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Regulation of K^+ Channels in the Basolateral Membrane of *Necturus* Oxyntic Cells

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Summary. Patch-clamp methods were used to study single-channel events in isolated oxyntic cells and gastric glands from *Necturus maculosa*. Cell-attached, excised inside-out and outside-out patches from the basolateral membrane frequently contained channels which had conductances of 67 ± 21 pS in 24% of the patches and channels of smaller conductance, 33 ± 6 pS in 56% of the patches. Channels in both classes were highly selective for K^+ over Na^+ and Cl^-, and shared linear current-voltage relations. The 67-pS channel was activated by membrane depolarization, whereas the activity of the 33-pS channel was relatively voltage independent. The larger conductance channels were activated by intracellular Ca^{2+} in the range between 5 and 500 nM, but unaffected by cAMP. The smaller conductance channels were activated by cAMP, but not Ca^{2+}. The presence of K^+ channels in the basolateral membrane which are regulated by these known "second messengers" can account for the increase in conductance and the hyperpolarization of the membrane observed upon secretagogue stimulation.

Key Words oxyntic cell · basolateral K^+ conductance · patch clamp · Ca^{2+}-activated K^+ channels · cAMP-regulated K^+ channels · acid secretion

Introduction

Acid secretion involves changes in the K^+ permeability of the parietal and oxyntic cell membranes. It is now established that secretion occurs via an exchange between cytosolic H^+ and luminal K^+. The exchange is catalyzed by an ATPase located in the apical membrane (Ganser & Forte, 1973; Sachs et al., 1976). Secretagogues such as carbachol and histamine are thought to affect cell function by elevating the intracellular levels of Ca^{2+} (Muallem & Sachs, 1985) and cAMP (Chew et al., 1980), respectively. Stimulation leads to morphological changes caused by the fusion of intracellular vesicles with the apical membrane (Helander & Hirschowitz, 1974) and an increase in acid output. During stimulation the conductance of the basolateral membrane also seems to increase, leading to a hyperpolarization of this membrane (Demarest & Machen, 1985), and the K^+ permeability in isolated parietal cells increases (Okada & Ueda, 1984).

Here single-channel recording methods are used to study the electrical properties of the basal surface of *Necturus* oxyntic cells. K^+ channels which are specifically activated by Ca^{2+} or cAMP were found. These may account for the hyperpolarization and conductance increase of the basolateral membrane during acid secretion.

Materials and Methods

Cell Preparations

Oxyntic cells and intact gastric glands were isolated from the fundic region of *Necturus maculosa* gastric mucosa using a technique similar to that described by Blum et al. (1971). The tissue with the underlying muscle layer removed, was stretched out as a flat sheet and subject to digestion by both pronase (1 mg/ml) and collagenase (1 mg/ml). Intact gastric glands were obtained after 60 min of digestion and isolated cells at 120 min. The suspension of either gastric glands or isolated cells was washed twice in albumin (10 mg/ml) before use.

Suspensions of isolated cells and gastric glands were placed in a transparent chamber and viewed under a Nikon Diaphot-TMD inverted microscope at 400× using Hoffman modulated optics. Oxyntic cells were distinguished quite readily from surface cells by appearance and size: they are rich in mitochondria, and have a larger diameter (50 μm) than surface cells (30 μm).

Solutions

In all experiments the pipette was filled with a KCl solution that contained (in mM): 110 K^+, 1 Na^+, 1 Mg^{2+}, 115 Cl^-, 1 Ca^{2+}, 10 EGTA, and buffered by 10 mM HEPES/KOH to pH 7.3. The Ca^{2+} concentration was calculated to be 5 nM. The bath solution

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bathing the cells was NaCl Ringer's (in mM): 110 Na^+, 2 K^+, 1 Mg^{2+}, 116 Cl^-, 1 Ca^{2+} and was buffered by 10 mM HEPES/NaOH to pH 7.3. Bath Ca^{2+} concentration was lowered to 5 and 500 nM in some experiments. Ca^{2+} concentrations were buffered using Ca^{2+}-EGTA, at concentrations determined using the stability constants of Martell and Smith (1974). The calculations took into account the pH and Mg^{2+} concentrations, and also assumed a 96% purity of EGTA (Sigma, St. Louis, Mo.) (*see* Miller & Smith, 1984; Findlay et al., 1985). At pH 7.3, for a free Ca^{2+} = 5 nM, $CaCl_2$ was 1.00 mM and EGTA was 10.0 mM; for a free Ca^{2+} = 500 nM, $CaCl_2$ was 1.16 mM and EGTA was 1.34 mM.

Stock solutions of dbcAMP (N6-2′-O-dibutyryladenosine 3′:5′-cyclic monophosphate monosodium salt, Sigma) and carbachol (Sigma) at concentrations of 20 mM and 10 mM were dissolved in NaCl Ringer's and kept at −20°C. A23187 (calcium-magnesium salt, Sigma) was dissolved in DMSO at a stock concentration of 2 mM, and stored at −20°C. When used, they were diluted by NaCl Ringer's into final concentrations of 1 mM, 10 μM and 2 μM, respectively.

Pronase (pronase E) and collagenase were purchased from E. M. Science Research Organics (Gibbstown, N.J.) and Cooper Biomedical (Malvern, Pa.).

To change the bath solution the new solution was perfused (20 μl/sec) into the experimental chamber (0.5 ml in volume) using a multi-staltic pump. A total of 5 min of perfusion was required to completely replace the contents of the chamber.

All experiments were performed at room temperature (20 to 23°C).

Patch-Clamp Methods

Fire-polished electrodes made of 100 μl hematocrit capillary (Blue Tip, VWR Scientific, San Francisco, Ca.), with tip resistances of approximately 5 MΩ were used in all experiments. In some cases, the patch pipettes were coated with Sylgard. Five to 10 gigaohm seals could be obtained on applying slight suction to the pipette. Occasionally seals would form instantaneously, but in most cases 2 to 5 min of continuous suction was required.

