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Whole-Cell K^+ Current Activation in Response to Voltages and Carbachol in Gastric Parietal Cells Isolated from Guinea Pig

Tohru Kotera, Akira Hashimoto, Shunji Ueda, and Yasunobu Okada
Departments of Physiology and Internal Medicine, Kyoto University, Faculty of Medicine, Kyoto 606, Japan

Summary. Patch-clamp studies of whole-cell ionic currents were carried out in parietal cells obtained by collagenase digestion of the gastric fundus of the guinea pig stomach. Applications of positive command pulses induced outward currents. The conductance became progressively augmented with increasing command voltages, exhibiting an outwardly rectifying current-voltage relation. The current displayed a slow time course for activation. In contrast, inward currents were activated upon hyperpolarizing voltage applications at more negative potentials than the equilibrium potential to K^+ (E_K). The inward currents showed time-dependent inactivation and an inwardly rectifying current-voltage relation. Tail currents elicited by voltage steps which had activated either outward or inward currents reversed at near E_K, indicating that both time-dependent and voltage-gated currents were due to K^+ conductances. Both outward and inward K^+ currents were suppressed by extracellular application of Ba^{2+}, but little affected by quinine. Tetraethylammonium inhibited the outward current without impairing the inward current, whereas Cs^+ blocked the inward current but not the outward current. The conductance of inward K^+ currents, but not outward K^+ currents, became larger with increasing extracellular K^+ concentration. A Ca^{2+}-mobilizing acid secretagogue, carbachol, and a Ca^{2+} ionophore, ionomycin, brought about activation of another type of outward K^+ currents and voltage-independent cation currents. Both currents were abolished by cytosolic Ca^{2+} chelation. Quinine preferentially inhibited this K^+ current. It is concluded that resting parietal cells of the guinea pig have two distinct types of voltage-dependent K^+ channels, inward rectifier and outward rectifier, and that the cells have Ca^{2+}-activated K^+ channels which might be involved in acid secretion under stimulation by Ca^{2+}-mobilizing secretagogues.

Key Words inward-rectifier K^+ channel · outward-rectifier K^+ channel · Ca^{2+}-activated K^+ channel · carbachol · acid secretion · parietal cell

Introduction

The K^+ conductance of the basolateral membrane is known to play an essential role in fluid secretion by a variety of Cl^--secreting epithelial cells (Greger, Schlatter & Gögelein, 1986; Petersen, 1986). In parietal or oxyntic cells, acid secretion is thought to be initiated by activation of the proton pump with concomitant increases in the membrane conductance, not only to Cl^- but also to K^+ (Demarest & Loo, 1990). In fact, microelectrode studies have shown that stimulation with acid secretagogues leads to an increase in the K^+ conductance in cultured rat parietal cells (Okada & Ueda, 1984; Ueda & Okada, 1989), isolated *Necturus* oxyntic cells (Demarest & Machen, 1985) and frog oxyntic cells (Schettino & Trischitta, 1989; Debellis, Curci & Frömter, 1990).

Recent patch-clamp studies have provided direct evidence for the presence of several K^+ channel species in the basolateral membrane: Ca^{2+}-activated K^+ channels and cyclic AMP-activated K^+ channels in *Necturus* oxyntic cells (Ueda, Loo & Sachs, 1987*b*) and inward-rectifier K^+ channels in rabbit parietal cells (Sakai et al., 1989). However, it has not been investigated as to whether these K^+ channel activities actually participate in the whole-cell K^+ conductance in mammalian parietal cells before and after stimulation with acid secretagogues. Applying the whole-cell recording technique to parietal cells isolated from the guinea pig, we have identified three different types of K^+ channel conductances which can be differentiated on the basis of their voltage sensitivity, calcium dependence and pharmacology. Stimulation with an acid secretagogue, carbachol, was found to activate Ca^{2+}-dependent K^+ currents as well as nonselective cationic currents.

A preliminary account of some of these results has been given in abstract form (Kotera et al., 1989).

Materials and Methods

Cell Preparations

The stomach was removed from the male guinea pig (weighing 250–300 g) under anesthesia with ether. The animal was then killed by an overdose of the anesthetic before it recovered con-

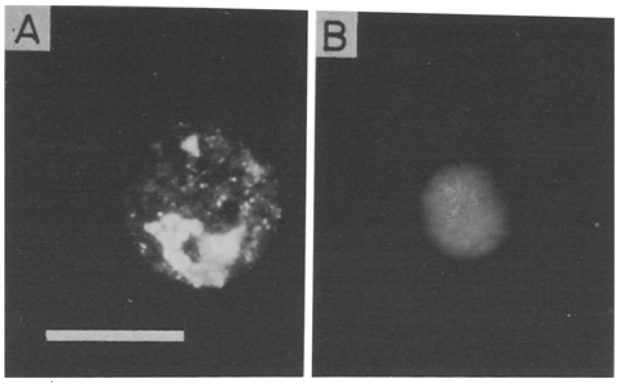

Fig. 1. Two types of gastric epithelial cells isolated from the guinea pig stomach under a fluorescence microscope in the presence of 0.1 mM Acridine orange. The larger cell type (*A*), but not the smaller one (*B*), was found to prominently take up the dye into the intracellular compartment (seen herein as more lucent canalicular structures) after 30-min stimulation with three secretagogues (0.1 mM histamine, 3 μM penta-gastrin plus 50 μM carbachol). Bar, 20 μM.

