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Regulation of Maxi-K^+ Channels on Pancreatic Duct Cells by Cyclic AMP-Dependent Phosphorylation

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Summary. Using the patch-clamp technique we have identified a Ca^{2+}-sensitive, voltage-dependent, maxi-K^+ channel on the basolateral surface of rat pancreatic duct cells. The channel had a conductance of ~200 pS in excised patches bathed in symmetrical 150 mM K^+, and was blocked by 1 mM Ba^{2+}. Channel open-state probability (P_o) on unstimulated cells was very low, but was markedly increased by exposing the cells to secretin, dibutyryl cyclic AMP, forskolin or isobutylmethylxanthine. Stimulation also shifted the P_o/voltage relationship towards hyperpolarizing potentials, but channel conductance was unchanged. If patches were excised from stimulated cells into the inside-out configuration, P_o remained high, and was not markedly reduced by lowering bath (cytoplasmic) Ca^{2+} concentration from 2 mM to 0.1 μM. However, activated channels were still blocked by 1 mM Ba^{2+}. Channel P_o was also increased by exposing the cytoplasmic face of excised patches to the purified catalytic subunit of cyclic AMP-dependent protein kinase. We conclude that cyclic AMP-dependent phosphorylation can activate maxi-K^+ channels on pancreatic duct cells via a stable modification of the channel protein itself, or a closely associated regulatory subunit, and that phosphorylation alters the responsiveness of the channels to Ca^{2+}. Physiologically, these K^+ channels may contribute to the basolateral K^+ conductance of the duct cell and, by providing a pathway for current flow across the basolateral membrane, play an important role in pancreatic bicarbonate secretion.

Key Words pancreatic duct cells · bicarbonate secretion · patch clamp · Ca^{2+}-activated K^+ channels · protein kinase-A · cyclic AMP-dependent phosphorylation

Introduction

Large conductance (BK or maxi-) K^+ channels, whose activity is regulated by membrane potential and intracellular Ca^{2+}, have been identified on the basolateral surface of many Cl^--secreting epithelial cells [30]. As well as recycling K^+ accumulated by the Na^+/K^+ ATPase, these channels play an important role in electrogenic chloride transport. Secretagogue-induced channel activation: (*i*) increases the local extracellular K^+ concentration, thus providing a more favorable K^+ gradient for the operation of Na/K/2Cl cotransporters on the basolateral surface; (*ii*) allows outward current flow at the basolateral membrane which balances electrogenic anion secretion at the apical pole of the cell; and (*iii*) hyperpolarizes the cell which increases the electrochemical driving force for Cl^- exit at the apical membrane [30].

This paper deals with the regulation of maxi-K^+ channels located on the basolateral membrane of rat pancreatic duct cells [2]. These cells secrete the bicarbonate ions found in pancreatic juice, probably by an electrogenic mechanism similar to that operating in Cl^--secreting epithelia [7, 16, 29]. As such their basolateral membrane possesses Na^+/K^+ ATPases [28], Na^+/H^+ exchangers [28, 36], a Ba^{2+}-sensitive K^+ conductance [28] and maxi-K^+ channels [2], while the apical membrane contains Cl^-/HCO_3^- exchangers [29, 36], together with a chloride conductance [29] and small conductance chloride channels [16, 17]. This spatial localization of transport elements implies a secretory model in which bicarbonate is accumulated across the basolateral membrane by backward transport of H^+ on the Na^+/H^+ exchangers, and then exits at the apical membrane via a Cl^-/HCO_3^- exchanger. The Cl^- necessary for operation of the anion exchanger is delivered to the lumen via the small conductance Cl^- channel [16].

As in the case of Cl^--secreting epithelia [30], the Cl^- and K^+ channels on the duct cell are probably the most important control points in the bicarbonate secretory mechanism. In this respect, we have already shown that activity of the apical Cl^- channel can be increased by the hormone secretin, which is the physiological regulator of pancreatic bicarbonate transport [7], and also by its intracellu-

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lar messenger cyclic AMP [16]. Here we show that maxi-K^+ channels are also activated following stimulation of the duct cell and, furthermore, that channel activity remains high after patch excision. We also show that these channels can be activated by exposing the cytoplasmic face of the plasma membrane to the catalytic subunit of cyclic AMP-dependent protein kinase. From these results we conclude that cyclic AMP-dependent protein phosphorylation activates maxi-K^+ channels via a stable modification of the channel protein itself, or a closely associated regulatory subunit.

Materials and Methods

Tissue Preparation

Most experiments were performed on inter- and intralobular ducts that had been isolated from the pancreas of copper-deficient rats, and maintained in organ culture for up to three days [1, 3]. Copper deficiency causes a noninflammatory atrophy of enzyme secreting acinar cells (80% by volume of the pancreas) but leaves the duct cells (4% by volume of the gland) structurally and functionally intact (*see* Ref. [3]). As a starting point for duct isolation this preparation has two advantages over the copper-replete rat pancreas: (*i*) the glandular content of potentially harmful digestive enzymes is markedly decreased; and (*ii*) the ratio of ductal to acinar tissue in the pancreas is markedly increased. We have previously shown that these isolated ducts possess morphological, biochemical and secretory characteristics typical of ducts within the intact copper-replete rat pancreas [1, 3].

The basolateral membrane of the epithelial cells was exposed by dissecting away surrounding connective tissue, while the apical plasma membrane was accessed by tearing a flap in the wall of the duct. Once prepared, ducts were immobilized in a perspex tissue bath (volume 1.5 ml) by means of a suction pipette and a fine glass rod. Bath solution changes were accomplished by gravity feed from a bank of reservoirs at a flow rate of 5 ml · min^{-1}.

After studies on intact ducts had established that K^+ channels were located only on the basolateral plasma membrane, some experiments were also performed on isolated duct cells [16]. Although morphological polarity is probably lost in this preparation, their use greatly facilitated giga-seal formation and also our ability to maintain cell-attached recordings over long periods.

Electrophysiology

Single-channel current recordings were made at 21–23°C from cell-attached and excised patches using the patch-clamp technique [19]. Recording pipettes were constructed from borosilicate glass (GC120F15, Clarke Electromedical, Pangbourne, UK), had resistances of between 8 and 15 MΩ, and were positioned using a Narishige MO-103 hydraulic manipulator (Narishige Scientific Instrument Laboratory, Tokyo, Japan). Seal resistances were between 10 and 30 GΩ.

Full details of the electrophysiological techniques employed in this study are described elsewhere [16]. Briefly, single-channel currents were amplified using an EPC-7 patch-clamp system (List Electronic, Darmstadt, FRG) and stored on a digital tape recorder. For viewing and analysis these data were low-pass filtered at 1–10 kHz (Fern EF5-02 in damped mode, Barr and Stroud, Glasgow, Scotland) and displayed on a digital storage oscilloscope (Model 1425, Gould Advance, Hainault, UK). Permanent records were made using a graphics plotter (Model 7470A, Hewlett Packard, San Diego, CA). Junction potentials were measured using a flowing 3M-KCl electrode [16], and the appropriate corrections applied to our data. The tissue bath was grounded, and potential difference across excised, inside-out, patches (V_m) is given as $V_{inside} - V_{outside}$. During cell-attached recording, the total voltage across the patch is equal to the cell membrane potential (V_m) minus the pipette potential (V_p). In this configuration we quote either V_p values or, when comparison of cell-attached I/V plots from different cells is required, calculated V_m values. For K^+-rich pipette solutions:

$$V_m = V_p - E_{rev}^{Na\ bath}. \quad (1)$$

For Na^+-rich pipette solutions:

$$V_m = -V_p + (E_{rev}^{Na\ bath} - E_{rev}^{K\ bath}) \quad (2)$$

where $E_{rev}^{Na\ bath}$ and $E_{rev}^{K\ bath}$ are the single-channel current reversal potentials in Na^+-rich and K^+-rich bath solutions, respectively. Because it was not always possible to obtain $E_{rev}^{Na\ bath}$ and $E_{rev}^{K\ bath}$ for the same patch, we subtracted a mean $E_{rev}^{K\ bath}$ ($n = 4$ patches) from each individual estimate of $E_{rev}^{Na\ bath}$. Outward current, the flow of positive charge from the inside to the outside of the membrane, is indicated as an upward deflection on all the records. Conductance data were obtained from linear current/voltage (I/V) plots by least-square regression analysis.

Open-state probability (P_o), mean open time (t_o), and mean closed time (t_c) were determined from 8-bit digitized data, sampled at either 5, 10 or 22 kHz, using a microcomputer (BBC Archimedes, Acorn Computers, Cambridge, UK) running a suite of programs based on a previously described algorithm [40]. Analysis of channel lifetimes was exclusively performed on patches which contained only one active channel, as judged by the absence of multiple current steps. The mean t_o and t_c were calculated by dividing the total open or closed time by the number of events [40]. This method will always overestimate t_o and t_c because some very fast events escape detection. We calculate that the maximum error would be about 7% for t_c, and about 2% for t_o.