Since the cells and glands were free to move around the chamber, excised patches were obtained from a cell-attached or whole cell configuration by squirting a stream of Ringer's solution from a Pasteur pipette at the cell or gland. About 40% of the cell-attached patches were successfully transformed into inside-out patches in this way.

Data Acquisition and Analysis

Single-channel currents from cell-attached or excised membrane patches were recorded using a patch-clamp amplifier as previously described (Horn et al., 1984). The current-measuring amplifier had a gain of 10 pA/mV. Single-channel activity was studied by holding the patches (cell-attached, inside-out, and outside-out) at constant DC potentials. The bath was held at earth potential. All potentials are expressed with respect to the pipette potential (V_p). Therefore, making V_p positive in cell-attached and excised inside-out patches corresponded to hyperpolarization of the cell membrane from the resting potential.

A Teac R-400 analog tape recorder (Montebello, Ca.) was used to store data. Data were digitized and analyzed using an IBM personal computer interfaced to the Tecmar Labmaster data acquisition system (Cleveland, Ohio). The tape was replayed, and the signal was filtered with an 8-pole Bessel filter (Model LP902, Frequency Devices, Haverhill, Mass.) with the corner frequency set either at 1 or 3 kHz. The signal was then digitized at the rate of 200 μsec and was stored in the computer. Each file consisted of 10.24 sec of continuous recorded data.

A modified version of the computer program "pClamp" (Axon Instruments, Burlingame, Ca.) was used to analyze single-channel events. The modification allowed us to set the baseline and threshold levels. A section of the digitized record was displayed on a graphics monitor, and the threshold level set at 50% of a channel opening. The program determined the open time, the closed time, and the single-channel currents, and except where stated 10 sec of recorded data were analyzed by the computer. As many as 1000 events were analyzed to determine the kinetics of single-channel events such as the mean open time and mean closed times. For probabilities in the range of 10^{-3} or less, more than 1 min of data was analyzed. In general 100 events or more were analyzed to measure single-channel currents as plotted in the current-voltage relations.

Open probability (P_o) was defined as the fraction of time for which a single channel is open. For the data presented in this paper, only patches containing single channels were analyzed. P_o was estimated by determining the sum of the durations of all the individual openings, and dividing by the total time analyzed.[1]

All statistics were expressed as a mean ± standard deviation of n observations, except where stated. Current voltage (I-V) curves were fitted by linear regression where possible.

Results

Isolated *Necturus* oxyntic cells, and cells in intact gastric glands sealed readily to patch pipettes. Most patches contained only a single channel. Channels with conductances of 67 ± 21 pS (range 45 to 135 pS) made up 24% of the patches while those with conductances 33 ± 6 pS (range 20 to 45 pS) were in 56% of the patches. The 67-pS channel was found to be activated by intracellular Ca^{2+} and the 33-pS channel was activated by intracellular cAMP. These two populations of channels made up the majority (80%) of channels observed and are the subject of this paper. The channels were observed in both the oxyntic cells of isolated gastric glands, and in isolated cells. Since the only surface accessible to patch pipettes in the glands was the basolateral membrane, we concluded that these channels should be located in this membrane.

Ca^{2+}-Activated K^+ Channels

Conductance and Selectivity

Typical records for channels activated by Ca^{2+} are shown in Fig. 1(*A*) for a single channel in a cell-attached patch. The patch was held at a number of

[1] P.D. Brown, D.D.F. Loo, and E.M. Wright. Calcium-activated potassium channels in the apical membrane of *Necturus* choroid plexus. [*Submitted*]

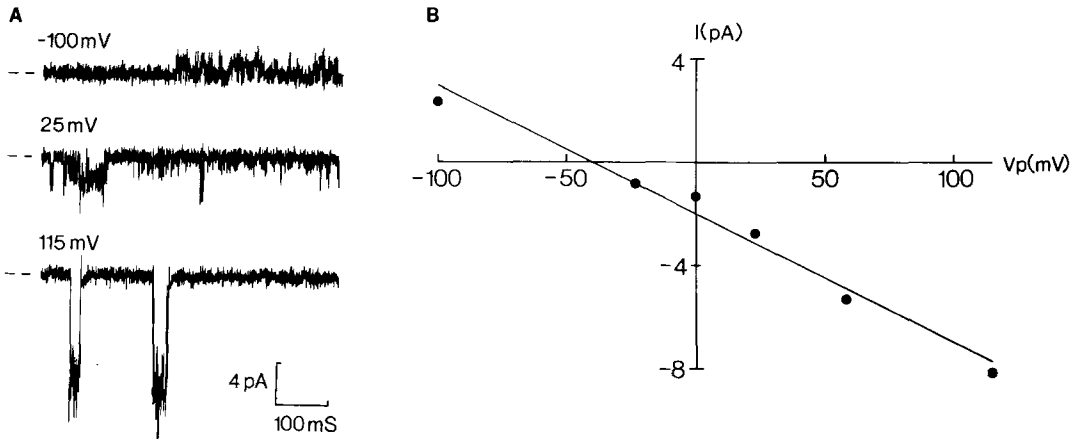

Fig. 1. (*A*) Current records for a single Ca^{2+}-activated K^+ channel. The channel was in a cell-attached patch on the basolateral membrane of an oxyntic cell from an intact gastric gland. Pipette potentials (V_p) are indicated by the numbers to the left of the current traces. The currents were filtered at 1 kHz. Positive V_p corresponds to hyperpolarizations away from the resting potential. Downward deflection of the current trace from the base line (dashed line) indicates current into the cell. (*B*) The *I-V* relations for events in (*A*). The straight line was obtained by linear regression and the slope was 49 ± 4 pS. Reversal potential was −44 mV. Pipette solution was (in mM): 110 K^+, 1 Na^+, 1 Mg^{2+}, 115 Cl^-, 1 Ca^{2+} and 10 EGTA. Pipette Ca^{2+} concentration was calculated to be 5 nM. Bath solution contained (in mM): 110 Na^+, 2 K^+, 1 Mg^{2+}, 116 Cl^-, and 1 Ca^{2+}. Both solutions were buffered by 10 mM HEPES to pH 7.3

