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Control of K^+ Conductance by Cholecystokinin and Ca^{2+} in Single Pancreatic Acinar Cells Studied by the Patch-Clamp Technique

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Summary. The effect of cholecystokinin (CCK) and internal Ca^{2+} on outward K^+ current in isolated pig pancreatic acinar cells has been investigated using the patch-clamp method for whole-cell current recording under voltage-clamp conditions. CCK (2×10^{-10} M) applied to the bath evoked a marked increase in the outward K^+ current associated with depolarizing voltage steps, and this effect was fully reversible and acutely dependent on the presence of external Ca^{2+}. When strongly buffered Ca^{2+}-EGTA solutions were used inside the cells CCK failed to evoke an effect. Increasing the internal Ca^{2+} concentration ($[Ca^{2+}]_i$) from 5×10^{-8} M to 10^{-7} and 5×10^{-7} M mimicked the effect of CCK. It would appear therefore that CCK controls K^+ conductance in the acinar cells via changes in the internal free ionized Ca^{2+} concentration.

Key Words pancreatic acinar cells · patch clamp · whole cell recording · single channel recording · K^+ channel · Ca^{2-} control · cholecystokinin

Introduction

Specific neurotransmitters and peptide hormones control membrane potential and conductance in the acinar cells of all mammalian exocrine glands so far investigated [13]. The increase in membrane conductance evoked by various agonists appears to be mediated via an increase in the intracellular concentration of ionized calcium ($[Ca^{2+}]_i$) [2, 4, 5, 10, 11, 13, 14]. Two types of Ca^{2+}-activated channels in plasma membranes from exocrine acini, have recently been studied directly with the help of the patch-clamp technique. A Ca^{2+}-activated and voltage-insensitive channel permeable to monovalent cations with a unit conductance of 30–35 pS was found in mouse and rat pancreatic acini, and this channel could be activated indirectly (via internal Ca^{2+}) by the peptide hormone cholecystokinin (CCK) [10]. More recently the large Ca^{2+}- and voltage-activated K^+ channel (200–250 pS) originally described in the chromaffin cells [8] and thereafter in many other tissues [6] was found in the acinar cells from several mammalian salivary glands [9] as well as the pig pancreas [11]. In the resting acinar cells from the pig pancreas it was possible to calculate that there are about 50 K^+ channels per cell by combining the results from patch-clamp single-channel and whole-cell current recordings [11].

In the patch-clamp whole-cell recording mode the cell interior will be equilibrated with the pipette filling solution [1, 3], and it should therefore be possible to investigate the whole cell K^+ currents at different $[Ca^{2+}]_i$. Using this method we now show that variations in $[Ca^{2+}]_i$ within the range 5×10^{-8} M to 5×10^{-7} M causes marked changes in the magnitude of the outward K^+ currents associated with depolarizing voltage steps. Using nominally Ca^{2+}-free pipette filling solutions with low (0.3–0.5 mM) concentrations of EGTA it is possible to evoke a marked increase in the K^+ conductance by external application of CCK. These effects are blocked if high concentrations (10 mM) of internal EGTA are used. The effect of sustained CCK stimulation is dependent on the presence of Ca^{2+} in the external (bathing) solution. CCK therefore controls K^+ conductance in the acinar cells via changes in the internal free ionized Ca^{2+} concentration.

Materials and Methods

Pig pancreata were obtained at the local abattoir. Small fragments (50 mg) were injected with physiological saline containing pure collagenase (Worthington) (100 units of activity per ml) and incubated with the same type of collagenase solution at 37 °C for 30 min (once or twice). At the end of this period there were large undigested lumps, clusters containing from a few up to 20–30 acinar cells as well as isolated single acinar cells. The cells were rinsed with collagenase-free physiological saline solution for 15 min to 1 hr before use.