Solutions and Chemicals

The Na^+-rich, extracellular type, solution had the following composition (in mM): 138.0 NaCl, 4.5 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 5.0 glucose, 10.0 HEPES at pH 7.4. The K^+-rich, intracellular-type, solution contained (in mM): 145.0 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 5.0 glucose, 10.0 HEPES at pH 7.4. For some experiments the Ca^{2+} concentration in this K^+-rich solution was stabilized using a buffer system which contained 2-mM ethyleneglycol-*bis*-(β-aminoethyl ether)-N,N′-tetraacetic acid. The free-Ca^{2+} and Mg^{2+} concentrations in these solutions were calculated [41] using the equilibrium constants given by Fabiato [11], and were either 0.1, 0.3, 1 or 3 μM for Ca^+, and 1 mM for Mg^{2+}.

The catalytic subunit (C-subunit) of cyclic AMP-dependent protein kinase was purified to apparent homogeneity from bovine adipose tissue as previously described [35], but in the absence of nonionic detergent. The purified enzyme had a specific activity

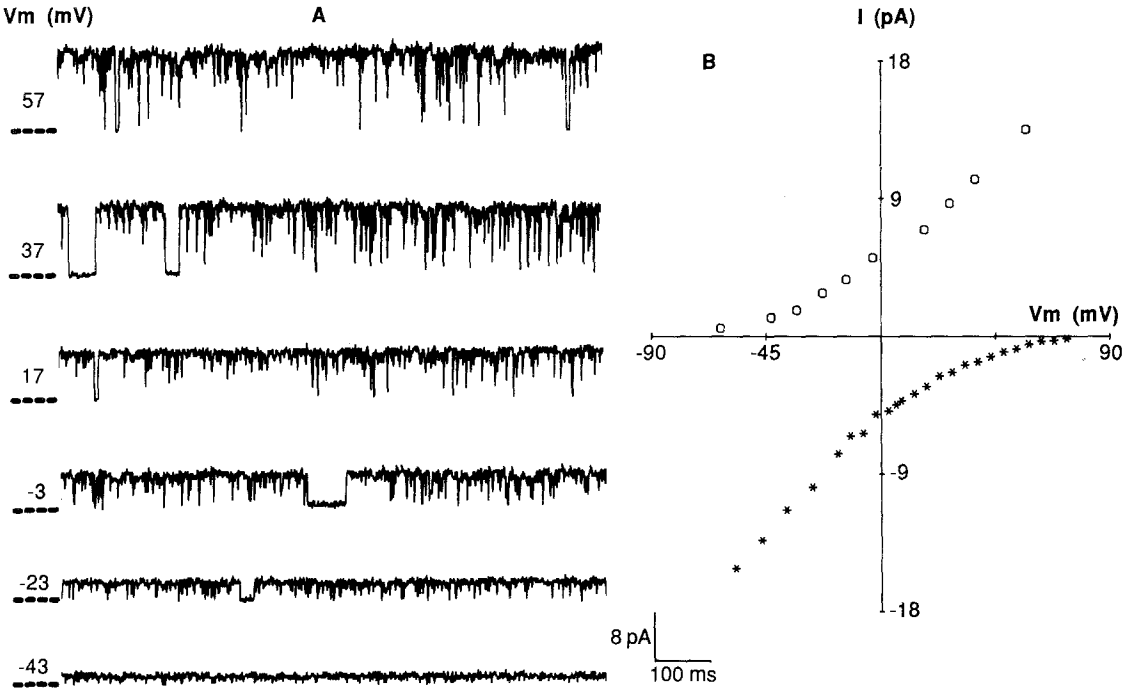

Fig. 1. Maxi-K⁺ channels in excised, inside-out, patches. (*A*) Typical single-channel currents recorded at the membrane potentials (V_m) indicated to the left of each trace. Dashed lines indicate the closed state of the channel. Solutions: pipette, Na^+-rich; bath, K^+-rich containing 2 mM Ca^{2+}. Low-pass filtered at 3 kHz. (*B*) Single-channel *I/V* plots. Solutions: (○) pipette, Na^+-rich; bath K^+-rich containing either 0.1 μM or 2 mM Ca^{2+}. Data from four patches with between one and four observations at each point. (*) pipette, K^+-rich; bath, Na^+-rich containing 2 mM Ca^{2+}. Data from nine patches with between one and nine observations at each point

of 200 U/mg protein, where 1 unit is defined as the incorporation of 1 nmol phosphate into 0.2 mg histone (type 2A) per min at 30°C. For electrophysiological experiments, aliquots were diluted in K^+-rich solution to give a final bath C-subunit concentration of 60–100 nM. In some experiments we inhibited C-subunit activity using a synthetic peptide which corresponds to residues 6–23 of the heat-stable inhibitor protein [32]. The final bath concentration of the inhibitor peptide was 20 μM, which reduces activity of 100 nM C-subunit by more than 99%.

A stock solution of 10 mM forskolin (Sigma, Poole, UK) was made up in ethanol and diluted 10,000-fold with the appropriate bath solution to give a final concentration of 1 μM. The final bath ethanol concentration was 0.01%, which alone had no effect on channel activity. Secretin, dibutyryl cyclic AMP and isobutylmethylxanthine were all made up as concentrated stocks in the appropriate bath solutions. The final bath concentrations were: secretin, 10 nM; dibutyryl cyclic AMP, 0.1 mM; and isobutylmethylxanthine, 0.1 mM. We employed two different protocols for stimulating the ductal epithelial cells. In some experiments cells were exposed to one, or a cocktail, of the stimulants throughout the experimental period which usually lasted between 0.5 and 6 hr. In other experiments, cells were briefly exposed to stimulants while cell-attached recording was in progress. All other chemicals were purchased from commercial sources and were of the highest purity available.

Statistics

Significance of difference between means was determined using Student's *t* test. The level of significance was set at $P < 0.05$. All values are expressed as mean ± SE (number of observations).

Results

Maxi-K⁺ Channels on Unstimulated Duct Cells

Figure 1*A* shows single-channel currents recorded from an inside-out patch excised from the basolateral membrane of a pancreatic duct cell. In this experiment the intracellular surface of the patch was bathed in a solution containing 2 mM Ca^{2+} and the channel was open for most of the time at all membrane potentials tested. With quasi-physiological ion gradients, that is with a K^+-rich solution bathing the cytoplasmic face and a Na^+-rich solution bathing the extracellular face of the membrane, there was a marked outward rectification of the single-channel currents; in fact inward currents were not detected (Fig. 1*B*). Under these conditions the reversal potential was clearly greater than −70 mV (Fig. 1*B*), and since K^+ is the only ion present with a negative equilibrium potential this immediately identifies the channel as being predominantly K^+-selective. When the orientation of the cation gradients was reversed the channel became an inward rectifier but, otherwise, the *I/V* relationship was unaffected (Fig. 1*B*). Changing the Ca^{2+} concentration on the inside-face of the membrane from 0.1 μM to

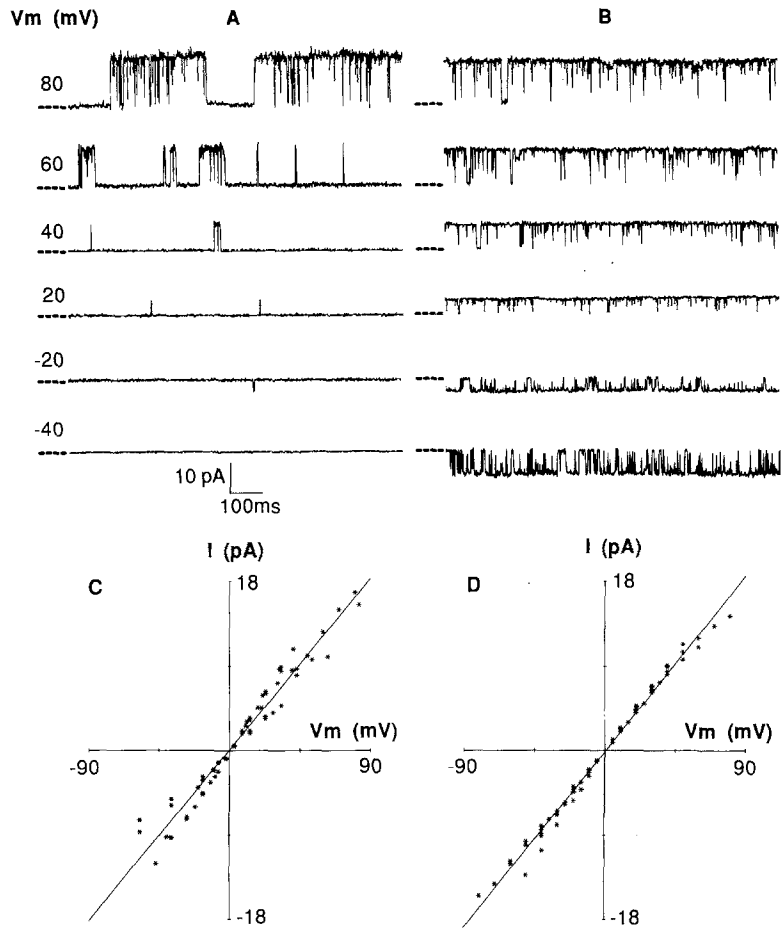

Fig. 2. Effect of Ca^{2+} and voltage on maxi-K^+ channels in excised, inside-out, patches. (*A* and *B*) Typical single-channel currents recorded from patches bathed in symmetrical K^+-rich solutions containing (*A*) 0.3 μM Ca^{2+} and (*B*) 2 mM Ca^{2+} on the cytoplasmic face of the membrane. Low-pass filtered at 3 kHz. (*C* and *D*) Single-channel *I/V* plots for patches bathed in (*C*) 0.3, 1.0 and 3.0 μM Ca^{2+} (five plots from three patches), and (*D*) 2 mM Ca^{2+} (five plots from five patches). The lines were fitted by least-squares regression analysis

2 mM had no effect on the conductance of the channel exposed to asymmetric Na^+ and K^+ solutions (Fig. 1*B*). On average each patch contained 1.75 ± 0.32 channels (n = 16 patches).