pipette potentials (V_p). At V_p = 25 mV, the downward deflections of the current trace indicate inward currents, i.e. cation movement from the pipette into the cell, or anion exit. Hyperpolarization of the cell membrane (V_p = 115 mV) increased the magnitude of the unitary current steps. Depolarization (V_p negative) decreased the unitary current steps, until they were indistinguishable from the background noise at the reversal potential (V_p approx. −30 mV). Further depolarization resulted in reversal of the unitary current steps (V_p = −100 mV). Apart from affecting the size of the current steps, potential also influenced channel opening. The frequency of individual openings decreased with hyperpolarization (Fig. 1*A*).

Single-channel currents from Fig. 1(*A*) are plotted against V_p in Fig. 1(*B*). Over the range of pipette potentials studied here the *I-V* relationship was linear. The straight line was fitted by linear regression analysis and has a slope of 49 ± 4 pS. Data from seven experiments gave a mean conductance of 67 ± 21 pS. The reversal potential for the channel in Fig. 1(*B*) was −44 mV, whereas pooled data gave −32 ± 10 mV (n = 7). This reversal potential was comparable to the value for resting potential obtained in isolated cells by microelectrode impalements (−44 ± 3 mV, Blum et al., 1971) and that obtained in the basolateral membrane of *Necturus* oxyntic cells in intact mucosa (−48.3 ± 1.2 mV, Demarest & Machen, 1985).

In excised patches, the *I-V* relationship was linear only in symmetrical (110 mM) KCl solutions (●, Fig. 2*A*). The conductance of the channel did not appear affected by excision and was 72 ± 4 pS (n = 3) which is similar to that obtained in cell-attached patches.

When bath K^+ was completely replaced by Na^+, the *I-V* relationship showed a large rectification (○, Fig. 2*A*). Current reversal was not observed even when V_p = −100 mV. This suggested that the channels are selective to K^+, as the line deviated towards the calculated K^+ reversal potential (V_p = −101 mV) and away from that for Cl^- (0 mV) or Na^+ (+119 mV). The line was drawn according to the constant field equation (Hodgkin & Katz, 1949) assuming P_{Na} and P_{Cl} to be zero. P_K was calculated from the limiting conductance (77 pS) of the channel at large hyperpolarizations. The close agreement between this curve and the data values suggests that the channel is permeable to K^+, not to Na^+ or Cl^-.

To obtain more information on channel selectivity, the bath solution was replaced by a solution that contained equal concentrations of K^+ and Na^+ (55 mM KCl and 55 mM NaCl). In this situation, the reversal potentials calculated for K^+, Na^+ and Cl^- were −17, +101 and 0 mV, respectively. The *I-V* relationship for such an experiment is shown in Fig. 2(*B*). The apparent reversal potential was at V_p = −17 mV. This value is close to the reversal potential for K^+, indicating that the channel was K^+ selective ($P_K/P_{Na} \gg 10$ and $P_K/P_{Cl} \gg 10$). The curve was again predicted using the constant field equation. The close agreement between experimentally

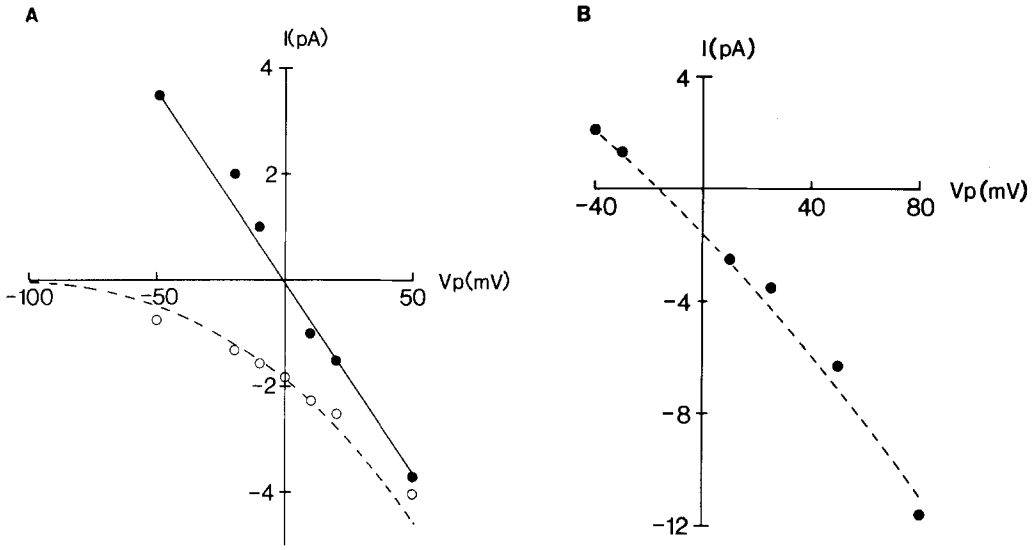

Fig. 2. *I-V* relationships for Ca^{2+}-activated K^+ channels in excised patches. (*A*) The solid circles are data from an excised outside-out patch held in symmetrical 110 mM KCl solutions (pipette and bath). The line drawn by linear regression has a slope of 77 ± 1 pS. The bath solution was then replaced with 110 mM NaCl Ringer's and currents measured (○). Under these conditions, the reversal potentials calculated for K^+, Na^+, and Cl^- are −101, +119 and 0 mV, respectively. The broken line shows currents predicted by the constant field equation: $I = -[P_K F^2 V_p/RT] \cdot [K_o - K_i \cdot \exp(-FV_p/RT)]/[1 - \exp(-FV_p/RT)]$, where F, R and T have their usual meanings. K_i = 110 mM and K_o = 2 mM are the pipette and bath K^+ concentrations. P_K is the single-channel K^+ permeability calculated from the limiting conductance. $P_K = 1.84 \times 10^{-13}$ cm^3 sec^{-1} per channel. (*B*) *I-V* relationship in an excised inside-out patch. The pipette contained 110 mM KCl and bath solution contained equal K^+ and Na^+ concentrations (55 mM KCl and 55 mM NaCl). The calculated reversal potentials for K^+, Na^+ and Cl^- are −17, +101 and 0 mV, respectively. The curve was drawn according to the constant field equation (as in Fig. 2*A*), but with P_K corresponding to a limiting conductance of 135 pS