Patch-clamp single-channel and whole cell current recordings were carried out on isolated cells at room temperature as

previously described by Neher and collaborators [1, 3, 12]. All single-channel recordings were carried out on excised inside-out membrane patches; i.e., the inside of the plasma membrane faced the bathing solution and the outside the pipette filling solution. The normally employed 'extracellular' solution contained (mM): 140 NaCl or 100 Na_2SO_4, 4.7 KCl, 2.5–5 $CaCl_2$, 1.15 $MgCl_2$, 10 glucose, 10 HEPES (pH 7.2 titrated with NaOH). The normal 'intracellular' solution had the following composition (mM): 145 KCl or 100 K_2SO_4, 1.15 $MgCl_2$, 10 glucose, 10 HEPES (pH 7.2, titrated with NaOH). The ionized Ca^{2+} concentration was adjusted with Ca^{2+}/EGTA buffers. Solutions having a nominal free ionized Ca^{2+} concentration of 5×10^{-8} M contained EGTA and calcium in the molar proportions 3 to 1, 10^{-7} M in the proportions 2 to 1 and the 5×10^{-7} M solutions contained EGTA and Ca in the proportion 1.2 to 1. In solutions with 'low' buffer capacity the EGTA concentration was 0.3 to 0.5 mM, whereas highly buffered solutions contained EGTA in a concentration of 5–10 mM.

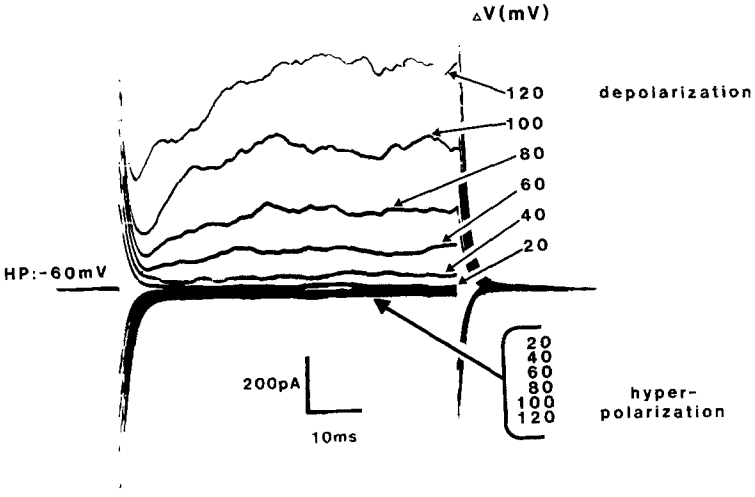

Fig. 1. Traces of whole-cell currents associated with depolarizing or hyperpolarizing voltage steps. The recording pipette was filled with a nominally Ca-free intracellular solution containing 1 mM EGTA. The holding potential was −60 mV. The upward deflections represent outward currents associated with depolarizing steps of 20, 40, 60, 80, 100 and 120 mV. The downward deflections represent the inward currents when hyperpolarizing steps of the same magnitudes were used