sciousness. The tissue was flooded with Ca^{2+}/Mg^{2+}-free Hanks' saline solution, which was composed (in mM) of 136.9 NaCl, 5.4 KCl, 0.3 Na_2HPO_4, 0.4 KH_2PO_4, 4.2 $NaHCO_3$, 5.6 glucose, 12.6 HEPES and 1 ethylenediaminetetraacetic acid (EDTA, Nacalai Tesque) (pH 7.4 adjusted with NaOH), supplemented with 1 mg/ml bovine serum albumin (BSA, Nacalai Tesque). The fundic mucosa was then scraped with a slide glass and minced with scissors in the above solution. After passing through a 20-gauge needle with gentle pressure, tissue fragments were centrifuged at 700 rpm for 5 min. The pellet was resuspended in M199 solution (Nissui Pharmaceutical) supplemented with 1 mg/ml BSA, 0.04–0.05 mg/ml collagenase (Yakult), 1.33 mg/ml dispase (Godoh Shusei) and 0.1 mg/ml streptomycin sulfate (Meiji Seika). The suspension was incubated for 10 min at room temperature (about 25°C) under continuous gassing with 95% O_2/5% CO_2. After centrifugation the cells were incubated in the Ca^{2+}/Mg^{2+}-free Hanks' saline solution containing BSA, which was supplemented with 0.154 mg/ml dithiothreitol (DTT: Nacalai Tesque), for 10–15 min. An additional two cycles of incubation was carried out in the BSA-containing Ca^{2+}/Mg^{2+}-free Hanks' saline solution (with no DTT added) for 5 min and then in the enzyme-containing M199 solution for 15 min at room temperature. After passing through a metal mesh (mesh size around 0.5 mm) the filtrate was centrifuged. The cell pellet was resuspended in a recovery solution, the composition (in mM) of which was 25 KCl, 10 KH_2PO_4, 3 $MgCl_2$, 70 L-glutamate, 20 taurine, 10 glucose, 0.5 EGTA, 10 HEPES and 70 KOH (pH 7.4), and stored on ice before experiments (for less than 7 hr).

Under a phase-contrast microscope the cell suspension was found to contain at least two cell populations; one with a cell diameter of 18 to 25 μm (22.6 ± 0.2 μm (100); Fig. 1*A*) and another with a diameter of 10 to 15 μm (12.3 ± 0.2 μm (50); Fig. 1*B*). By applying the Acridine orange staining method (Berglindh et al., 1980) the acid accumulating activity was monitored, as described previously (Okada & Ueda, 1984). The larger cells were found to preferentially take up the dye into the intracellular canalicular compartment after stimulation with secretagogues under a fluorescence microscope (Nikon Optiphoto) (Fig. 1*A*). Thus, the larger cells were identified as parietal cells. More than 50% of the cells in the preparation were parietal cells. For electrical experiments the isolated cells were added to a plastic dish (Corning 25010), the bottom of which was coated with concanavalin A (Sigma Chemical), and the cells with larger diameters were selected under a phase-contrast microscope (Nikon TMD).

Current Recordings

Whole-cell current recordings using tight-seal patch electrodes were carried out, as described by Hamill et al. (1981), with a patch-clamp amplifier (LIST EPC7). The patch electrodes were pulled from hematocrit capillaries (Nichiden Rika Glass) in two stages on a vertical puller (Narishige). The resistance of patch electrodes filled with pipette solutions was 5–10 MΩ. The Ag-AgCl wires for the patch electrode and for the reference electrode were exposed to the same solution. The pool for the reference electrode was electrically connected to the bath solution by a salt bridge.

To record voltage-dependent current activation, a series of command pulses (20- or 40-mV steps of 1- or 2-sec duration) was applied at 4-sec intervals from the holding potential at −50 or −30 mV. Voltage pulses were generated by a computer-aided pulse generator (Shoshin EM, Type OI-8). Capacitance (10–30 pF) and series resistance (less than 25 MΩ) were compensated electronically as best as possible. The leak current component was estimated by applying pulses of +2 and 4 mV from the holding potential at the equilibrium potential of K^+ (E_K). To record the voltage-dependence of tail currents, deactivation of outward and inward currents which had been induced by 2-sec and 100-msec voltage steps to +30 and −140 mV, respectively, was initiated by steps back to various potentials for 1 sec at regular intervals of 4 sec. To monitor current activation during stimulation with secretagogues, the cell potential was continuously clamped, alternating between 0 and −30 mV at 4- to 6-sec intervals. Step pulses (*see above*) or ramp waves (±100 mV, 3 sec) were applied from a pulse generator (Shoshin OI-8) to monitor the current-voltage relationship of ionomycin- and carbachol-induced responses.

To change the ionic composition or to apply ion channel blockers, the bath solution around the cell was superfused with a fast microperfusion system (Carbone & Lux, 1987). Errors caused by liquid junction potentials due to changes in the extracellular ionic composition were experimentally determined and corrected.

Solutions and Chemicals

To eliminate the Cl^- current contribution, all experiments were carried out using Cl^--free pipette and bathing solutions. The composition of a control bathing solution was (in mM): 4.2 K-aspartate, 137.5 Na-gluconate, 2.9 Ca-gluconate, 0.85 $MgSO_4$, 20 mannitol, 10 NaHEPES and 10 HEPES (pH 7.4). To increase the K^+ concentration (to 18 or 42 mM), Na-gluconate was replaced with K-gluconate. The control pipette solution contained (in mM): 147 K-aspartate, 0.113 $CaSO_4$, 5.4 $MgSO_4$, 3 Na_2-ATP (Sigma), 0.05 Na_2-GTP (Sigma), 10 NaHEPES and 10 HEPES (pH 7.4), as well as 0.2 mM ethylene glycol bis(β-aminoethyl-ether)-N,N,N′,N′-tetraacetic acid (EGTA: Nacalai Tesque) (*p*Ca 7). When necessary, the free Ca^{2+} concentration in the pipette solution was buffered at *p*Ca 6 with 0.2 mM EGTA or 5 mM 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid