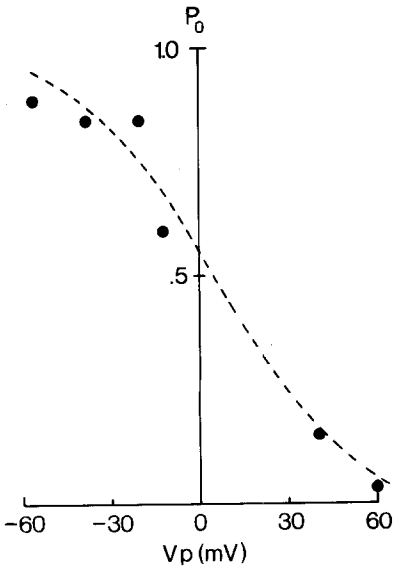

Fig. 3. Dependence of P_o on pipette potential (V_p). Data is from an excised inside-out patch. Pipette solution was 110 mM KCl and bath solution was also 110 mM KCl with 500 nM Ca^{2+}. The smooth curve was drawn through the data using the equation: $P_o = 1/[1 + \exp(A(V_p - V_o)]$ (Wong et al., 1982). $A = 4.73 \times 10^{-2}$ mV^{-1} and V_o = 4 mV

obtained and theoretical values in Figs. 2(*A*) and 2(*B*) indicated that the conductive properties of the channels may be described by the constant field equation.

Voltage Dependence of Opening and Closing

In Fig. 3, the dependence of open probability (P_o) on voltage is shown in an inside-out patch (bath Ca^{2+} was 500 nM). The dependence of P_o on potential appeared sigmoidal, and with P_o increasing with depolarization (V_p negative). At the resting potential (which corresponds to V_p = 32 mV), a 10-mV hyperpolarization decreased P_o by 0.1. A sigmoidal dependence of P_o on potential has been observed in Ca^{2+}-activated K^+ channels from several tissues including rat muscle (Moczydlowski & Latorre, 1983), clonal anterior pituitary cells (Wong et al., 1982) and choroid plexus.[2]

In cell-attached patches, channel activity varied from cell to cell. Between different channels, there was a wide range in the resting P_o (1.4×10^{-3} to 1.6

[2] *See* footnote 1, p. 32.

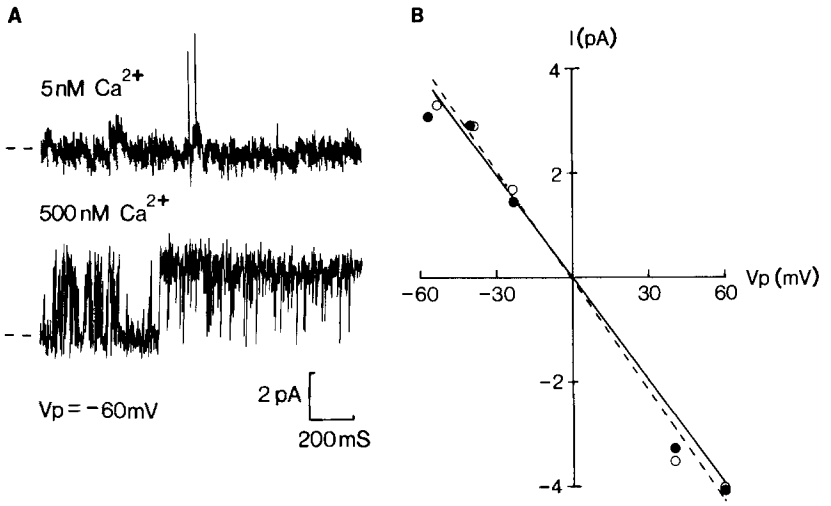

Fig. 4. Activation of K^+ channel by intracellular Ca^{2+}. *A* shows the single-channel current records (filtered at 1 kHz) in an excised inside-out patch with symmetric KCl solutions (110 mM KCl in the pipette and bath solutions). Bath Ca^{2+} was increased from 5 to 500 nM. The dashed line indicates baseline. $V_p = -60$ mV. *B* shows the *I-V* relations where bath Ca^{2+} was 5 nM (○) and 500 nM (●). The dashed and solid lines were obtained by linear regression on the open and closed circles. The slopes were 72 ± 8 and 67 ± 6 pS, respectively

$\times 10^{-1}$), mean open time (0.6 to 1.4 msec) and mean closed times (10 to 623 msec). However, the dependence of P_o on voltage was relatively constant from cell to cell. Data analyzed from three patches showed that for a 10-mV hyperpolarization from the resting potential, open probability decreased by 10%. This is in agreement with the values obtained from excised patches (Fig. 3).

Dependence of Channel Activity on Ca^{2+}

The effect of Ca^{2+} was to increase both the number of channel openings and the duration of each opening. In Fig. 4(*A*) single-channel currents are shown from an inside-out patch held at $V_p = -60$ mV. P_o increased from 0.0004 to 0.92 when bath Ca^{2+} was increased from 5 to 500 nM (Fig. 4*A*). The frequency of events increased 90-fold (from 30 to 2600 openings/min), and mean open time increased 20-fold (from 0.9 ± 0.4 to 18.1 ± 26.8 msec). Ca^{2+} affected P_o at every V_p studied, and the effect was a function of V_p; i.e. at $V_p = -22$ mV, the increase in P_o was 200-fold (from 0.005 to 0.86), at $V_p = +60$ mV, the increase was only 10-fold (from 0.003 to 0.032). Ca^{2+} had no effect on single-channel conductance (Fig. 4*B*). The conductances at 5 and 500 nM were 72 ± 8 and 67 ± 6 pS, respectively.