Figure 2*A* and *B* show single-channel currents recorded from inside-out patches bathed in symmetrical K^+-rich solutions. When the cytoplasmic face of the membrane was exposed to 0.3 μM Ca^{2+}, the single-channel open-state probability (P_o) was markedly voltage dependent, being increased by depolarization (Fig. 2*A*). Elevating the Ca^{2+} concentration to 2 mM increased P_o such that the channel was open for most of the time at voltages between −40 and 80 mV (Fig. 2*B*). Under these conditions the most frequent closing event was a rapid flickering from the fully open state, although occasionally longer closures were observed (Fig. 2*B*). Variations in Ca^{2+} had no effect on the *I/V* relationship which was linear in symmetrical K^+-rich solutions, reversed at V_m = 0 mV, and gave single-channel conductances of 203 ± 5.6 pS (0.3 to 3 μM Ca^{2+}, n = 5) (Fig. 2*C*) and 208 ± 2.6 pS (2 mM Ca^{2+}, n = 5) (Fig. 2*D*). Taken together, the results in Figs. 1 and 2 identify the channel as a voltage-dependent, Ca^{2+}-activated, maxi-K^+ channel. It was found only in patches obtained from the basolateral surface of the duct cell.

Figure 3*A* summarizes the interactions of Ca^{2+} and voltage on channel activity in excised, inside-out, patches. When the cytoplasmic face of the membrane was exposed to 2 mM Ca^{2+}, P_o remained close to 0.9 over the voltage range +80 to −55 mV, but decreased sharply at more hyperpolarizing potentials (Fig. 3*A*). Under these conditions the voltage required to give a P_o of 0.5 was −66 mV. Lowering the bath Ca^{2+} concentration shifted the P_o/voltage plots to the right and increased the voltage required to give a P_o of 0.5 to −4 mV at 3 μM Ca^{2+}, and +70 mV at 0.3 μM Ca^{2+} (Fig. 3*A*). When the bath contained 0.1 μM Ca^{2+}, P_o remained very low over the potential range −3 to +60 mV (Fig. 3*A*). Thus, changing the bath Ca^{2+} concentration shifted the P_o/voltage curves along the voltage axis; however, it had no appreciable effect on the voltage dependence of P_o.

Figure 3*B* and *C* show semilog plots of the mean closed (t_c), and mean open times (t_o) of the channel as a function of voltage and bath Ca^{2+} concentra-

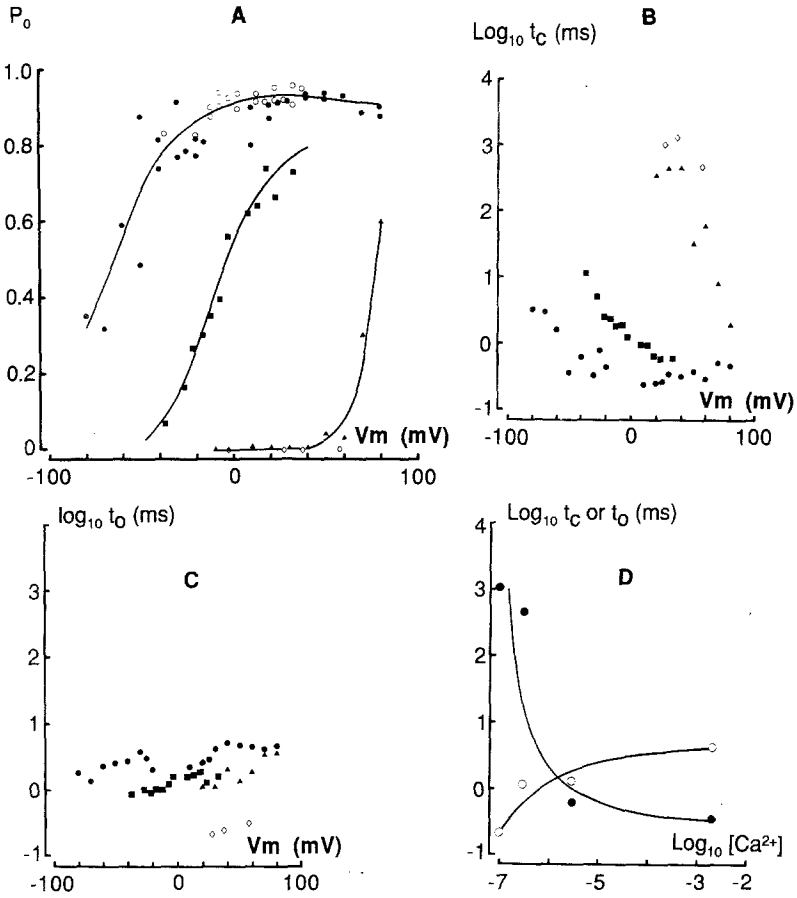

Fig. 3. Analysis of the effects of Ca^{2+} and voltage on open-state probability (P_o), mean closed time (t_c), and mean open time (t_o) of maxi-K^+ channels in inside-out patches. Bath Ca^{2+} concentrations: (○,●) 2 mM, (■) 3 μM, (▲), 0.3 μM, (◇) 0.1 μM Ca^{2+}. Open symbols: pipette, Na^+-rich; bath K^+-rich. Filled symbols: pipette and bath K^+-rich. (*A*) P_o. Data from eight patches. (*B*) Semilog plot of t_c from six patches. The t_c plateau observed with 2 mM Ca^{2+} is not due to a bandwidth limitation. Bandwidth was 200 μsec. (f = 5 kHz) in these experiments. (*C*) Semilog plot of t_o from six patches. (*D*) Effect of bath Ca^{2+} concentration on t_o (○) and t_c (●) at V_m = +30 mV. Constructed from the data in *B* and *C*

tion. These data reveal that at a fixed Ca^{2+} concentration, membrane depolarization caused only a small increase in t_o but markedly reduced t_c. The minimal duration of t_c was about 0.3 msec, and this limiting value was reached at membrane potentials around 0 mV in the presence of 2 mM Ca^{2+} (Fig. 3*B*). Thus the large, voltage-dependent, increases in P_o shown in Fig. 3*A* mainly result from the channel spending a shorter period of time in the closed state. A similar conclusion is reached if t_o and t_c are plotted against bath Ca^{2+} concentration at a fixed voltage (Fig. 3*D*). A change in bath Ca^{2+} from 0.1 μM to 2.0 mM (when V_m is clamped at +30 mV) caused t_o to increase 20-fold, whereas t_c decreased by a factor of 3,400 (Fig. 3*D*).

Figure 4*A–D* details the characteristics of maxi-K^+ channel activity in cell-attached patches. The current traces shown in Fig. 4*A* were obtained with a K^+-rich solution in the recording pipette, and show that membrane depolarization increased channel P_o. However, channel openings were only rarely observed at voltages close to the resting membrane potential of the cell (Fig. 4*A*). Similar results were obtained with Na^+-rich pipette solutions.

Typical cell-attached *I/V* relationships for maxi-K^+ channels are shown in Fig. 4*B*. With Na^+-rich solutions in both pipette and bath there was a marked outward rectification of the single-channel currents, and inward currents were not detected even at strong hyperpolarizing potentials. Replacing the Na^+-rich bath solution with a K^+-rich solution shifted the cell-attached *I/V* relationship to the left (Fig. 4*B*). The magnitude of this shift, estimated from reversal potentials determined by extrapolation of the outward currents, was −49 ± 8 mV (n = 7, the mean of four $E_{rev}^{K\ bath}$ determinations was subtracted from each of seven $E_{rev}^{Na\ bath}$ values). This equals the membrane potential assuming the cells were fully depolarized by the K^+-rich bath solution.