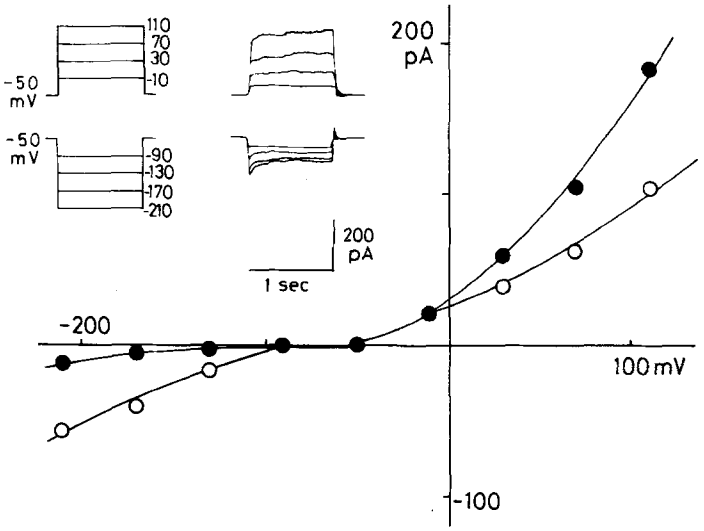

Fig. 2. The current-voltage relations of outwardly and inwardly rectifying K^+ currents recorded in an isolated guinea pig parietal cell. Control bathing and pipette solutions contained 4.2 and 147 mM K^+, respectively. Open circles represent instantaneous currents recorded at 50 msec after command pulse applications. Filled circles represent steady currents at the end of pulses. The linear leak component was subtracted. The holding potential was −50 mV. The series resistance (less than 25 MΩ) was regularly checked and compensated during the experiment. Similar data were observed in 25 experiments. *Insets:* Traces of whole-cell currents (right panel) in response to depolarizing and hyperpolarizing command pulses (pulse protocols, left panel). There is no leak correction. Same current profiles could be repeatedly observed in the same cell.

(BAPTA, Dojindo Laboratories), at *p*Ca 7 with 5 mM BAPTA and at *p*Ca > 10 with 10 mM EGTA or 5 mM BAPTA by adding appropriate amounts of $CaSO_4$. When ATP and GTP were removed from the pipette solution, the $MgSO_4$ concentration was reduced to 1.35 mM.

Carbachol (CCh, Sigma), histamine (Nacalai Tesque), dibutyryl cyclic AMP (Sigma) and ionomycin (Calbiochem) were dissolved in the 42 mM K^+ bathing solution and applied to the cell by adding an aliquot ($\frac{1}{100}$ volume) of these solutions to the bath. Other chemicals employed were quinine HCl, TEA-Cl (Nacalai Tesque) and atropine sulfate (Merck).

Data are given as means ± SEM with the number of observations in parentheses. All experiments were conducted at room temperature (23–25°C). Statistical significance was evaluated by Student's *t* test.

Results

Outwardly Rectifying Potassium Currents

In most parietal cells dialyzed with a control pipette solution (Cl^--free, K^+-rich *p*Ca 7), outward whole-cell currents were found to be activated by applications of depolarizing command potentials (of ≥ −10 mV) from a holding potential (at −50 mV) in a control bathing solution (4.2 mM K^+), as shown in Fig. 2 (*inset*, upper traces). The outward current recorded at a positive potential exhibited a slow time course for full activation (1–3 sec). The conductance progressively increased as voltage steps became more positive exhibiting outward rectification (Fig. 2). Similar outward currents were observed when intracellular ATP and GTP were removed from the control pipette solution (20 observations).

A 10-fold increase in the extracellular K^+ concentration ($[K^+]_o$) induced a shift of the outward current-voltage curve to the right within several seconds (by around 40 mV), though the slope conductance of steady outward currents at +100 mV was little affected by the $[K^+]_o$ change (3.4 ± 0.6 nS (8) at 4.2 mM K^+ and 2.8 ± 0.3 nS (6) at 42 mM K^+). These currents were absent when the cells were internally dialyzed with K^+-free Cs^+ solutions.

A tail current analysis was conducted to determine the ionic mechanism underlying outward currents by using the pulse protocol shown in Fig. 3*A* (top panel). The tail currents of outward currents at 4.2 mM $[K^+]_o$ were found to reverse at −80.7 ± 4.2 mV (3) (Fig. 3*A*, middle panel). The reversal potential shifted to −28.7 ± 1.1 mV (3), when $[K^+]_o$ increased to 42 mM (Fig. 3*A*, bottom panel). Since their reversal potentials are close to E_K, it appears that outward currents were carried by K^+ ions.

As shown in Fig. 4*A*, the voltage-gated outward K^+ current was mostly abolished by a K^+ channel blocker, Ba^{2+} (10 mM). The Ba^{2+} effect was, at least partially, reversible when Ba^{2+} was rinsed out. Another K^+ channel blocker, TEA (10 mM), irreversibly suppressed the outward K^+ current (Fig. 4*B*). In the presence of TEA, remaining outward currents exhibited time-dependent inactivation. This fact may be related to a possibility that TEA blocks channels only when they are open (Hille, 1984). Alternatively, these inactivating outward currents may represent the outward component of TEA-resistant inwardly rectifying K^+ channel currents. However, such inactivating outward currents were never observed in the presence of Ba^{2+} which did not completely block inwardly rectifying K^+ currents (*see* Fig. 5*A*). Extracellular applications of Cs^+ (4.2 mM) and quinine (0.1 mM) did not affect the outward K^+ current (seven observations, *data not shown*).