The regulation of the channel by Ca^{2+} was further studied in cell-attached patches by the addition of the calcium ionophore A23187. Figure 5 shows single-channel current records from a cell-attached patch bathed in 110 mM NaCl Ringer's solution (control). The bath solution was then exchanged for one containing 2 μM A23187. There was an increase in the number of single-channel events which was similar to that shown in Fig. 4*A* for an increase in intracellular Ca^{2+}. A summary of the results is

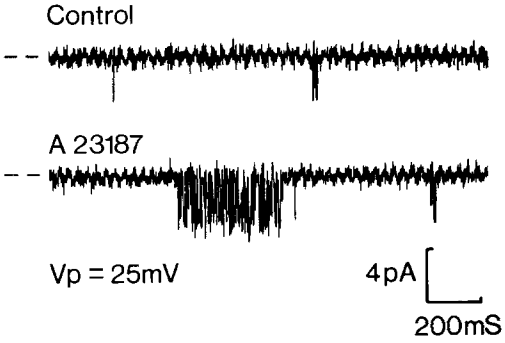

Fig. 5. The effect of A23187 on the activity of the 67-pS channel. The experiment was performed on a cell-attached patch in an oxyntic cell from a gastric gland. The single-channel current records are shown in control conditions and after the addition of 2 μM of A23187 to the bath. Pipette solution was 110 mM KCl Ringer's (5 nM Ca^{2+}) and the bath solution was 110 mM NaCl Ringer's (1 mM Ca^{2+}). Current records were filtered at 3 kHz

shown in Table 1. P_o increased at every pipette potential tested, by between 1.2- to 7.2-fold. The mean increase at $V_p = 60$ mV was 3.1 ± 1.3-fold ($n = 3$), while at $V_p = 25$ mV it was 3.8 ± 3.1-fold ($n = 3$). The conductance of the channels was not affected by A23187; it was 65 pS ($n = 2$), and 71 pS ($n = 2$) in the absence and presence of A23187.

The effect of A23187 on P_o was qualitatively the same as that of Ca^{2+} on excised inside-out patches (*data not shown*). The addition of A23187 caused increases in both the frequency of openings and the mean open time, and a decrease in the mean closed time.

The action of carbachol which is known to elevate intracellular Ca^{2+} (Muallem & Sachs, 1985; Muallem et al., 1986) was examined in a cell-at-

tached patch on an oxyntic cell in a gastric gland. After the perfusion of a Ringer's solution containing 10 μM carbachol, P_o increased approximately 100-fold, from 0.021 to 0.23, at V_p = 115 mV. The result is consistent with the activation of this channel by intracellular Ca^{2+}. Moreover, the cholinergic receptors are retained in this preparation.

Two experimental protocols were employed to determine the cAMP sensitivity of Ca^{2+}-activated K^+ channels. In cell-attached patches, A23187 was first added and then this solution was completely replaced with a solution containing dbcAMP. The converse experiment of first perfusing the tissue with a solution containing dbcAMP and then with a solution containing A23187 was also performed. In these two experiments, A23187 always increased P_o of these channels (2.7-fold) while cAMP had little effect: in the two experiments the change was 1.1-fold. In these experiments, the channels did not increase their sensitivity to cAMP after they had been stimulated by A23187. Conversely, prior exposure to cAMP did not alter the increase in P_o induced by the addition of A23187. Hence we conclude that this channel had very little sensitivity to cAMP.

Table 1. Changes in open probability with A23187 stimulation[a]

Channel	Conductance (pS)	V_p (mV)	P_o (control) $\times 10^{-3}$	P_o (A23187) $\times 10^{-3}$	% increase in P_o $\times$ 100%
3/4/3	60	60	0.8	2.5	3.1
		40	1.2	3.2	2.7
		25	0.4	2.9	7.2
3/13/4	75	60	1.2	2.0	1.7
		25	1.3	3.6	2.9
		0	2.9	7.0	2.5
8/21/2	110	60	0.4	1.6	4.4
		25	0.9	1.1	1.2
		10	0.4	1.4	3.5

[a] Differences in open probability (P_o) for channels in cell-attached patches in the presence and absence of 2 μM A23187 to the bath. Bath solution was 110 mM NaCl Ringer's (1 mM Ca^{2+}).

cAMP-regulated K^+ Channels

Conductance and Selectivity

Figure 6(*A*) shows typical current records for a cAMP-activated K^+ channel in a cell-attached patch. Compared to the Ca^{2+}-activated K^+ channel, the current steps were smaller and the frequency of openings did not change with potential.

The kinetic behavior was complex. Individual channel openings were very brief (approx. 1 msec). Occasionally bursts of activity were observed which consisted of a series of openings and closings. The duration of the bursts varied from 75 msec to over 1 sec.