When the recording pipette contained a K^+-rich solution the cell-attached *I/V* relationship was linear (Fig. 4*B*). Intracellular [K^+] has not been measured in pancreatic duct cells; however, assuming pipette [K^+] is close to intracellular [K^+], the reversal potential determined under these conditions will be close to the membrane potential of the cell. The mean value for eight cells was −33 ± 6.7 mV, and is not significantly different from the value estimated

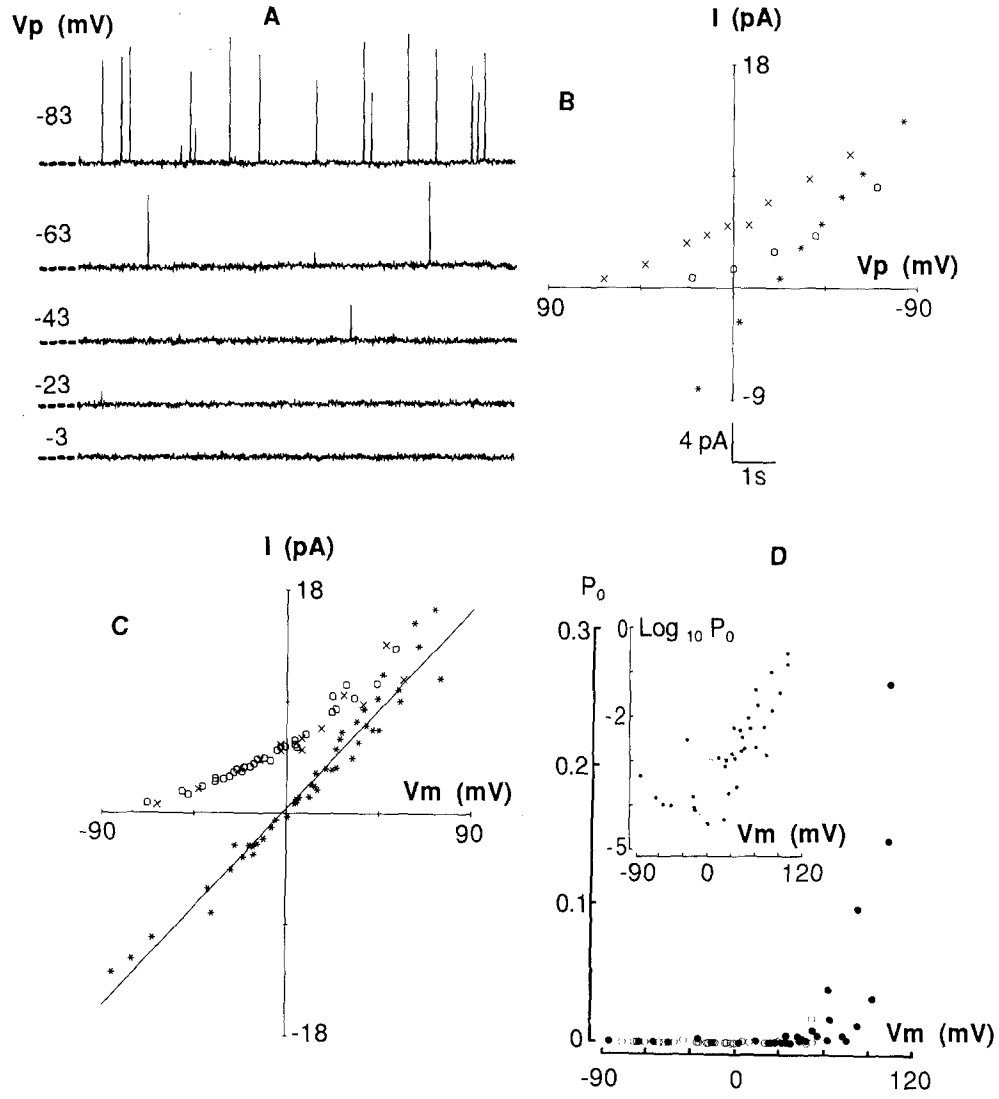

Fig. 4. Maxi-K^+ channels in cell-attached patches on unstimulated duct cells. (*A*) Typical single-channel currents recorded at pipette potentials (V_p) shown to the left of each trace. Low-pass filtered at 2 kHz. Solutions: pipette, K^+-rich; bath, Na^+-rich. (*B*) *I/V* plots. (*) pipette, K^+-rich; bath, Na^+-rich. (O) pipette and bath, Na^+-rich. (×) pipette, Na^+-rich; bath, K^+-rich. The two plots with Na^+-rich pipette solutions (O,×) were obtained from the same patch. (*C*) Summary of cell-attached *I/V* data. Same solutions as *B*. (*) eight patches; (O) seven patches; (×) four patches. To facilitate comparison of *I/V* plots from different cells the voltage axis has been scaled as V_m instead of V_p. V_m was calculated as described in Materials and Methods. (*D*) Effects of V_m on P_o. (O) Na^+-rich pipette solution, eight patches. (●) K^+-rich pipette solution, six patches. Inset shows a semilog plot of the same data

using Na^+-rich pipette solutions (*see above*). These estimated membrane potentials fall within the range (−33 to −63 mV at 37°C) of those previously measured in duct cells with microelectrodes [15, 28].

All eight cell-attached *I/V* plots obtained with K^+-rich pipette solutions, and the 11 obtained with Na^+-rich solutions, are shown in Fig. 4*C*. To allow comparison of plots obtained from different cells, the voltage axis has been scaled as membrane potential rather than pipette potential. These combined results give a cell-attached single-channel conductance of 180 ± 4 pS (n = 8) with K^+-rich pipette solutions. This is significantly lower (P = 0.01) than the value of 203 ± 5.6 pS obtained from excised patches bathed in symmetrical K^+-rich solutions containing between 0.3 and 3 μM Ca^{2+} (Fig. 2*C*).

Figure 4*D* depicts the relationship between membrane potential and the activity of maxi-K^+ channels on intact duct cells. Over the potential range −90 to +30 mV, P_o varied widely in different cells but was generally less than 0.001. However, channel activity increased steeply at membrane potentials above +30 mV, with P_o reaching a value of 0.26 at +102 mV which was the highest potential tested (Fig. 4*D*). Comparison of these cell-attached

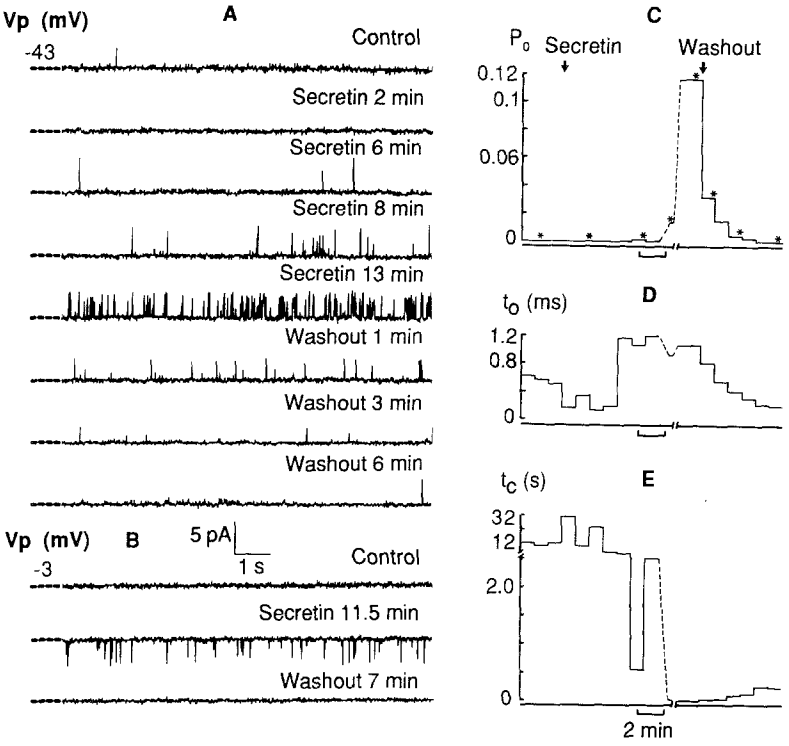

Fig. 5. Activation of maxi-K+ channels by secretin. (*A*) Typical single-channel currents recorded from a cell-attached patch at V_p = −43 mV before (*Control*), during exposure to 10 nM secretin (*Secretin*), and after washout of the hormone from the tissue bath (*Washout*). The traces show 10 sec of data, and start at the times indicated after addition, or washout, of the hormone. Solutions: pipette, K+-rich; bath, Na+-rich. Low-pass filtered at 2 kHz. (*B*) Same cell as *A*, but currents recorded at V_p = −3 mV. (*C–E*) Effects of secretin on P_o (*C*), t_o (*D*), and t_c (*E*). Asterisks indicate when records shown in *A* were obtained, and dashed lines the periods when V_p was varied from −43 mV (*see B*). One of these periods lasted 4 min and is indicated by a break in the time axes. Also note the change of scale on the vertical axis in *E*. The arrows marked '*Secretin*' and '*Washout*' indicate when secretin was added to the bath, and when bath perfusion with a Na+-rich, hormone-free, solution was started. P_o was calculated over 60-sec periods, except for one 15-sec segment of data

results (Fig. 4*D*) with inside-out data (Fig. 3*A*) suggests that the intracellular Ca^{2+} concentration in unstimulated duct cells is less than 0.3 μM.

Maxi-K+ Channels on Stimulated Duct Cells

The effect of secretin on maxi-K+ channel activity in a cell-attached patch is shown in Fig. 5*A–E*. For most of this experiment the membrane was voltage clamped at a depolarizing potential (V_p = −43) (Fig. 5*A*), however, some current records were also obtained at V_p = −3 mV, i.e., when the voltage across the patch was close to the resting membrane potential of the cell (Fig. 5*B*). At both potentials channel activity was very low prior to stimulation, however, adding 10 nM secretin to the bath caused a gradual increase in activity and this effect was reversed after hormone washout (Fig. 5*A* and *B*). Activation most probably results from an increase in P_o of individual channels, rather than an increase in channel number, because the maximum number of simultaneous current steps did not change following stimulation. Because secretin would not have had access to its receptors on the membrane patch, the enhanced channel activity must have been caused either by depolarization of the cell or by an intracellular messenger. In this experiment depolarization can be ruled out as a major factor because there was little or no change in the amplitude of the single-channel currents following secretin stimulation (Fig. 5*A*).