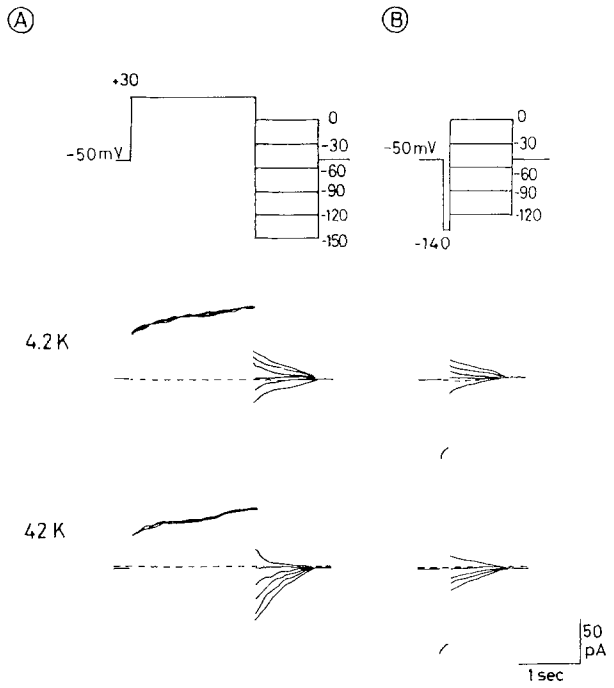

Fig. 3. Tail current analyses of the outwardly (*A*) and inwardly rectifying K^+ currents (*B*) according to the double-pulse protocol. Representative traces of current responses at 4.2 mM (middle panels) and 42 mM K^+ (bottom panels) to double pulses (top panels). Isolated parietal cells were dialyzed with a control pipette solution. Records were corrected for leak conductances. Zero-current levels are indicated by broken lines. Their reversal potentials were close to E_K (−91 and −32 mV at 4.2 and 42 mM K^+, respectively).

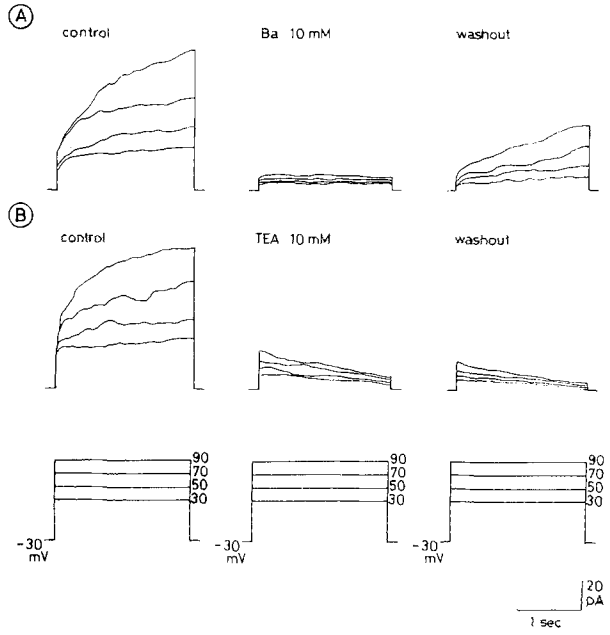

Fig. 4. Effects of K^+ channel blockers on outwardly rectifying K^+ currents. Representative traces of whole-cell current responses to depolarizing command pulses (bottom panels) before (left traces), 1–3 min after applications (middle traces) of and 1–6 min after washout (right traces) of 10 mM Ba^{2+} (*A*) and TEA (*B*). Isolated parietal cells were dialyzed with a control pipette solution and bathed in a 42 mM K^+ solution. The holding potential (−30 mV) was close to E_K. The leak component of the current was subtracted. Ba^{2+} and TEA induced significant inhibition of the current (by 88.3 ± 5.1 and 73.7 ± 3.1% (3), respectively, at +90 mV; $P < 0.05$).

To study Ca^{2+} dependency of the outward K^+ current, the cytosolic free Ca^{2+} concentration was controlled by using pipette solutions with different *p*Ca values. The outward K^+ current was virtually unaffected by cytosolic *p*Ca changes in the range between 6 and 10 (the Table).

Inwardly Rectifying Potassium Currents

As shown in Fig. 2 (*inset*, lower traces), applications of hyperpolarizing command pulses of more negative potentials than E_K (−90 mV) rapidly activated inward currents. The inward currents displayed a time-dependent inactivation kinetics. The conductance was dependent on the voltage, exhibiting inward rectification at large negative potentials (Fig. 2). Deprivation of intracellular ATP and GTP from the control pipette solution did not affect the inward current (20 observations).

Upon raising $[K^+]_o$ the inward current profile was rapidly altered. The peak current-voltage curve shifted to the right and crossed the zero-current axis at voltages approximately equal to E_K (−85, −55 and −30 mV at 4.2, 18 and 42 mM K^+, respectively, in one cell in which whole-cell recordings could be maintained stable despite successive changes of three different extracellular solutions). At 4.2, 18 and 42 mM $[K^+]_o$, the slope conductances of inward currents between −110 to −130 mV were 215 ± 34, 400 ± 50 and 596 ± 81 pS (7), respectively, being approximately proportional to the square root of $[K^+]_o$ ($\propto [K^+]_o^{0.45}$). Such $[K^+]_o$ dependency of inward rectifier K^+ conductances has been reported in a variety of cell species (Ohmori, 1978; Hagiwara & Yoshii, 1979; Gallin & Sheehy, 1985; Lindau & Fernandez, 1986; Sauvé et al., 1986; Sakai et al., 1989).

Tail current analyses conducted by a pulse protocol shown in Fig. 3*B* (top panel) clearly indicated that the inward current was also carried by K^+ ions, since the reversal potentials were −86.7 ± 2.7 and −34.0 ± 2.5 mV (3) at 4.2 and 42 mM $[K^+]_o$, respectively (Fig. 3*B*, lower two panels).