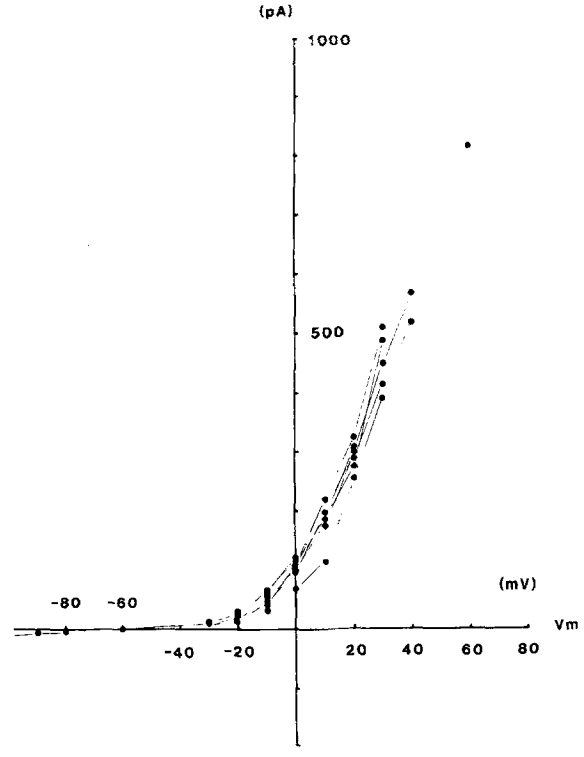

Fig. 2. The relationship between the change in steady-state current and the membrane potential when the potential is changed from the holding potential of −60 mV to higher or lower values. Results from six separate experiments of the type shown in Fig. 1 are combined.

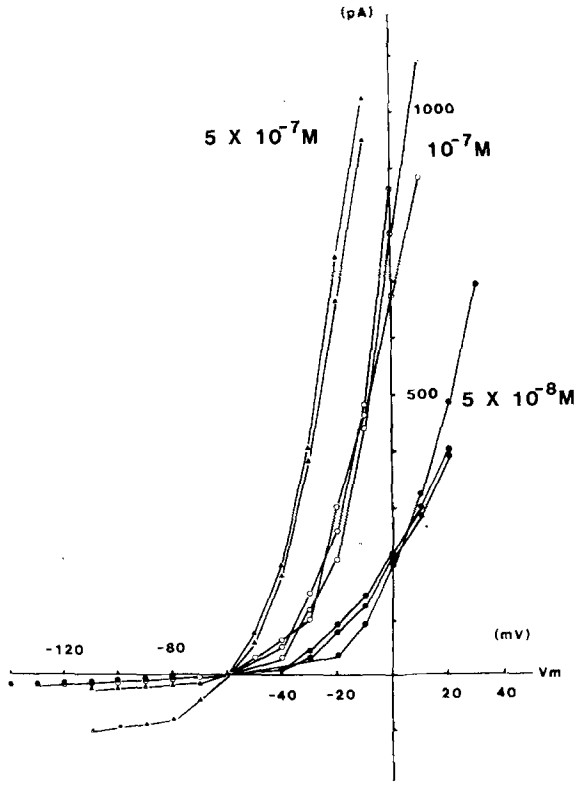

Fig. 3. Whole cell currents at different $[Ca^{2+}]_i$ (strongly buffered Ca-EGTA solution) plotted as function of membrane potential. These experiments were carried out in the Cl-rich solutions.

Results

Whole-Cell Current Recordings with Internal Ca^{2+}-Free Solution

Figure 1 shows a typical example of the currents evoked by voltage jumps in an isolated pig pancreatic acinar cell when the recording pipette was filled with a nominally Ca^{2+}-free solution containing 1 mM EGTA. The holding potential was −60 mV, which is close to the normal resting potential of these cells [11]. Depolarizing voltage steps evoked outward currents of markedly increasing amplitude with increasing step size. Hyperpolarizing voltage steps hardly evoked any inward currents. The relationship between the steady state (after 40 msec) current change and the voltage is shown in Fig. 2. The results from six cells from different tissues are plotted together showing that the *I/V* relationships in different cells were quantitatively very similar.

The Effect of Varying the Internal Ionized Ca^{2+} Concentration

We used three different pipette-filling solutions, identical except for the Ca^{2+}/EGTA concentration ratios. The *I/V* relationship at $[Ca^{2+}]_i = 5 \times 10^{-8}$ M was slightly different from the one obtained with the Ca^{2+}-free solution since the outward currents at the same potentials were larger at the higher $[Ca^{2+}]_i$. Thus a 60-mV depolarizing step (to 0 mV) evoked an outward current of about 100 pA with the 'Ca^{2+}-free' solution (Fig. 2) but about 200 pA using $[Ca^{2+}]_i = 5 \times 10^{-8}$ M (Fig. 3). Very marked increases in the outward currents were observed with $[Ca^{2+}]_i = 10^{-7}$ M and even more so at $[Ca^{2+}]_i = 5 \times 10^{-7}$ M (Fig. 3). When the higher $[Ca^{2+}]$ were used the outward currents associated with depolarizing voltage steps increased markedly over the first minutes after the membrane patch under the pipette had been disrupted. The change in the *I/V* relationship over the first 4 min in one particular cell is illustrated in Fig. 4. The changes were particularly dramatic in the first minutes and may well relate to the time it takes for $[Ca^{2+}]$ in the isolated cell to equilibrate with the pipette solution. In all further experiments (also the ones shown in Fig. 3) measurements were only carried out 5 min after the whole-cell recording configuration had been established.