These effects of secretin are summarized in Fig. 5*C* which shows K+ channel P_o (at V_p = −43 mV) over the whole experiment from which the current traces in Fig. 5*A* were obtained. Measured over 3 min prior to addition of secretin the P_o was very low, about 0.00005. This subsequently increased 20-fold to 0.00086 after 6-min exposure to the hormone, and by 280-fold to 0.014 after 8-min exposure. In this experiment the maximum P_o observed in the presence of secretin was 0.116. This was reached 13 min after addition of the hormone and represents a 2,300-fold increase in channel activity above control levels (Fig. 5*C*). These large, hormone-induced, changes in P_o mainly resulted from a very substantial reduction in t_c, whereas t_o only increased by a factor of 2 (Fig. 5*D* and *E*).

After secretin washout the P_o declined slowly and eventually fell to 0.0009 after 6 min, however, this is still some 20-fold higher than the control value (Fig. 5*C*). At this point t_o had returned to prestimulation values (Fig. 5*D*) but t_c, although increasing, was only about 2% of the control value (Fig. 5*E*). Acute exposure to stimulants increased maxi-K+ channel activity in four cells (three with secretin, one with forskolin), and the average maximum increment in channel activity was 748-fold (range 37–2,300) above control levels. Attempts to

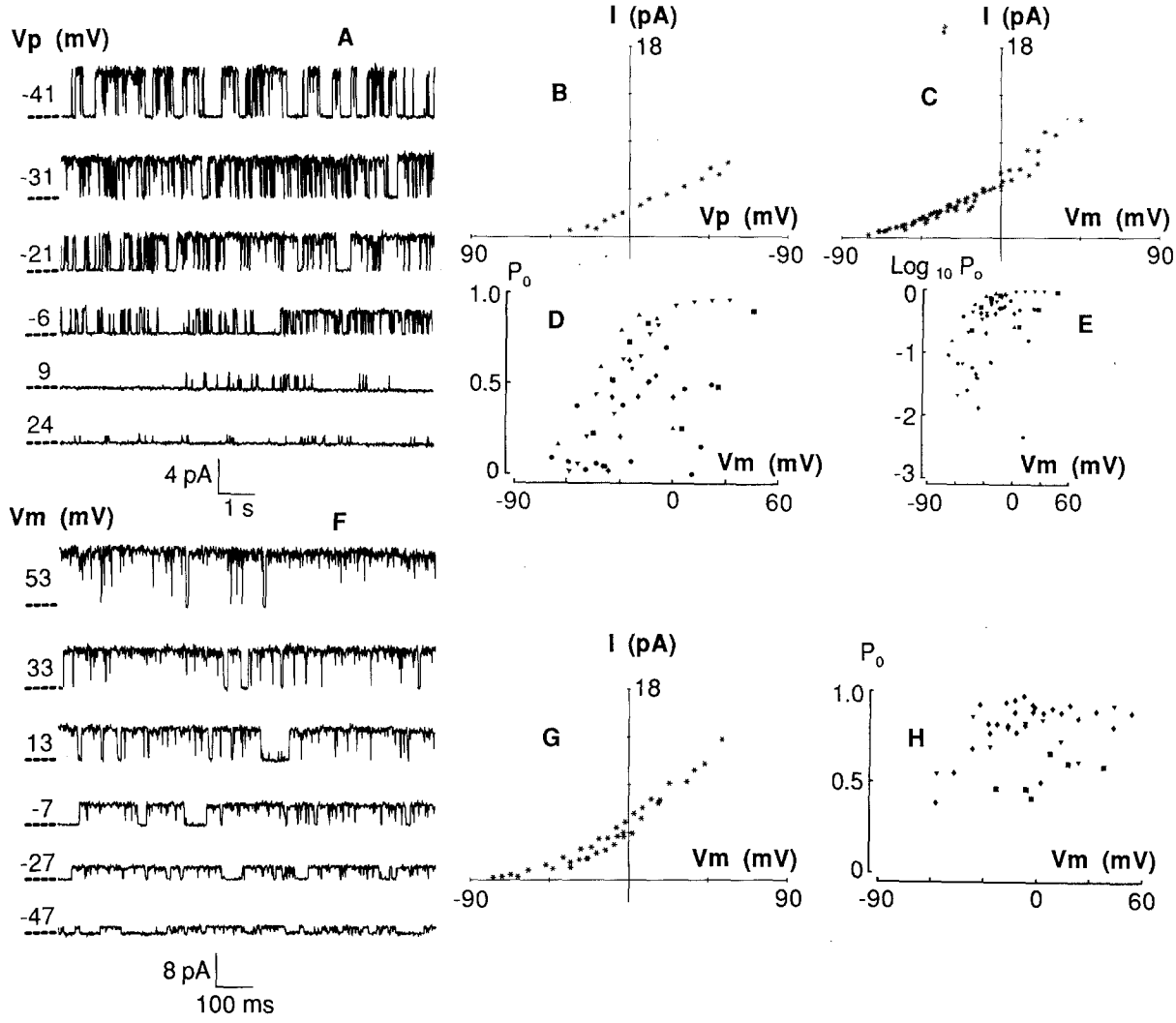

Fig. 6. Stimulant-induced activation of maxi-K^+ channels on intact cells is retained after patch excision. (*A–E*) Cell-attached patches. (*A*) Typical single-channel currents recorded from a cell that had been exposed to 1 μM forskolin. Solutions: pipette, Na^+-rich; bath, Na^+-rich. Low-pass filtered at 2 kHz. (*B*) *I/V* plot for channel shown in *A*. (*C*) Summary of *I/V* data obtained from seven prestimulated cells. Same conditions as *A*. V_m was calculated as described in Materials and Methods. (*D*) P_o as a function of V_m for seven prestimulated cells. Each symbol represents a different cell. (*E*) Semilog plot of the data in *D*. (*F–H*) Inside-out patches. (*F*) Same experiment as *A*. After completion of cell-attached recording the patch was excised, and the bath medium changed to a K^+-rich solution containing 0.1 μM Ca^{2+}. Low-pass filtered at 3 kHz. (*G*) *I/V* plots. Patches excised from four prestimulated cells. Same conditions as *F* but with either 0.1 (n = 3), 1, or 3 μM Ca^{2+} in the bath solution. (*H*) P_o as a function of V_m in four patches excised from different prestimulated cells. Solutions: pipette, Na^+-rich; bath, K^+-rich containing either 0.1 (◆, n = 3), 1 (▼), or 3 μM Ca^{2+} (■)

stimulate channel activity in two other cells were unsuccessful.

Figure 6*A–E* details the characteristics of maxi-K^+ channels on duct cells that had been exposed to 1 μM forskolin, 0.1 mM isobutylmethylxanthine and 0.1 mM dibutyryl cyclic AMP prior to patch clamping. Comparison of the cell-attached currents recorded from these cells (Fig. 6*A*), with those recorded from unstimulated cells (Fig. 4*A*), indicates that stimulation had markedly increased channel activity. Furthermore, the P_o/voltage relationship was much more variable in prestimulated cells and was shifted towards hyperpolarizing potentials (*compare* Fig. 6*D* and *E* with Fig. 4*D*). However, the *I/V* plot was unaffected (*compare* Fig. 6*B* and *C* with Fig. 4*B* and *C*), and the membrane potential (−54 ± 8.5 mV, n = 7) was not statistically different from the value calculated for unstimulated cells using Na^+-rich pipette solutions (*see above*).

When cell-attached patches on prestimulated cells were excised into low Ca^{2+} solutions, K^+ channel activity remained high (Fig. 6*F–H*). The current traces in Fig. 6*F* were obtained from the same patch as those in Fig. 6*A* immediately after excision into the inside-out recording configuration, and after the bath medium had been changed to a