Currents from the channel in Fig. 6(*A*) are plotted as a function of V_p in Fig. 6(*B*), and the *I-V* relationship was linear. In this experiment the single-channel conductance was 27 ± 1 pS, and results from 14 experiments gave 33 ± 6 pS. The reversal potential in Fig. 6(*B*) was −35 mV, the mean value was −33 ± 16 mV (n = 14). The *I-V* relationship for the channels in excised inside-out patches in symmetrical 110 mM KCl solutions was also linear. The

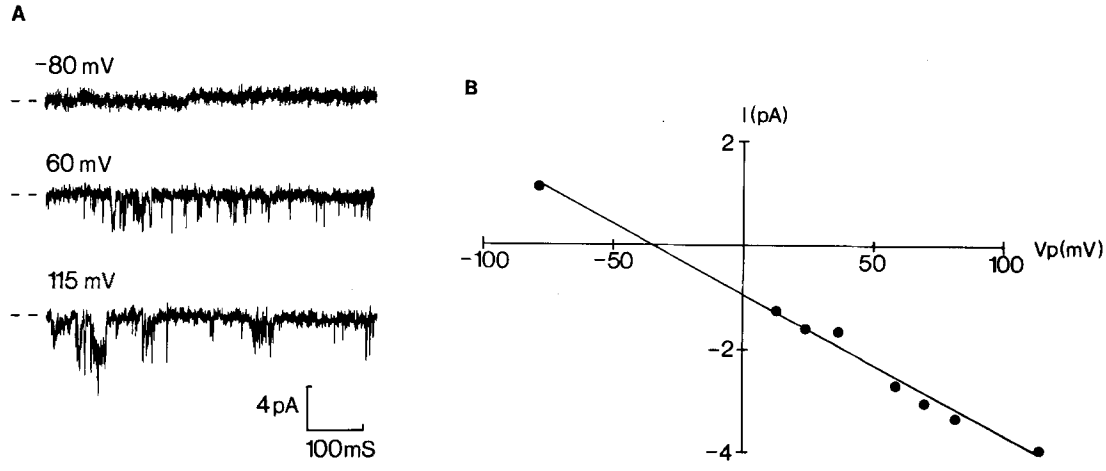

Fig. 6. (*A*) Current records from a cAMP-regulated channel in a cell-attached patch on an isolated oxyntic cell. The currents were filtered at 1 kHz. V_p is indicated by the figures on the left of the traces. The dashed line indicates when the channel was closed. (*B*) The *I-V* relationship for the channel is shown. The line was drawn by linear regression with a slope of 27 ± 1 pS and reversal potential of −36 mV. Pipette solution is 110 mM KCl (5 nM Ca^{2+}) and bath solution is 110 mM NaCl Ringer's (1 mM Ca^{2+})

value obtained for the single-channel conductance was 28 ± 4 pS (n = 7), similar to that in the cell-attached patches.

The selectivity of the channel was determined from the reversal potential observed in inside-out patches, where the pipette solution contained 110 mM KCl Ringer's and bath solution was 55 mM KCl and 55 mM NaCl Ringer's. Data from two separate patches are shown in Fig. 7, where the smooth curve is the constant field prediction for K^+ current. The apparent reversal potential obtained was −17 mV which is the calculated reversal potential for K^+. The Na^+ and Cl^- reversal potentials were +101 and 0 mV, respectively, so that the channel appeared impermeable to Na^+ and Cl^-. This conclusion was also supported by experiments in biionic conditions (110 mM KCl in the pipette and 110 mM NaCl in the bath solutions), where current reversal was not observed even when V_p = −100 mV. (The K^+ reversal potential in this case was −101 mV.)

Voltage Dependence of Opening and Closing

Because of their extremely low activity and small size of the unitary currents in excised patches, we were unable to study the dependence of open probability on voltage in those experiments. In cell-attached patches, as with the Ca^{2+}-activated K^+ channels, from cell to cell there were large differences in P_o (8×10^{-3} to 8.5×10^{-1}), mean open time (1.0 to 802.1 msec) and closed time (17 to 272 msec). Data pooled from six patches showed a large scatter (*data not shown*). The trend was that hyperpolarizations from the resting potential tended to slightly increase P_o. We can conclude that the voltage dependence of the 33-pS channel is very slight compared to the 67-pS channel and is in the opposite direction, i.e. hyperpolarization tended to slightly activate the 33-pS channel.

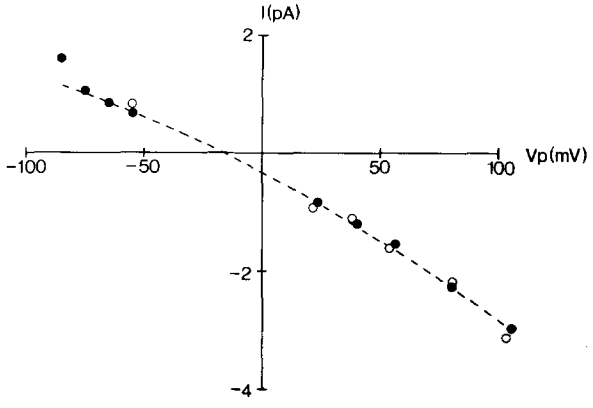

Fig. 7. The *I-V* relationships for cAMP-regulated channels in an inside-out patch. Results from two experiments are shown. The pipette solution was 110 mM KCl and bath solution was 55 mM KCl and 55 mM NaCl. Bath Ca^{2+} was 1 mM. The curve was drawn assuming the constant field equation (as in Fig. 2*B*) with a limiting conductance of 30 pS. Reversal potential was −17 mV

Regulation by cAMP

Figure 8 shows current traces in an unstimulated patch (control). Replacing the bath solution with one containing 1 mM dbcAMP caused P_o to increase. Table 2 shows the results for cAMP stimula-

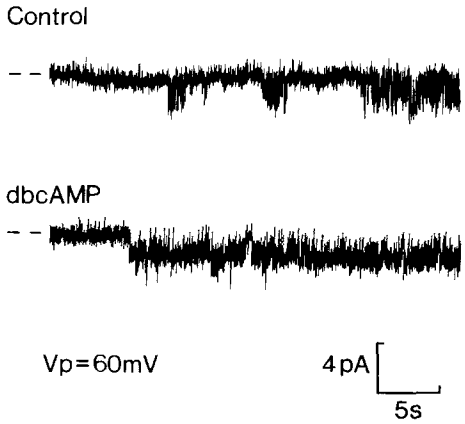

Fig. 8. Stimulation of the 33-pS channel by dbcAMP. Single-channel current records (filtered at 100 Hz) from a cell-attached patch from an oxyntic cell in a gastric gland. The upper trace shows the channel activity under control conditions. Bath solution was 110 mM NaCl Ringer's with 1 mM Ca^{2+}. The lower trace shows the channel activity after the bath solution was exchanged for one containing 1 mM dbcAMP. The dashed line indicates baseline level. Pipette solution was 110 mM KCl with 5 mM Ca^{2+} and V_p = 60 mV

