the possibility that our recordings were contaminated with acinar cells. The average capacitance of the duct cells was 7 ± 0.2 pF (Mean \pm SEM, n=23), which was much smaller than those

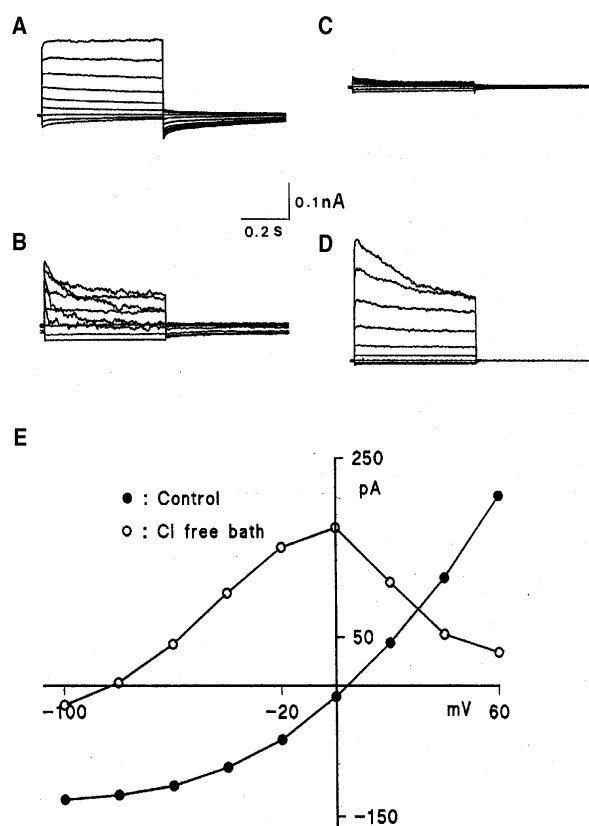


Fig. 2. Identification of K^+ currents in single pancreatic duct cells. Command potentials from -100 mV to +60 mV with 500 ms duration were applied by 20 mV step potentials. (A) A current profile recorded in a normal bath solution with KCl rich pipette. The Ca^{2+} concentration in the pipette was 500 nm. An outward rectifying current was observed in the resting state. (B) By changing bath solution to low Cl^- (9 mM), in which NaCl was replaced with L-aspartic acid (Na Salt), the currents generated by positive command potentials more than 0 mV were decreased and inactivated time-dependently. (C) The residual currents in low Cl^- bath solution were almost completely inhibited by 5 mM TEA, which is known as a K^+ channel blocker. (D) The current was recovered after washout with normal bath solution. (E) A current-voltage (I-V) relationship of the current in normal and Cl^- free (low Cl^-) bath solution.

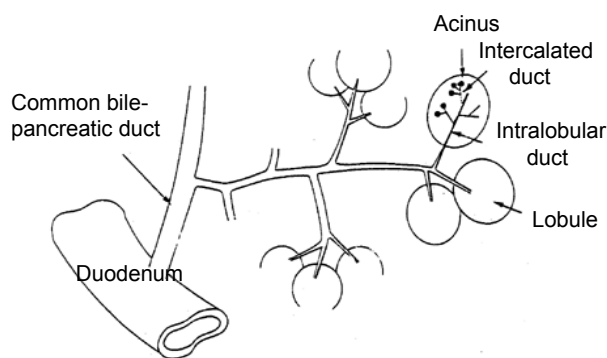


Fig. 1. A schematic diagram for the anatomy of acini and ducts in guinea pig pancreas. Single pancreatic duct cells were obtained from the intralobular or interlobular ducts in our experiments.

of acinar cells (10 ± 0.2 pF, $n=18$).