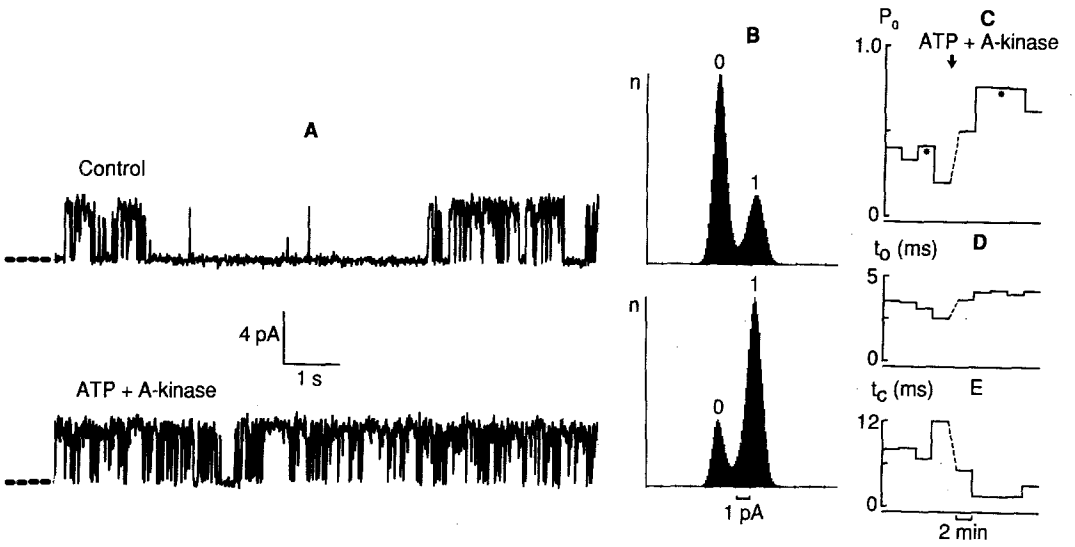

Fig. 7. Activation of maxi-K^+ channels by the purified catalytic subunit of cyclic AMP-dependent protein kinase. (*A*) Typical single-channel currents recorded from an inside-out patch excised from an unstimulated duct cell. V_m was −3 mV. Top record, control. Lower record, starts 7 min after addition of 0.25 mM ATP and 60 nM catalytic subunit of protein kinase to the bath. Solutions: pipette, Na^+-rich; bath, K^+-rich containing 1 μM Ca^{2+}. Low-pass filtered at 3 kHz. (*B*) Current amplitude histograms derived by analysis of 60-sec recordings, including the data shown in *A*. Top histogram, control. Lower histogram, ATP + A-kinase. The closed and open states of the channel are labeled 0 and 1, respectively. (*C–E*) Effects of ATP + A-kinase on P_o (*C*), t_o (*D*) and t_c (*E*). Asterisks indicate when data shown in *A* and *B* were obtained, and the arrow when ATP + A-kinase were added to the bath. Dashed line indicates period of access to the screened cage

K^+-rich solution containing 0.1 μM Ca^{2+}. These records should be compared with those in Fig. 2*A*, which were obtained from a patch excised from an unstimulated cell and bathed in 0.3 μM Ca^{2+}. Channel P_o is clearly much higher in the prestimulated patch, despite a lower Ca^{2+} concentration on the cytoplasmic face of the membrane. As in the cell-attached configuration, the *I/V* relationships for maxi-K^+ channels in patches excised from prestimulated and unstimulated duct cells were identical (*compare* Figs. 6*G* and 1*B*). Patches derived from prestimulated cells contained on average 1.5 ± 0.4 channels (n = 8 patches). This is not significantly different from the value obtained for unstimulated cells (1.8 ± 0.3), which suggests that stimulation does not affect channel density.

Figure 6*H* summarizes the relationship between P_o and voltage in four inside-out patches that had been excised from stimulated cells into bath solutions containing either 0.1, 1 or 3 μM Ca^{2+}. A comparison of these data with the results obtained using patches excised from unstimulated cells (Fig. 3*A*) indicates that channel activity is now much less affected by a reduction in bath Ca^{2+} concentration. In fact, the P_o/voltage plots for channels in 'stimulated' patches exposed to 0.1 μM Ca^{2+} on the cytoplasmic face of the membrane are essentially the same as those for channels in 'unstimulated' patches exposed to 2 mM Ca^{2+} (*compare* Figs. 3*A* and 6*H*).

These results obtained with excised patches show that membrane depolarization, and/or a direct effect of intracellular Ca^{2+}, cannot account for stimulant-induced channel activation on intact duct cells. However, because secretin increases the cyclic AMP level in isolated pancreatic ducts [3], and forskolin activates the catalytic subunit of adenylate cyclase, an alternative explanation is phosphorylation of the maxi-K^+ channel, or an associated regulatory subunit, by cyclic AMP-dependent protein kinase.

Activation of Maxi-K^+ Channels in Excised Patches by the Catalytic Subunit of Cyclic AMP-Dependent Protein Kinase (C-Subunit)

The upper current trace in Fig. 7*A* was obtained from an inside-out patch excised from an unstimulated cell and exposed to quasi-physiological ion gradients with 1 μM Ca^{2+} on the cytoplasmic face of the membrane. Since the membrane was voltage clamped at V_m = −3 mV, the single-channel currents are outward. The lower current trace was recorded 7 min after adding 0.25 mM ATP and 60 nM of purified C-subunit to the bath solution. This caused a marked increase in channel activity. The 60-sec current amplitude histograms in Fig. 7*B* show that there was only one active channel in this patch, and that P_o increased from 0.326 to 0.781 after exposure to ATP/protein kinase-A. This level

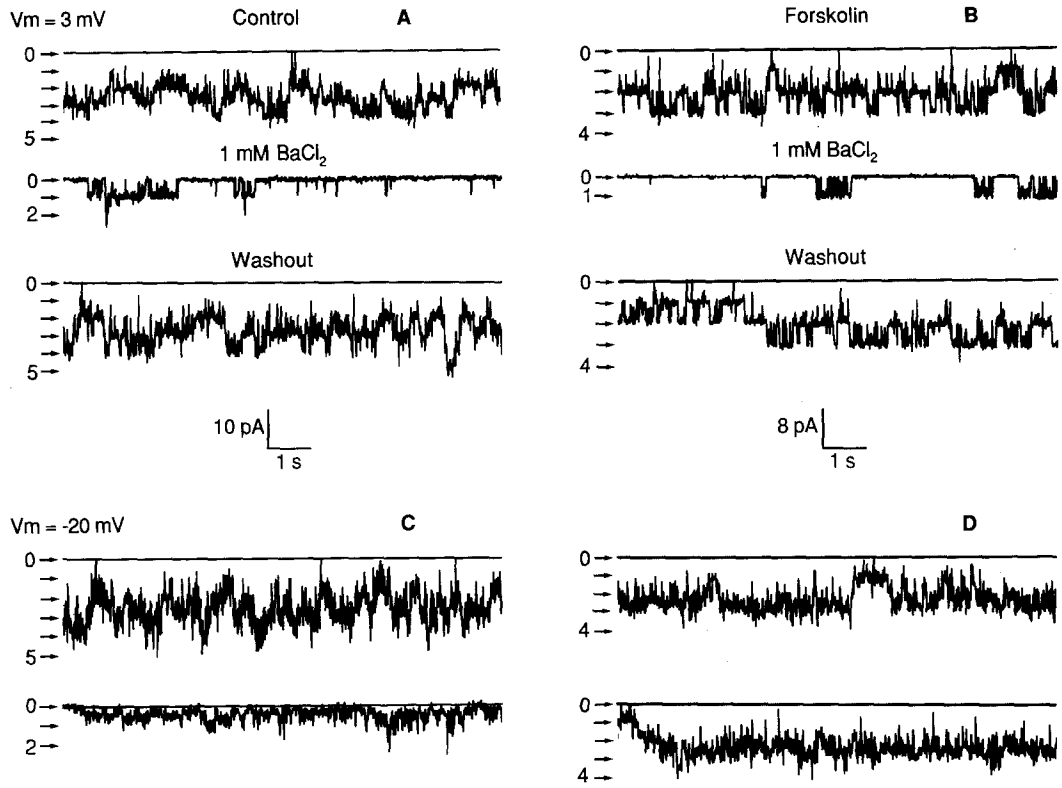

Fig. 8. Barium block of maxi-K^+ channels in inside-out patches. (*A*) Unstimulated cell. Solutions: pipette, K^+-rich; bath, Na^+-rich containing 2 mM Ca^{2+}. Top record, control. Middle record, 1 mM Ba^{2+} in the bath solution. Lower record, after Ba^{2+} washout. Arrows indicate number of channels open simultaneously. (*B*) Forskolin-stimulated cell. Same conditions as *A*. Top record, control. Middle record, 1 mM Ba^{2+}. Lower record, after Ba^{2+} washout. (*C* and *D*) Same patches as *A* and *B* but with a K^+-rich solution containing 2 mM (upper records) or 0.1 μM Ca^{2+} (lower records) in the bath. $V_m = -20$ mV. All records low-pass filtered at 2 kHz

of channel activity is similar to that found in inside-out patches excised from stimulated cells and bathed in 1 μM Ca^{2+} (Fig. 6*H*).

These effects of C-subunit are summarized in Fig. 7*C* which plots K^+ channel P_o for the whole experiment from which the current traces in Fig. 7*A* were obtained. Channel activity was clearly elevated 4 min after addition of C-subunit to the bath solution. P_o averaged 0.336 over the 8-min control period; 0.499 over the first 2 min after addition of C-subunit; and 0.724 over the remaining 8 min of the experiment. Thus, ATP/protein kinase-A caused a maximum twofold increase in channel activity which resulted largely from a reduction in t_c, together with a small increase in t_o (Fig. 7*D* and *E*). Overall, three out of five attempts to activate K^+ channels in excised patches with ATP/protein kinase-A were successful, the average maximum increase in channel activity being 28-fold (range 2- to 58-fold). In two separate control experiments, 0.25 mM ATP alone had no effect on channel activity. In three further experiments, we exposed patches to ATP/protein kinase-A plus 20 μM inhibitor peptide for an average time of 13 min and detected no changes in K^+ channel activity. Finally, three patches were exposed to 100 nM bovine serum albumin for 8 min, and again there was no effect on the channel.