Ba^{2+} reversibly inhibited the inward K^+ current (Fig. 5*A*), whereas TEA was ineffective (three ob-

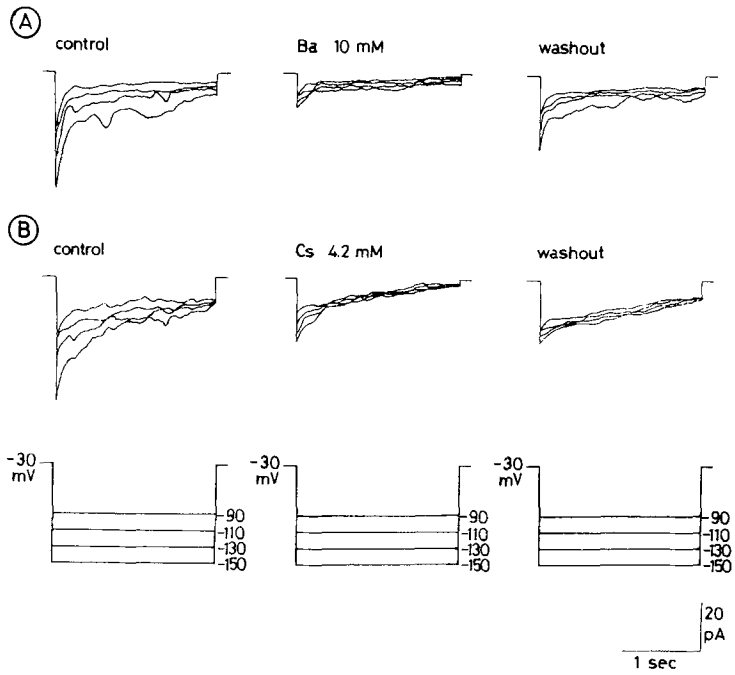

Fig. 5. Effects of K^+ channel blockers on inwardly rectifying K^+ currents. Representative traces of whole-cell current responses to hyperpolarizing command pulses (bottom panels) before (left traces), 1–3 min after applications (middle traces) of and 1–5 min after washout (right traces) of 10 mM Ba^{2+} (*A*) and 4.2 mM Cs^+ (*B*). Isolated parietal cells were dialyzed with a control pipette solution and bathed in a 42 mM K^+ solution. The holding potential (−30 mV) was close to E_K. The leak component of the current was subtracted. Ba^{2+} and Cs^+ significantly inhibited the current (by 76.0 ± 3.6 and 55.0 ± 2.0% (5), respectively, at −120 mV; $P < 0.05$).

servations, *data not shown*). This TEA insensitivity of the inwardly rectifying K^+ channel in guinea pig parietal cells is in contrast to the sensitivity of the channel in rabbit parietal cells (Sakai et al., 1989). The extracellular application of 4.2 mM Cs^+ partially blocked the inward K^+ current even in the presence of 42 mM K^+ (Fig. 5*B*). Quinine (0.1 mM) had no significant effect on the inward K^+ current (four observations, *data not shown*).

The inward K^+ current was insensitive to cytosolic *p*Ca changes in the range between 6 and 10 (the Table).

Table. Effects of intracellular *p*Ca on the outward and inward K^+ currents

*p*Ca	Outward (pA)	Inward (pA)
6	92.5 ± 2.2 (4)	−68.8 ± 5.6 (4)
7	93.8 ± 2.2 (8)	−67.8 ± 3.0 (9)
>10	96.3 ± 4.6 (4)	−69.0 ± 3.0 (5)

Peak outward and inward currents were measured at +90 and −150 mV, respectively, in isolated parietal cells of similar cell size. The holding potential was −50 mV. The leak component was subtracted. Cells were dialyzed with pipette solutions containing 0.2 or 10 mM EGTA and bathed in a 42 mM K^+ solution. The *p*Ca values were calculated by taking the purity of EGTA and the activity coefficient of H^+ into account. Differences between each experimental group were not significant. Investigation of effects of $[Ca^{2+}]_i$ over *p*Ca 6 was not possible, because the whole-cell configuration could not be stably attained. When cells were dialyzed with pipette solutions containing 5 mM BAPTA, similar results were obtained at *p*Ca 6, 7 and >10 (three observations).

Calcium-Activated Potassium Currents and Nonselective Cation Currents

When the cell potential was alternately clamped at 0 and −30 mV, CCh (0.1 mM) was found to transiently evoke activation of outward currents at 0 mV (117.5 ± 11.0 pA (4)) and of inward currents at −30 mV (−145.0 ± 19.2 pA (4)) in parietal cells bathed in a 42 mM K^+ solution, as shown in Fig. 6*A*. The inward current response started earlier than the outward current response. Atropine (1–50 μM) abolished both currents (six observations), showing that they are muscarinic in nature.

Under Cl^--free conditions, the current required to clamp the cell at −30 mV, which is close to E_K in a 42 mM K^+ solution, is a measure of nonselective cation currents mostly carried by Na^+, whereas the current at 0 mV (equals the equilibrium potential for monovalent cations, Na^+ plus K^+) is a measure of K^+ currents. Thus, it appears that CCh induced activation of K^+ currents as well as nonspecific cation currents.

Cytosolic Ca^{2+} is known to be increased by CCh in guinea pig parietal cells (Tsunoda, Yodozawa & Tashiro, 1989). Therefore, cytosolic Ca^{2+} rises may be causatively involved in CCh-induced responses. In fact, cytosolic Ca^{2+} chelation with 10 mM EGTA completely blocked the CCh response in all four experiments (Fig. 6*B*).