The Effect of Cholecystokinin

In three experiments in which Ca^{2+}-free solutions with high (10 mM) EGTA concentrations were used no effects on the outward currents associated with depolarizing voltage steps were observed immediately after CCK application (2×10^{-10} M) or after several minutes of continued stimulation. It was necessary, however, to have EGTA in the pipette solution as otherwise $[Ca^{2+}]$ would have increased gradually. A compromise was therefore attempted by using a nominally Ca^{2+}-free solution with a relatively low EGTA concentration of 0.3–0.5 mM. Figure 5*A* shows current traces from an experiment of this type in which stimulation with CCK was carried out. After 1–2 min of stimulation there was an increase in the outward current at the holding potential and the changes in the outward currents associated with depolarizing voltage steps were markedly enhanced. These effects were largely reversible within a few minutes after reintroducing the normal CCK-free bathing solution. Figure 5*B* shows the plots of the *I/V* relationships. CCK-evoked increases in the outward currents were observed in five different acinar cells.

The effect of sustained CCK stimulation was acutely dependent on the presence of external Ca^{2+} as seen in Fig. 6. Removal of external Ca^{2+} very

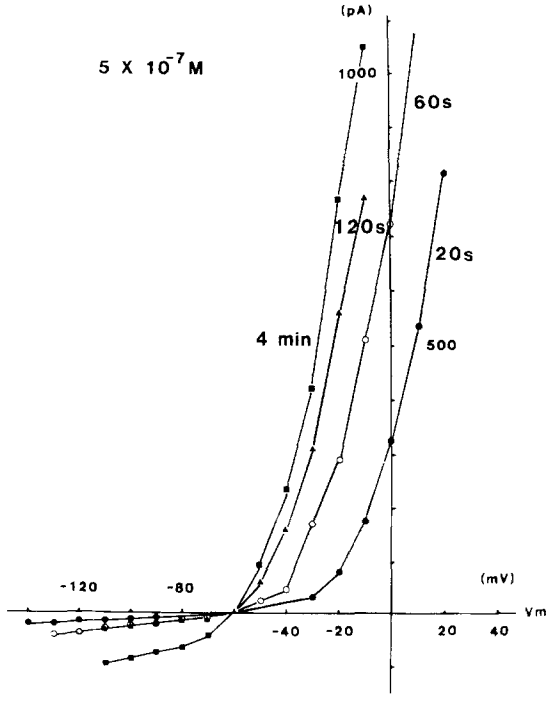

Fig. 4. Time-development of voltage-activated K^+ current when internal (pipette) solution contains intracellular K-chloride solution with $[Ca^{2+}] = 5 \times 10^{-7}$ M (strongly buffered with 10 mM EGTA). The steady-state whole-cell currents are shown as a function of membrane potential. ●, 20 sec after rupturing patch membrane; ○ 60 sec after patch membrane rupture; ▲, 120 sec after breaking patch membrane and ■, 4 min after the rupture of the patch membrane