Effects of Barium on Maxi-K^+ Channel Activity

Figure 8*A* shows that maxi-K^+ channels in a patch excised from an unstimulated duct cell were blocked by 1 mM Ba^{2+}, and that this effect was fully reversed after Ba^{2+} washout. Similar results were obtained for channels in a patch excised from a cell that had been stimulated with 1 μM forskolin (Fig. 8*B*). From these results it is apparent that stimulant-induced phosphorylation does not affect the ability of Ba^{2+} to block maxi-K^+ channel activity. The current traces in Fig. 8*C* and *D* were obtained from the same patches after reversal of Ba^{2+} blockade, and show that lowering the Ca^{2+} concentration on the cytoplasmic face of the membrane from 2 mM to 0.1 μM markedly reduced channel activity in the 'unsti-

mulated' patch, but had little effect on channels in the 'stimulated patch.'

Discussion

Using the patch-clamp technique we have identified a large conductance K^+ channel on the basolateral surface of rat pancreatic duct cells. Channel P_o was increased by membrane depolarization and by an elevation in cytoplasmic Ca^{2+} concentration, and blocked by barium. Large conductance or maxi-K^+ channels [21] with similar properties have previously been described in muscle cells [4, 21], nerve cells [12], endocrine cells [14, 24, 42], and in a variety of epithelial cell types (exocrine gland acini [13, 25, 26, 30, 37], enterocytes [27, 34], choroid plexus [6, 8], and renal collecting duct [20]).

We found that bath Ca^{2+} concentrations in excess of 0.1 μM were required to significantly activate maxi-K^+ channels in patches excised from unstimulated duct cells. This is in marked contrast to the effect of Ca^{2+} on the P_o of these channels in acinar cells of the pig [26] and guinea pig pancreas [37], the mandibular salivary gland [25], the lacrimal gland [13], and rat enterocytes [27]. In all these tissues channel activity is detectable with 0.1 μM Ca^{2+} on the cytoplasmic face of the membrane, even at hyperpolarizing potentials, and full activation occurs at voltages around +20 mV [13, 26, 27]. In terms of Ca^{2+} sensitivity, the pancreatic duct cell channel more closely resembles maxi-K^+ channels in cultured skeletal muscle [4], choroid plexus [6, 8] and pancreatic islet cells [14]. In these tissues depolarizing potentials as high as +50 mV have little effect on channel activity with 0.1 μM Ca^{2+} on the cytoplasmic face of the membrane. Maxi-K^+ channels in anterior pituitary cells [42], *Necturus* enterocytes [34], and renal collecting duct [20] fall into an intermediate category in terms of Ca^{2+} sensitivity.

Secretin is the physiological regulator of pancreatic bicarbonate transport and there is good evidence to suggest that this hormone uses cyclic AMP, rather than Ca^{2+} as an intracellular messenger [7]. Exposing duct cells to secretin during cell-attached recording markedly increased maxi-K^+ channel activity, and this effect was fully reversed when the hormone was washed from the tissue bath. Activation probably results from an increase in P_o of individual channels, rather than an increase in channel number, because the maximum number of simultaneous current steps did not change following stimulation. This increase in P_o largely results from a decrease in the time that the channel spends in the closed state, together with a relatively small increase in open time. A similar mechanism underlies the effects of Ca^{2+} and voltage on channel P_o in patches excised from unstimulated cells; however, it is unlikely that stimulant-induced channel activation can be explained by depolarization or an increase in intracellular Ca^{2+} since (*i*) activation occurred without any significant alteration in the size of single-channel currents, and (*ii*) secretin has no detectable effect on intracellular Ca^{2+} in pancreatic duct cells (J.I. Gillespie & B.E. Argent, *unpublished observations*). Receptor-gated channel activation can also be excluded because secretin would not have had access to the internal surface of the isolated membrane patches. More likely, channel activation results from an increase in intracellular cyclic AMP since forskolin, which activates the catalytic subunit of adenylate cyclase, also increased channel activity. However, neither secretin nor forskolin had any effect on channel conductance in cell-attached patches.

In addition to causing an increase in channel activity, stimulation shifted the cell-attached P_o/voltage relationship to the left, such that channel activity was observed at hyperpolarizing membrane potentials. This is strikingly different from the behavior of the channel on unstimulated cells where P_o remained below 0.001 until the membrane potential was depolarized to more than +30 mV. Furthermore, when patches on stimulated cells were excised, channel activity remained high. This observation suggested a stable modification of the channel protein, or an associated regulatory subunit, and confirmed that stimulant-induced channel activation was not caused by a direct effect of raised intracellular Ca^{2+}. However, stimulation did affect the response of the channel to Ca^{2+}. Channels in patches excised from stimulated cells were almost fully activated by 0.1 μM Ca^{2+} over the potential range −30 to +60 mV, and even at −60 mV the P_o was 0.4 under these experimental conditions. Comparable levels of channel activity were only observed in patches derived from unstimulated cells when the cytoplasmic face of the membrane was bathed in 2 mM Ca^{2+}. As with intact cells, stimulation did not affect channel conductance in excised patches and, furthermore, did not affect the ability of Ba^{2+} to block channel activity. Since Ba^{2+} enters the channel, and probably binds to the selectivity filter [39], this result may indicate that the modulation site is outside the ion-conducting pore.

To test if the effects of stimulants resulted from cyclic AMP-dependent phosphorylation, we exposed the cytoplasmic face of patches excised from unstimulated cells to ATP and the purified C-subunit of cyclic AMP-dependent protein kinase. This caused a marked increase in maxi-K^+ channel activity. Furthermore, channel P_o values after activation by cyclic AMP-dependent protein kinase were comparable to those in patches excised from stimulated

cells, and exposed to the same Ca^{2+} concentration on the cytoplasmic face of the membrane. These results provide direct evidence for the activation of single maxi-K^+ channels in pancreatic duct cells by protein phosphorylation and suggest that the effects of secretin and forskolin on the intact cell are caused by protein phosphorylation mediated by cyclic AMP. While we cannot determine whether the K^+ channel protein itself is phosphorylated or whether a separate regulatory subunit (or a membrane-bound kinase) is involved, activation in excised patches does indicate that the phosphorylated protein remains closely associated with the channel when this is removed from the cell. A cytoplasmic phosphatase may be required for channel deactivation because channel activity in cell-attached patches was quite rapidly reversed on removal of stimulants, but remained high if patches were excised from stimulated cells.

The very low P_o of the channel on unstimulated duct cells may indicate that it does not contribute to the resting, Ba^{2+}-sensitive, K^+ conductance of the basolateral membrane [28]. However, we have provided clear evidence for a stimulant-induced increase in channel activity which could have two roles in bicarbonate transport. First, it would tend to hyperpolarize the duct cell, offsetting the depolarizing effect of an increased apical anion conductance [15, 29] and maintaining a driving force for electrogenic anion exit at the apical membrane. Second, increased K^+ efflux across the basolateral membrane would act to balance charge movement across the apical membrane during secretion. In this respect the basolateral K^+ conductance of the duct cell fulfills exactly the same role as the regulated K^+ conductance in Cl^--secreting epithelia [30]. As is thought to occur in these epithelia [30], some of the released K^+ would be expected to diffuse into the duct lumen via the leaky paracellular pathway. Such an effect would explain the dose-dependent increase in pancreatic juice K^+ concentration (from 5 to 10 mM) that has been shown to occur following stimulation of the rat pancreas with secretin [33]. Although it has been reported that Ba^{2+} has no effect on secretin-stimulated fluid secretion from the perfused rat pancreas [9], this could be explained by poor uptake of Ba^{2+} into the duct cell since it is thought to block the channel from the inside face of the plasma membrane [39].

It is well known that cyclic AMP can modulate the electrical activity of excitable cells, and some of these effects involve modulation of K^+ channel activity [23]. Phosphorylation catalyzed by protein kinase-A closes Ca^{2+}- and voltage-independent (S) K^+ channels in *Aplysia* sensory neurons (*see* ref. [5]) but activates Ca^{2+}- and voltage-dependent K^+ channels in *Helix* ganglia [10], rat brain synaptosomes [12] and smooth muscle cells cultured from rat aortas [31]. In the instances where channel activation occurs, this results from an increase in single-channel P_o, without any effect on channel conductance or channel numbers, and is associated with an increase in the sensitivity of the channel to Ca^{2+} [10, 12, 31].

There is also evidence to suggest that cyclic AMP-dependent phosphorylation modulates K^+ channels in epithelia. The P_o of a 33-pS K^+ channel on *Necturus* oxyntic cells, which is Ca^{2+} insensitive and weakly activated by hyperpolarization, can be increased by exposure of the cells to dibutyryl cyclic AMP [38]. Furthermore, a Ca^{2+}- and voltage-sensitive K^+ channel on cultured avian kidney cells, which has a conductance of 107 pS, can be activated by forskolin and antidiuretic hormone [18]. Recently, it has also been shown that maxi-K^+ channels on lacrimal acinar cells can be activated by vasoactive intestinal polypeptide through a cyclic AMP-dependent mechanism, and that these effects can be mimicked by exposure of excised patches to protein kinase-A [22]. Our results provide the first demonstration that cyclic AMP-dependent phosphorylation can alter the Ca^{2+} responsiveness of maxi-K^+ channels in epithelia. This might be a general mechanism by which Ca^{2+}-activated, voltage-dependent, maxi-K^+ channels are modulated, and could explain the wide variation in Ca^{2+} sensitivity reported for this channel in different tissues.