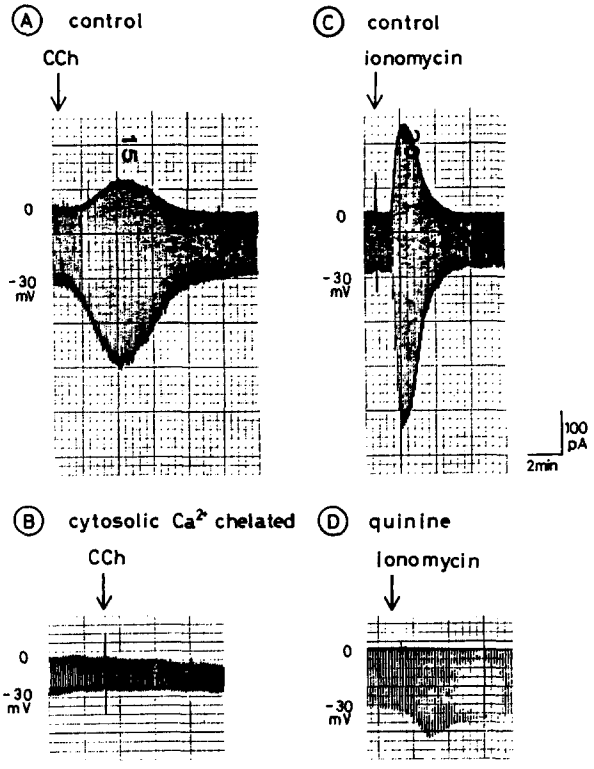

Fig. 6. Ca^{2+}-activated currents recorded upon stimulation (at arrows) with 0.1 mM CCh (*A* and *B*) and 1 μM ionomycin (*C* and *D*). Isolated parietal cells were dialyzed with a control pipette solution (pCa 7: A, C and D) or a Ca^{2+}-chelated pipette solution with 10 mM EGTA (pCa >10: *B*), bathed with a 42 mM K^+ bathing solution in the absence (*A–C*) or presence of 0.1 mM quinine (4-min treatment: *D*), and alternately voltage-clamped to 0 and −30 mV. There is no leak correction.

As shown in Fig. 6*C*, a Ca^{2+} ionophore, ionomycin (1 μM), induced similar current activation at both 0 and −30 mV (187.8 ± 22.7 and −322.5 ± 27.5 pA (4), respectively). When ATP and GTP were deprived from the pipette solution dialyzing the cytosol, both CCh- and ionomycin-induced responses became sustained (eight observations), presumably due to reduced activities of cellular Ca^{2+} storing and/or pumping mechanisms which are dependent on nucleotides. In the presence of quinine (0.1 mM), ionomycin failed to induce significant outward K^+ currents at 0 mV (Fig. 6*D*). In contrast, inward cation currents at −30 mV were still induced by ionomycin, though the amplitude reduced (to −60.0 ± 7.0 pA (3) by pretreatment with quinine for 4 to 6 min) (Fig. 6*D*). TEA (10 mM), Ba^{2+} (10 mM) and Cs^+ (4.2 mM) added to the bathing solution did not affect the ionomycin-induced responses (eight observations, *data not shown*).

At the peak response to ionomycin, the conductance was found to greatly increase. Outward currents became prominent at more positive potentials (Fig. 7*A*, filled circles). The voltage-dependent component of outward currents without and with ionomycin were 32.5 ± 1.9 pA (8) and 150.0 ± 10.6 pA (4), respectively, at 42 mM K^+ and +30 mV. Quinine (0.1 mM) reduced the voltage-dependent outward current recorded at the peak response to ionomycin to 87.5 ± 9.0 pA (4) at +30 mV even after application for a short time (1 min, $P < 0.05$). The voltage-independent linear component was also increased by ionomycin from 0.83 ± 0.12 (9) to 7.59 ± 0.68 nS (5) at 42 mM K^+ (Fig. 7*A*, broken lines). The current-voltage curves became rapidly shifted to the left by one-tenth decrease in $[K^+]_o$ (Fig. 7*A*, open circles). The zero-current potential was −14.4 ± 2.1 mV (4) at 4.2 mM K^+ and −8.8 ± 1.5 mV (5) at 42 mM K^+. The results suggest that the permeability ratio between K^+ and Na^+ (P_K/P_{Na}) is around 1.5 in ionomycin-stimulated whole-cell membranes in the zero-current steady state. A similar permeability ratio was obtained from CCh-stimulated membranes, in which the zero-current potential was −9.3 ± 1.1 mV (3) at 42 mM K^+. In the presence of quinine (0.1–0.2 mM, 5–15 min), a current-voltage relation of ionomycin-induced currents became linear (Fig. 7*B*). The zero-current potential for an ionomycin-dependent current component (the difference between currents before and after application of ionomycin) was −2.2 ± 0.9 mV (10) at 42 mM K^+. The P_K/P_{Na} value for quinine-resistant ionomycin-induced currents is around 1.0 in the steady state, indicating the nonselective nature for monovalent cations.

Discussion

The presence of Ba^{2+}-sensitive K^+ conductance in the basolateral membrane of resting mammalian parietal or amphibian oxyntic cells has been shown by vesicle studies (Muallem et al., 1985), by transepithelial electrical measurements (Rangachari, 1975; McLennan, Machen & Zeuthen, 1980; Zeiske, Machen & van Driessche, 1983) and by intracellular recordings with conventional microelectrodes (Schettino & Trischitta, 1989; Debellis et al., 1990). In the present study using the whole-cell recording technique, two types of voltage- and time-dependent K^+ channel currents, outward rectifier and inward rectifier, were observed in the guinea pig parietal cells at rest (Figs. 2 and 3). The two current species differed in their voltage dependence, kinetics, $[K^+]_o$ dependency and pharmacology (Figs. 4 and 5).