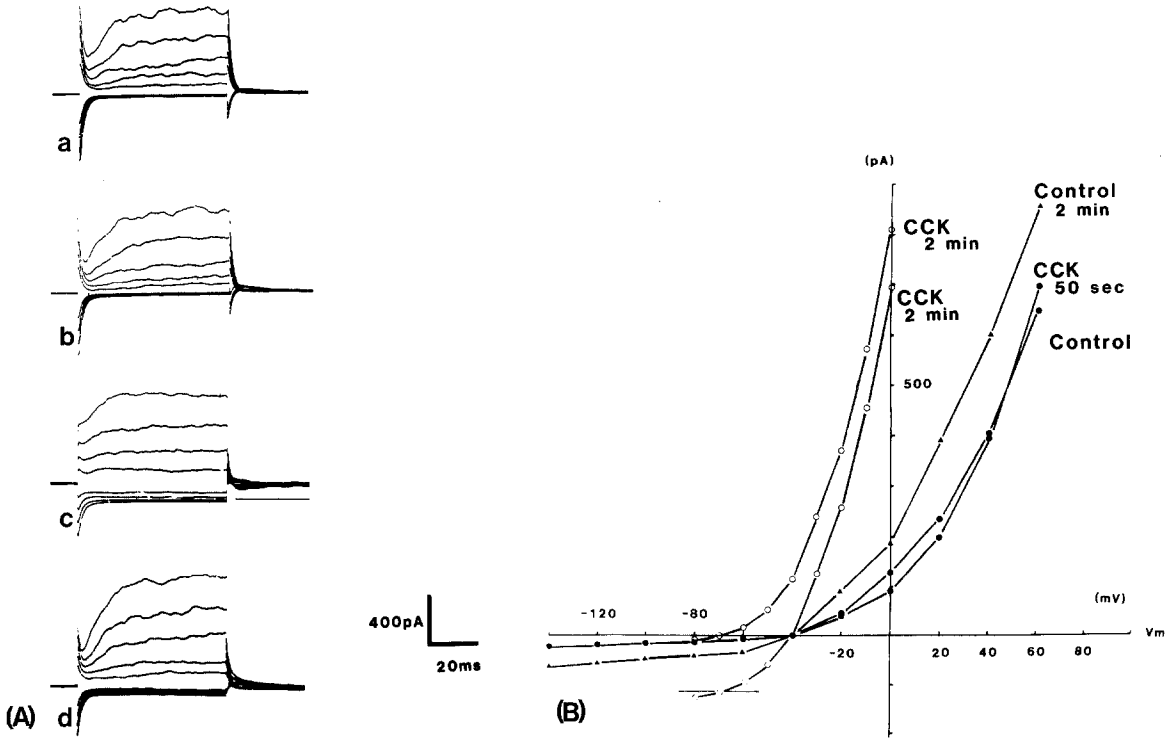

Fig. 5. The effect of CCK (2×10^{-10} M) on whole-cell currents. (*A*): Examples of whole-cell current recordings from one cell. The pipette was filled with standard K-sulphate solution without added Ca and a low EGTA concentration (0.5 mM). External medium was standard Na-sulphate solution with 5 mM Ca^{2+}. (*a*) Control before CCK application, (*b*) 50 sec after start of CCK exposure, (*c*) 2 min after start of CCK exposure, and (*d*) 2 min after return to control solution (without CCK). Holding potential was −40 mV throughout. In *a*, *b* and *d* the voltage steps were: ±20, 40, 60, 80 and 100 mV. In *c* the voltage steps were ±10, 20, 30 and 40 mV (note the increased outward current at holding potential). (*B*): Plots of the steady-state whole-cell currents as a function of membrane potential. Two plots are shown for the situation existing 2 min after start of CCK stimulation. The voltage steps were made from a holding potential of −40 mV, but the results were also recalculated on the basis of a 'graphical' readjustment of the holding potential to −70 mV

quickly abolished the CCK-evoked increase in current and subsequent removal of CCK had little further effect (Fig. 6).