Fig. 2 A shows a current profile recorded in a normal bath solution with KCl rich pipette. The Ca^{2+} concentration in the pipette was 500 nM. An outward rectifying current was observed in resting state. When we changed bath solution to low Cl⁻ (9 mM) (Fig. 2B), in which NaCl was replaced with L-aspartic acid (Na Salt), the currents generated by positive command potentials more than 0 mV were decreased and inactivated time-dependently. The remnant currents in low Cl⁻ bath solution were almost completely inhibited by 5 mM TEA (Fig. 2C), which is known as a K⁺ channel blocker. The current inhibited by TEA was recovered after washout with normal bath solution (Fig. 2D). We found that the outward rectifying current was also decreased by addition of 1 mM Ba^{2+} to the bath (data not shown). Fig. 2E shows current-voltage (I-V) relationship of this current in normal and low Cl⁻ bath solution. The negative shift of reversal potential (E_{rev}) from 16 ± 2 mV ($n=7$) to -75 ± 4 mV ($n=4$) in low Cl⁻ bath solution suggests that the current is dominantly K⁺

selective, since the value is very close to E_{K} (-80 mV).

We then characterized this outward rectifying K⁺ current. First, we examined whether this current was activated by intracellular Ca^{2+} . To record pure K⁺ current, the currents were recorded in low Cl⁻ (29 mM) bath solution with low Cl⁻ (22 mM) pipette solution at three different pipette Ca^{2+} concentrations. Fig. 3 shows each current profile recorded with 0 (A), 100 nM (B) and 500 nM (C) Ca^{2+} in pipette. As shown in Fig. 3D, the I-V relationships in the above 100 nM and 500 nM Ca^{2+} concentrations in pipette indicated a significant ($P < 0.05$) increase of the outward current to 76.8 ± 7.9 pA ($n=4$) and 107.9 ± 35.5 pA ($n=6$), respectively. The maximum current with 0 nM Ca^{2+} in pipette was only 27.8 ± 3.7 pA ($n=6$). All currents are normalized and compared at the +100 mV membrane potential.

We next examined the effects of some blockers, including iberiotoxin, Ba^{2+} and TEA, on this current with 100 nM Ca^{2+} pipette solution. Fig. 4A shows a whole-cell recording with 100 nM Ca^{2+} in pipette as a control. When 100 nM iberiotoxin was added into the bath solution, the current was almost completely inhibited (Fig. 4B). The result of these experiments is summarized in Fig. 4C. The maximum currents, recorded at +100 mV command potential, were inhibited by $40.4 \pm 4\%$ ($n=3$), $87.0 \pm 3.5\%$ ($n=5$) and $82.5 \pm 3\%$ ($n=9$) by 1 mM Ba^{2+} , 5 mM TEA and 100 nM iberiotoxin, respectively.

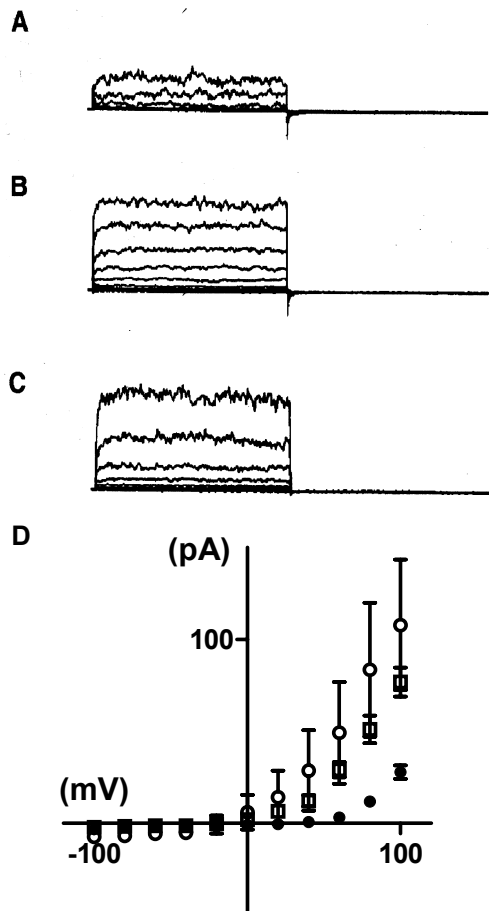


Fig. 3. Current profiles recorded with 0 (A), 100 (B) and 500 nM (C) Ca^{2+} in pipette. Command potentials from -100 mV to +100 mV with 500 ms duration were applied by 20 mV step potentials. (D) The I-V relationships of (A), (B) and (C). 100 nM (square) or 500 nM (open circle) Ca^{2+} in pipette significantly ($P < 0.05$) increased outward current (76.8 ± 7.9 pA, $n=4$ or 107.9 ± 35.5 pA, $n=6$) at +100 mV command potential, compared to those in 0 nM (dark circle) Ca^{2+} (27.8 ± 3.7 pA, $n=6$).