Upon stimulation with acid secretagogues, increases in the K^+ conductance were observed with

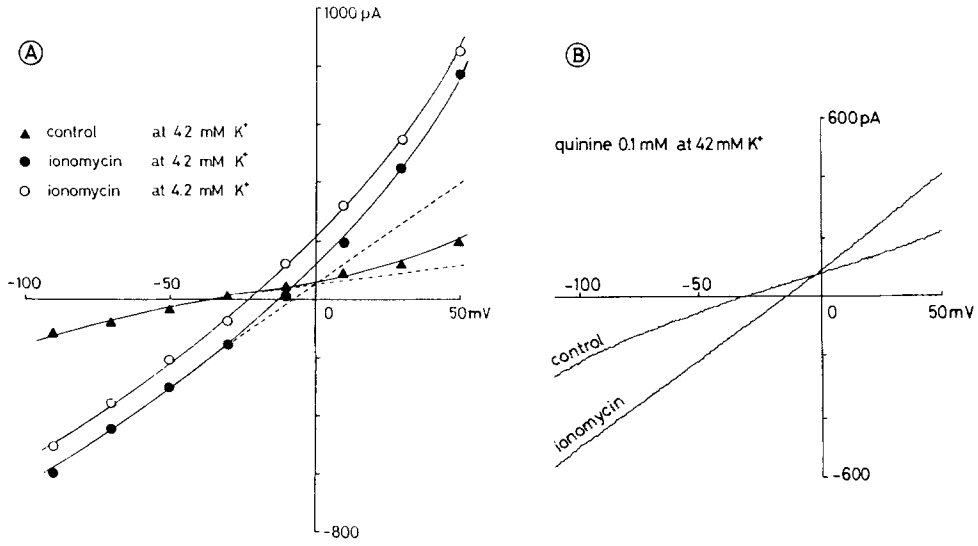

Fig. 7. Effects of ionomycin on current-voltage relations of steady whole-cell currents recorded in parietal cells in the absence (*A*) and presence of quinine (*B*). Cells were dialyzed with a *p*Ca 7 pipette solution devoid of ATP and GTP. (*A*) Current responses to step voltage pulses. Triangle represents control; circles represent 1 μM ionomycin (1–3 min). Open symbols represent 4.2 mM K^+; filled symbols represent 42 mM K^+. Broken lines represent voltage-independent linear components. (*B*) Current responses to ramp waves before and 1 min after application of 1 μM ionomycin in the presence of 0.1 mM quinine at 42 mM K^+.

conventional microelectrodes in rat parietal cells (Okada & Ueda, 1984; Ueda & Okada, 1989), in *Necturus* oxyntic cells (Demarest & Machen, 1985) and in frog oxyntic cells (Schettino & Trischitta, 1989; Debellis et al., 1990). In the present study, stimulation with an acid secretagogue, CCh, was found to induce additional whole-cell current activation (Fig. 6*A*). It is concluded that these currents were activated by a rise in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$); because (i) using fura-2 CCh has been shown to increase $[Ca^{2+}]_i$ in parietal cells of a variety of animal species (Chew & Brown, 1986; Negulescu & Machen, 1988; Black et al., 1989; Ueda & Okada, 1989), including the guinea pig (Tsunoda et al., 1989; H. Tatsuta, S. Ueda & Y. Okada, *unpublished*), (ii) a Ca^{2+} ionophore, ionomycin, induced similar current activation (Figs. 6*C* and 7*A*), and (iii) cytosolic Ca^{2+} chelation abolished CCh-induced current activation (Fig. 6*B*). The Ca^{2+}-induced currents (outward at 0 mV and inward at E_K) may be due to parallel activation of K^+ channels and nonselective cation channels. Alternatively, cytosolic Ca^{2+} increases may activate cation channels which are permeated by K^+ more preferentially than Na^+. The latter possibility, however, is unlikely, because (i) the onset of the CCh-induced inward current response was earlier than that of the outward current response (Fig. 6*A*), (ii) the ionomycin-induced outward current response recorded at 0 mV was more sensitive to a K^+ channel blocker (quinine) than the inward current response recorded at E_K (Fig. 6*D*), and (iii) ionomycin-induced currents, in the presence of quinine, showed a nearly linear current-voltage relation with P_K/P_{Na} of around 1.0 (Fig. 7*B*). The Ca^{2+}-induced outward K^+ channel is distinct from the outwardly rectifying K^+ channel, which operates at rest, because quinine blocked the former current (Fig. 6*D*) but not the latter current, and Ba^{2+} or TEA preferentially inhibited the latter (Fig. 4).

Location of the three K^+ channel species could not precisely be determined by the whole-cell recording technique. Rapid responses of these current profiles to changes in the K^+ concentration of the bath solution, to which the basolateral cell surface was more readily exposed, might suggest that the three types of K^+ channels are located on the basolateral membrane (Fig. 8). In fact, recent patch-clamp studies directly showed the presence of Ca^{2+}-activated K^+ channels in *Necturus* oxyntic cells (Ueda, Kotera & Okada, 1987*a*) and of inward-rectifier K^+ channels in rabbit parietal cells (Sakai et al., 1989) in their basolateral membranes.

Ca^{2+}-induced activation of K^+ channels residing in the basolateral membrane has been known to play a role in Cl^-- and fluid-secretion by acinar cells (Petersen, 1986; Cook & Young, 1989; Petersen & Maruyama, 1989) and airway epithelial cells (Greger et al., 1986; McCann & Welsh, 1990). Similarly, the Ca^{2+}-activated K^+ channel may participate in HCl secretion induced by Ca^{2+}-mobilizing secretagogues (Fig. 8). The K^+ channel would bring about