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References

1. Argent, B.E., Arkle, S., Cullen, M.J., Green, R. 1986. Morphological, biochemical and secretory studies on rat pancreatic ducts maintained in tissue culture. *Q. J. Exp. Physiol.* **71**:633–648
2. Argent, B.E., Arkle, S., Gray, M., Greenwell, J.R. 1987. Two types of calcium-sensitive cation channels in isolated rat pancreatic duct cells. *J. Physiol. (London)* **386**:82P
3. Arkle, S., Lee, C.M., Cullen, M.J., Argent, B.E. 1986. Isolation of ducts from the pancreas of copper-deficient rats. *Q. J. Exp. Physiol.* **71**:249–265
4. Barrett, J.N., Magleby, K.L., Pallotta, B.S. 1982. Properties of single calcium-activated potassium channels in cultured rat muscle. *J. Physiol. (London)* **331**:211–230
5. Belardetti, F., Siegelbaum, S.A. 1988. Up- and down-regulation of single K^+ channel function by distinct second messengers. *Trends Neurosci.* **11**:232–238
6. Brown, P.D., Loo, D.D.F., Wright, E.M. 1988. Ca^{2+}-activated K^+ channels in the apical membrane of *Necturus* choroid plexus. *J. Membrane Biol.* **105**:207–219
7. Case, R.M., Argent, B.E. 1989. Pancreatic secretion of electrolytes and water. *In:* Handbook of Physiology. The Gastrointestinal System, Vol. III (Sec. 6). S.G. Schultz, J.G.

Forte, and B.B. Rauner, editors. pp. 383–417. Oxford University Press, New York
8. Christensen, O., Zeuthen, T. 1987. Maxi K^+ channels in leaky epithelia are regulated by intracellular Ca^{2+}, pH and membrane potential. *Pfluegers Arch.* **408:**249–259
9. Evans, L.A.R., Pirani, D., Cook, D.I., Young, J.A. 1986. Intraepithelial current flow in rat pancreatic secretory epithelia. *Pfluegers Arch.* **407(Suppl. 2):**S107–S111
10. Ewald, D.A., Williams, A., Levitan, I.B. 1985. Modulation of single Ca^{2+}-dependent K^+-channel activity by phosphorylation. *Nature (London)* **315:**503–506
11. Fabiato, A. 1981. Myoplasmic free calcium concentration reached during the twitch of an intact isolated cardiac cell and during calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned cardiac cell from the adult rat or rabbit ventricle. *J. Gen. Physiol.* **78:**457–497
12. Farley, J., Rudy, B. 1988. Multiple types of voltage-dependent Ca^{2+}-activated K^+ channels of large conductance in rat brain synaptosomal membranes. *Biophys. J.* **53:**919–934
13. Findley, I. 1984. A patch-clamp study of potassium channels and whole-cell currents in acinar cells of the mouse lacrimal gland. *J. Physiol. (London)* **350:**179–195
14. Findlay, I., Dunne, M.J., Petersen, O.H. 1985. High-conductance K^+ channel in pancreatic islet cells can be activated and inactivated by internal calcium. *J. Membrane Biol.* **83:**169–175
15. Gray, M.A., Greenwell, J.R., Argent, B.E. 1988. Ion channels in pancreatic duct cells: Characterization and role in bicarbonate secretion. *In:* Cellular and Molecular Basis of Cystic Fibrosis. G. Mastella and P.M. Quinton, editors. pp. 205–216. San Francisco Press, San Francisco
16. Gray, M.A., Greenwell, J.R., Argent, B.E. 1988. Secretin-regulated chloride channel on the apical plasma membrane of pancreatic duct cells. *J. Membrane Biol.* **105:**131–142
17. Gray, M.A., Harris, A., Coleman, L., Greenwell, J.R., Argent, B.E. 1989. Two types of chloride channel on duct cells cultured from human fetal pancreas. *Am. J. Physiol.* **257:**C240–C251
18. Guggino, S.E., Suarez-Isla, B.A., Guggino, W.B., Sacktor, B. 1985. Forskolin and antidiuretic hormone stimulate a Ca^{2+}-activated K^+ channel in cultured kidney cells. *Am. J. Physiol.* **249:**F448–F455
19. Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100
20. Hunter, M., Lopes, A.G., Boulpaep, E., Giebisch, G. 1986. Regulation of single potassium ion channels from apical membrane of rabbit collecting tubule. *Am. J. Physiol.* **251:**F725–F733
21. Latorre, R., Miller, C. 1983. Conduction and selectivity in potassium channels. *J. Membrane Biol.* **71:**11–30
22. Lechleiter, J.D., Dartt, D.A., Brehm, P. 1988. Vasoactive intestinal peptide activates Ca^{2+}-dependent K^+ channels through a cAMP pathway in mouse lacrimal cells. *Neuron* **1:**227–235
23. Levitan, I.B. 1985. Phosphorylation of ion channels. *J. Membrane Biol.* **87:**177–190
24. Marty, A. 1981. Ca-dependent K channels with large unitary conductances in chrommafin cell membranes. *Nature (London)* **291:**497–500
25. Maruyama, Y., Gallacher, D.V., Petersen, O.H. 1983. Voltage and Ca^{2+}-activated K^+ channel in basolateral acinar cell membranes of mammalian salivary glands. *Nature (London)* **302:**827–829
26. Maruyama, Y., Petersen, O.H., Flanagan, P., Pearson, G.T. 1983. Quantification of Ca^{2+}-activated K^+ channels under hormonal control in pig pancreas acinar cells. *Nature (London)* **305:**228–232
27. Morris, A.P., Gallacher, D.V., Lee, J.A.C. 1986. A large conductance, voltage- and calcium-activated K^+ channel in the basolateral membrane of rat enterocytes. *FEBS Lett.* **206:**87–92
28. Novak, I., Greger, R. 1988. Electrophysiological study of transport systems in isolated perfused pancreatic ducts: Properties of the basolateral membrane. *Pfluegers Arch.* **411:**58–68
29. Novak, I., Greger, R. 1988. Properties of the luminal membrane of isolated perfused rat pancreatic ducts. Effect of cyclic AMP and blockers of chloride transport. *Pfluegers Arch.* **411:**546–553
30. Petersen, O.H. 1986. Calcium-activated potassium channels and fluid secretion by exocrine glands. *Am. J. Physiol.* **251:**G1–G13
31. Sadoshima, J.-I., Akaike, N., Kanaide, H., Nakamura, M. 1988. Cyclic AMP modulates Ca-activated K channel in cultured smooth muscle cells of rat aortas. *Am. J. Physiol.* **255:**H754–H759
32. Scott, J.D., Fischer, E.H., Takio, K., Demaille, J.G., Krebs, E.G. 1985. Amino acid sequence of the heat-stable inhibitor of the cAMP-dependent protein kinase from rabbit skeletal muscle. *Proc. Natl. Acad. Sci. USA* **82:**5732–5736
33. Sewell, W.A., Young, J.A. 1975. Secretion of electrolytes by the pancreas of the anaesthetised rat. *J. Physiol. (London)* **252:**379–396
34. Sheppard, D.N., Giraldez, F., Sepúlveda, F.V. 1988. Kinetics of voltage- and Ca^{2+} activation and Ba^{2+} blockade of a large-conductance K^+ channel from *Necturus* enterocytes. *J. Membrane Biol.* **105:**65–75
35. Stralfors, P., Belfrage, P. 1982. Properties and purification of the catalytic subunit of cyclic AMP-dependent protein kinase of adipose tissue. *Biochim. Biophys. Acta* **721:**434–440
36. Stuenkel, E.L., Machen, T.E., Williams, J.A. 1988. pH regulatory mechanisms in rat pancreatic ductal cells. *Am. J. Physiol.* **254:**G925–G930
37. Suzuki, K., Petersen, O.H. 1988. Patch-clamp study of single-channel and whole-cell K^+ currents in guinea pig pancreatic acinar cells. *Am. J. Physiol.* **255:**G275–G285
38. Ueda, S., Loo, D.D.F., Sachs, G. 1987. Regulation of K^+ channels in the basolateral membrane of *Necturus* oxyntic cells. *J. Membrane Biol.* **97:**31–41
39. Vergara, C., Latorre, R. 1983. Kinetics of Ca^{++}-activated K^+ channels from rabbit muscle incorporated into planar bilayers: Evidence for a Ca^{++} and Ba^{++} blockade. *J. Gen. Physiol.* **82:**543–568
40. Vivaudou, M.B., Singer, J.J., Walsh, J.V., Jr. 1986. An automated technique for analysis of current transitions in multilevel single-channel recordings. *Pfluegers Arch.* **407:**355–364
41. Wolf, H.U. 1973. Divalent metal ion buffers with low pH sensitivity. *Experientia* **29:**241–249
42. Wong, B.S., Lecar, H., Adler, M. 1982. Single calcium-dependent potassium channels in clonal anterior pituitary cells. *Biophys. J.* **39:**313–317

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