Table 2. Increases in open probability with cAMP stimulation[a]

Channel	Conductance (pS)	V_p (mV)	P_o control	P_o cAMP	% increase in P_o × 100%
3/19/2	23	60	0.22	0.49	2.3
4/15/3	22	60	0.10	0.23	2.4
		40	0.02	0.25	10.2
		25	0.02	0.07	3.9
4/17/2	37	60	0.22	0.26	1.2
		40	0.26	0.33	1.3

[a] Differences in open probability (P_o) in a cell-attached patch in the absence and presence of 1 mM cAMP. Pipette solution was 110 mM KCl Ringer's (5 nM Ca^{2+}). Bath solution in the controlled condition was 110 mM NaCl Ringer's (1 mM Ca^{2+}).

Table 3. Summary of the properties of the K^+ channels in the basolateral membrane of *Necturus* oxyntic cells

Conductance:	67 pS	33 pS
Voltage dependence:	depolarization activated	voltage independent
Selectivity:	$P_K/P_{Na} > 10$	$P_K/P_{Na} > 10$
Ca^{2+} sensitivity:	yes	no
cAMP sensitivity:	no	yes

tion on three patches. In each case, P_o increased by a similar degree at each potential studied (V_P = 25, 40 and 60 mV). This was compatible with the lack of potential sensitivity of the channel. The increase for each patch was relatively constant; in a total of six patches the average increase was 3.2 ± 1.9-fold (range 1.2- to 5.5-fold). cAMP had no effect on single-channel conductance. In three experiments, the control value was 28 ± 9 pS, and the conductance of the channels after stimulation by cAMP was 26 ± 10 pS.

The addition of A23187 or Ca^{2+} had very little effect on this channel. In an excised inside-out patch, increasing the bath Ca^{2+} from 5 to 500 nM, increased P_o slightly from 0.039 to 0.098, at V_p = 50 mV. In cell-attached patches, the increase of P_o with cAMP was not affected if A23187 had previously been added to the bath. Conversely, after the channels had first been stimulated by cAMP, on replacing the bath with a solution containing A23187, P_o was restored to control values. In both of the above experiments on the effect of A23187 on the cAMP-stimulated channel, P_o decreased by 0.7-fold. Thus we conclude that this channel had very little sensitivity to Ca^{2+}.

Discussion

In the work described above, evidence has been presented for the presence of at least two types of K^+ channels in the basolateral membrane of *Necturus* oxyntic cells. The two types of channels can be distinguished on the basis of conductance, voltage dependence and activation by cAMP or Ca^{2+} (Table 3). A cAMP-activated K^+ channel has a conductance of 33 pS and is relatively voltage independent, whereas a channel activated by Ca^{2+} has larger conductance, 67 pS, and is activated by depolarization of the membrane. The wide range of conductance of the Ca^{2+}-activated K^+ channels raises the possibility that there may be more than one class of these channels. In studies comparing the effects of cAMP on the large channel, or Ca^{2+} on the small channel there was little cross reactivity of the channels.

The activation of the channel by intracellular Ca^{2+} and its high selectivity to K^+ are common properties of Ca^{2+}-activated K^+ channels found in a variety of tissues (Latorre & Miller, 1983). Such channels can be blocked by Ba^{2+} (Latorre & Miller, 1983), and in a preliminary experiment, we have found that 5 mM Ba^{2+} at the intracellular surface of the membrane completely blocks the channel. Thus the Ca^{2+}-activated K^+ channels in the basolateral membrane of *Necturus* oxyntic cells are similar to those of other tissues.

Patch-clamp studies have been carried out on secretory tissue from elsewhere in the digestive tract. Basolateral Ca^{2+}-activated K^+ channels have been found in the salivary gland and pancreas (Maruyama et al., 1983; Susuki et al., 1985). There is as yet no evidence for cAMP-regulated K^+ channels, but cAMP may not be important in zymogen secretion in these tissues. In the gastric parietal cell, however, the binding of histamine to the H_2 receptor and subsequent elevation of cAMP is a well-established pathway to secretion (Chew et al., 1980).

K^+ channels that are cAMP regulated have been found in several neuronal preparations. For instance, the bag cell neuron of *Aplysia* appears to have three types of K^+ channels that are inhibited by cAMP (Kaczmarek & Strumwasser, 1984). In the R15 neurone of *Aplysia,* serotonin and cAMP appear to activate an anomalously rectifying K^+ channel (Levitan, 1985). In the same cell dopamine affects other ionic currents without the mediation of cAMP (Adams & Levitan, 1982). Thus the activation of separate channels by separate second messengers may be a common occurrence in many tissues, so that the *Necturus* oxyntic cell is not unique in this respect.

cAMP-activated K^+ channels have not been as well characterized as Ca^{2+}-activated K^+ channels, primarily because the former have smaller conductances and are generally unstable in excised membrane patches. The cAMP-activated K^+ channels in *Necturus* oxyntic cells appeared to be different from some of those found in neuronal preparations in that 1) they are not sensitive to Ca^{2+}, in contrast to the channels found in snail neurons (DePeyer et al., 1982), and 2) they are not activated by depolarization, in contrast to those found in *Aplysia* bag cell neurons (Kaczmarek & Strumwasser, 1984).