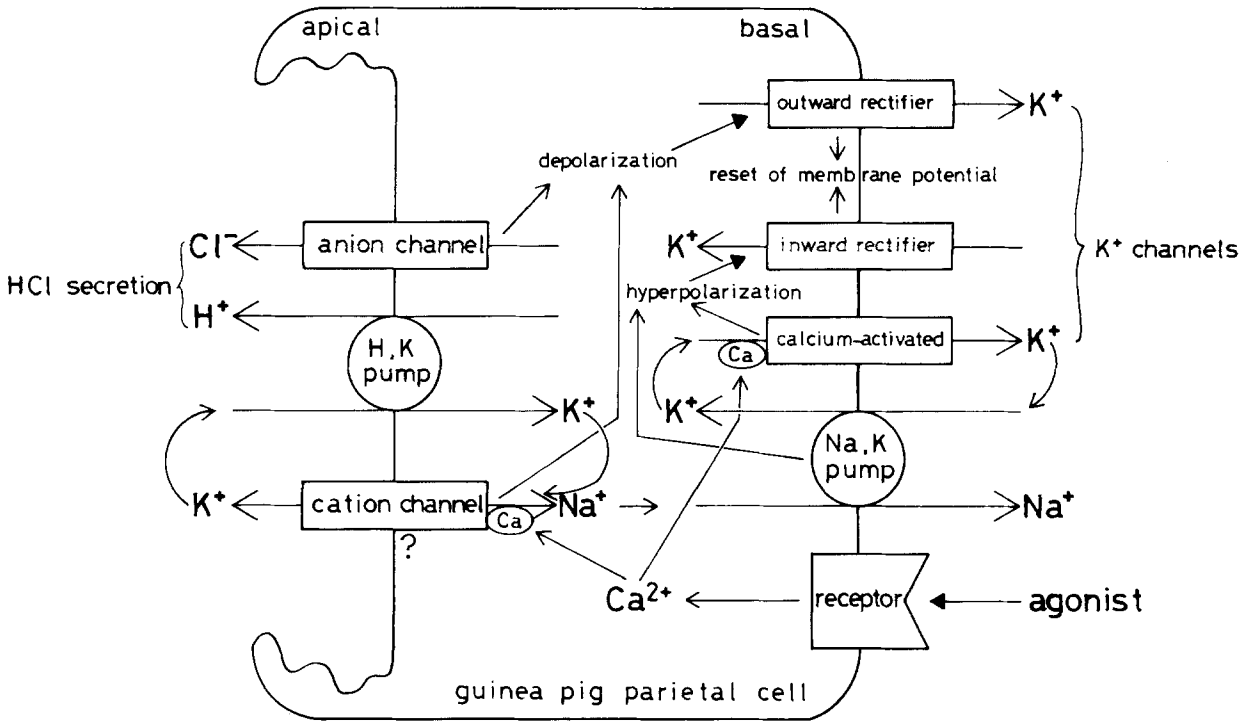

Fig. 8. A schematic model illustrating possible roles of outward-rectifier, inward-rectifier and Ca^{2+}-activated K^+ channels in HCl secretion by the guinea pig parietal cell stimulated with a Ca^{2+}-mobilizing secretagogue. Cyclic AMP-mediated mechanisms, most of which remain to be investigated, are not incorporated in this model. (*See* text for details.)

the back diffusion of K^+ ions, thereby balancing the K^+ influx via Na^+,K^+ pump which should be activated during acid secretion. The outward K^+ current may also match the inward Cl^- current through the luminal Cl^- channels (Ueda et al., 1987; Demarest, Loo & Sachs, 1989) to maintain electroneutrality during HCl secretion. Furthermore, hyperpolarizations thus induced in the whole-cell membrane should not only offset the depolarizing effect of activation of apical anion channels but also provide a driving force for Cl^- exit via the channels.

Stimulation with CCh or ionomycin induced activation of nonselective cation currents (Figs. 6 and 7). Similar Ca^{2+}-induced activation of nonselective cation channels has been observed in acinar cells by muscarinic stimulation (Maruyama & Petersen, 1982) and by a Ca^{2+} ionophore A23187 (Marty, Tan & Trautmann, 1984). A recent patch-clamp study showed that the basolateral membrane of rabbit parietal cells has nonselective cation channels (Sakai et al., 1989). However, this channel was observed in the resting state and did not show any sensitivity to cytosolic Ca^{2+}. Our preliminary study has shown that another type of single-channel currents through nonselective cation channels becomes induced by stimulation with acid secretagogues in the apical membrane of cultured rat parietal cells (Ueda, Kotera & Okada, 1991). Thus, there is a possibility that the nonselective cation channel resides in the apical membrane of guinea pig parietal cells. If it is the case, the K^+-permeable cation channel might deliver K^+ ions, which are necessary for operation of the H^+, K^+-ATPase (pump), to the lumen (Fig. 8). If not the case, unidentified K^+-selective channels might be activated at the apical membrane upon acid secretion, since membrane vesicle studies have shown that the apical membrane K^+ permeability becomes augmented after stimulation with acid secretagogues (Wolosin & Forte, 1984; Gunther, Bassilian & Rabon, 1987).

Inward- and outward-rectifier K^+ channels may be involved in stabilizing the membrane potential in parietal cells, as in the case in excitable cells (Hille, 1984). When Cl^- channels and/or nonselective cation channels are preferentially activated, resultant depolarizations, in turn, may activate outward-rectifier K^+ channels, thereby restoring the membrane potential (Fig. 8). On the other hand, activation of Ca^{2+}-dependent K^+ channels and/or electrogenic Na^+,K^+ pump should result in a negative shift of the membrane potential in acid-secreting cells. Hyperpolarizations thus induced may activate inward-rectifier K^+ channels, thereby leading to reset of the membrane potential of stimulated parietal cells

(Fig. 8). If operation of Ca^{2+}-activated K^+ channels gives rise to K^+ accumulation in a narrow region at the outside surface of the basolateral membrane, currents through inward-rectifier K^+ channels in the membrane exposed to high $[K^+]_o$ could more readily be activated. Re-uptake of the accumulated K^+ ions could be induced by the operation of inward-rectifier K^+ channels at the local membrane where the E_K value is, by any chance, more positive than the whole-cell membrane potential.

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