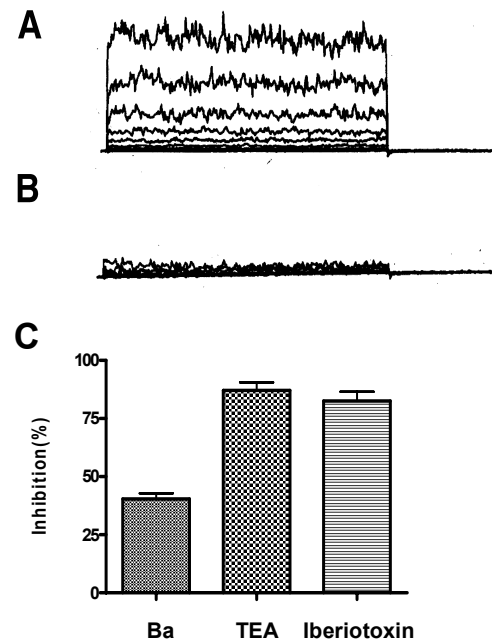


Fig. 4. The inhibitory effect of iberiotoxin on this current. Command potentials from -100 mV to +60 mV with 500 ms duration were applied by 20 mV step potentials. (A) control (B) An inhibition of K⁺ current by 100 nM iberiotoxin. (C) A summarized result. The maximum currents recorded with 100 nM Ca^{2+} pipette solution were inhibited by $40.4 \pm 2.4\%$ ($n=3$), $87.0 \pm 3.5\%$ ($n=5$) and $82.5 \pm 4.0\%$ ($n=9$) with 1 mM Ba^{2+} , 5 mM TEA and 100 nM iberiotoxin, respectively.

DISCUSSION

Studies on stimulus-secretion coupling in exocrine acini at the cellular level were started early of 1980s. In contrast, however, studies on secretory mechanism in ducts were relatively delayed. The delayed study on the duct cell physiology may have been due to technical difficulty to isolate pure intra or interlobular ducts from gland tissues. Recently, the advanced experimental techniques, microdissection of ducts, made it possible to isolate pure ducts from the gland tissues under the stereomicroscope. The main ionic channels involved in modification of the primary fluid in ducts are Cl^- and K^+ channels. Not only Cl^- channel but K^+ channel also has been suggested to play a role in ductal secretion. K^+ efflux through the K^+ channels evoked by secretagogues results in intracellular electrical negativity, which is necessary for the initiation and sustained secretion of Cl^- or HCO_3^- (Galiotta et al, 1994; Cotton, 1998).

In our experiments, the current profile of K^+ in whole cell recording was outward rectifying. It was activated by 100 nM and 500 nM Ca^{2+} in pipette solution and inhibited by Ba^{2+} and TEA, both of which are well known K^+ channel blockers. Furthermore, the inhibition of the currents by 100 nM iberiotoxin, which is a specific blocker for Ca^{2+} -activated K^+ channel, strongly suggests that this current is dependent on intracellular Ca^{2+} . Based on our results, the current which we recorded here appears to be a Maxi- K^+ channel, although the conductance size of this channel was not confirmed by single channel patch clamp. Maxi- K^+ channel is Ca^{2+} -sensitive and voltage-dependent. The opening probability (p_o) of this channel in the resting state is very low, but markedly increased by secretin, cAMP and forskolin (Gray et al, 1990). In our study, the current was activated by high concentration of Ca^{2+} (more than 100 nM) and activated only at the depolarizing pulse more than +60 mV command potential, indicating that the channel is hardly open at the resting state.

According to the presently described results, the K^+ channel in the pancreatic duct cell is regulated by intracellular Ca^{2+} . The physiological role of this K^+ current appears to be involved in secretagogue-induced transepithelial Cl^- transport, since recycling of K^+ ions is prerequisite for the transepithelial Cl^- transport. Further researches on the regulation of this channel by various secretagogues, not in resting state, might be necessary to understand the underlying mechanism of secretory modulation in duct cells.

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