The presence of K^+ channels in the basolateral membrane of *Necturus* oxyntic cells and their activation by secondary messengers helps clarify the nature of the hyperpolarization of this surface with

secretagogue stimulation (Demarest & Machen, 1985). Stimulation by carbachol and histamine will activate K^+ channels by elevating Ca^{2+} and cAMP. The opening of the channels will cause a hyperpolarizing shift in the membrane potential so that it is nearer the equilibrium potential for K^+. Stimulation of the Na^+,K^+-ATPase could also contribute to this hyperpolarization. A hyperpolarization towards K^+ equilibrium potential indicates that the resting basolateral membrane must contain conductive pathways for ions other than K^+. These pathways are also responsible for establishing the resting potential of these cells. Changes in Cl^- activity at the basolateral membrane have been shown to induce changes of transmucosal potential (Shoemaker et al., 1967), suggesting the membrane may be permeable to Cl^-. In the present experiments, the reversal potential of the oxyntic cells was −32 mV, in agreement with resting potential measurements using microelectrodes. This resting potential is much lower than the Nernst potential for K^+, which is −101 mV (assuming intracellular K^+ to be 110 mM). This suggests that there is a rather large Cl^- permeability. In preliminary experiments, we have observed Cl^--selective channels with conductance of about 30 pS, but their occurrence was rare (less than 5 percent of the patches). The majority of the Cl^- channels may have very small conductances, and hence were not detected in our experiments.

The basolateral membrane of the surface cell also hyperpolarizes in the presence of secretagogues (Shoemaker et al., 1970). It may be that this hyperpolarization is also due to the activation of K^+ channels and that activation of basolateral K^+ pathways is a common result of secretagogue stimulation in *Necturus* gastric mucosa.

In the present experiments, the Ca^{2+}- and cAMP-activated K^+ channels were found to be independent in that prior stimulation of a patch membrane first with either cAMP or Ca^{2+} did not affect the sensitivity of the channel to the other secondary messenger. This is of interest because the potentiating interactions in terms of acid secretion between cAMP- and Ca^{2+}-mediated agonist have been well documented (Soll & Grossman, 1978). In the present experiments, we found no evidence of interaction on the level of single K^+ channels. Interaction between second messengers presumably involves other mechanisms.

Acid secretion involves ion pathways in both the apical and basolateral membranes. Cl^- is secreted across the oxyntic cell layer of *Necturus* gastric mucosa (Demarest et al., 1986). In the mammal HCl is the predominant secretion (160 mM) but between 15 to 25 mM KCl is also secreted (such data is not available for *Necturus* tissue). Activation of HCl secretion results in a simultaneous secretion of H^+ and Cl^-, with increased short-circuit current (Shoemaker et al., 1970). The relationship of these K^+ channels that are activated by either cAMP or Ca^{2+} as second messengers to acid secretion is presented schematically in Fig. 9, which illustrates a stimulated oxyntic cell. Activation of acid secretion by either cAMP or Ca^{2+} has resulted in activation of the H^+,K^+-ATPase which accounts for acid secretion. Activation of the ATPase requires a supply of K^+ to its luminal surface which is obtained by activation of K^+ and Cl^- pathways in the membrane adjacent to the ATPase (Sachs et al., 1976; Cuppoletti & Sachs, 1984; Wolosin & Forte, 1985). This KCl effux activation and K^+ recycling results in KCl secretion that accompanies HCl secretion. In the basolateral membrane, the Na^+ for H^+ exchange (Paradiso et al., 1984; Muallem et al., 1985) and the Cl^- for HCO_3^- exchange pathways (Muallem et al., 1985) also increase as a function of cell stimulation. The result is an influx of NaCl into the cell, thus providing the cell Cl^- necessary for KCl efflux, and the Na^+ necessary for activation of the Na^+,K^+-ATPase. The present model differs from the model proposed for pancreatic and salivary cells

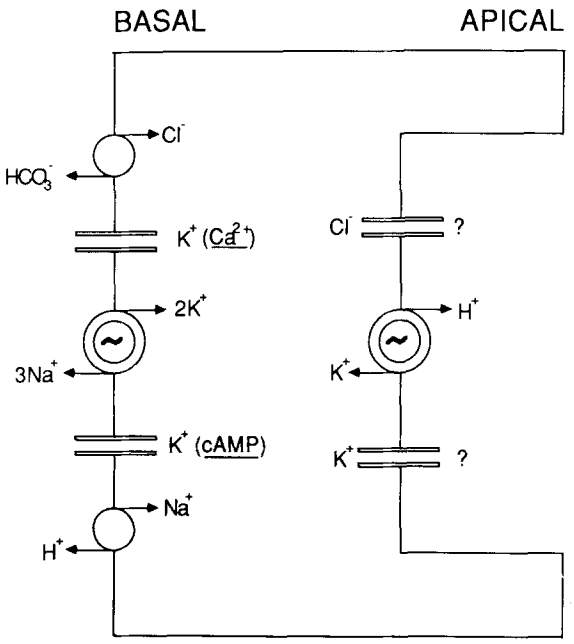

Fig. 9. A model illustrating the transport pathways involved in HCl secretion in the oxyntic cell. All of the pathways are activated with Ca^{2+} and cAMP stimulation. The symbols (↺), (⊚) and (=) represent exchange pathways, pumps and conductances, respectively. Existence of separate K^+ and Cl^- conductances in the apical membrane is postulated

where the intracellular Cl^- required for secretion is provided by the cotransport of $NaKCl_2$ across the basolateral membrane (Petersen & Maruyama, 1984).

In the oxyntic cell, the activity of the Na^+,K^+-ATPase maintains cell K^+ and compensates for the loss of K^+ across both the apical and basolateral membranes. During secretion Cl^- entry is electroneutrally coupled to HCO_3^- exit across the basolateral membrane. The activation of basolateral K^+ channels would result in the hyperpolarization of the cell and thus increase the driving force for Cl^- exit across the apical membrane. This mechanism of Cl^- secretion, where the activation of basolateral K^+ channels provides a driving force for apical Cl^- secretion, may also occur in other Cl^--secreting epithelia.

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