Single-Channel Current Recordings

A previous study [11] has demonstrated the presence of the large voltage and Ca^{2+}-activated K^+ channel in the plasma membrane of pig pancreatic acinar cells. In the previous experiments recordings were only made in the presence of solutions with high Cl concentration, but since many of the whole-cell recordings in this study were carried out in sulphate solutions it seemed important also to check the single-channel currents in the same type of solutions. Figure 7 summarizes all our data on single-channel current-voltage (*i/V*) relations under the different ionic conditions and shows examples of single-channel current traces at different $[Ca^{2+}]_i$ and in the presence and absence of Cl^-. The *i/V* relations were not markedly different in the presence or absence of Cl^- and variations in $[Ca^{2+}]_i$ in the range 10^{-8} to 5×10^{-7} M did not change the *i/V* relation, although the probability of channel opening did increase with increasing $[Ca^{2+}]_i$ as described in detail previously [11].

Discussion

Our results demonstrate that the outward K^+ current in pig pancreatic acinar cells can be regulated by internal Ca^{2+} as well as by external application of the pancreatic secretagogue CCK. Since the CCK effect was dependent on the presence of external Ca^{2+} and could not be evoked when the internal Ca^{2+} concentration was clamped at a low level with high concentrations of EGTA, it seems likely that CCK acts via an increase in $[Ca^{2+}]_i$. The relatively

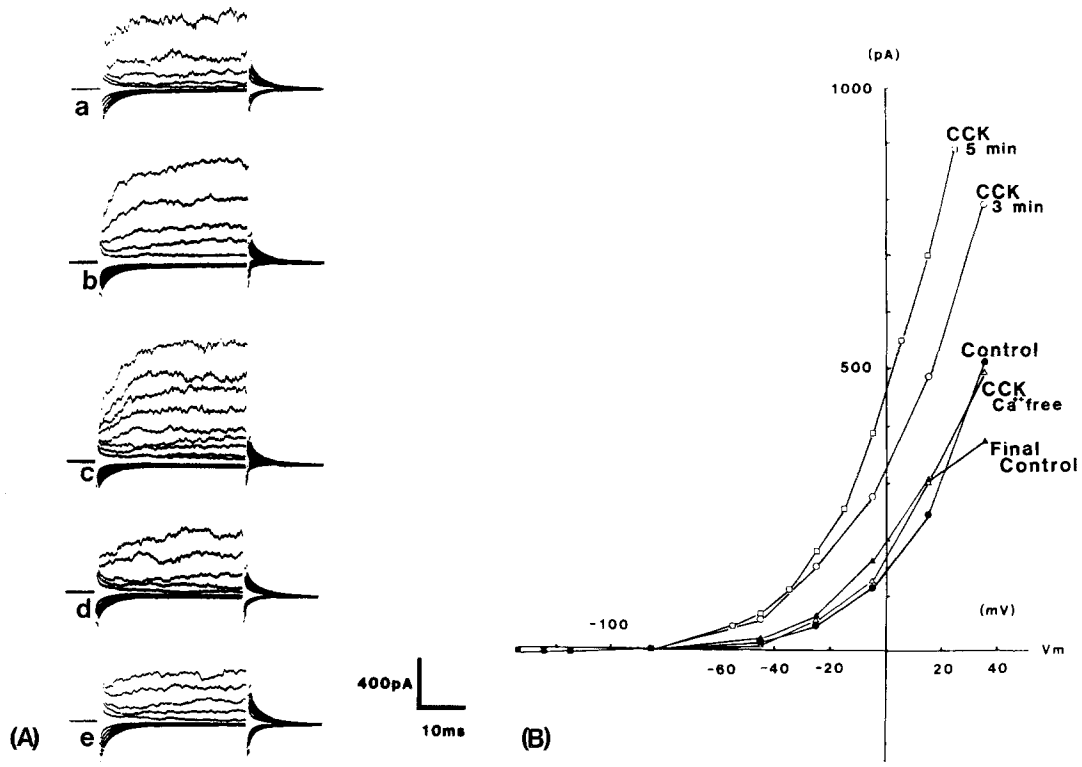

Fig. 6. Ca^{2+}-dependency of CCK effect (*A*): Examples of whole-cell current recordings from one cell. Usual Na-sulphate/*K*-sulphate solutions. No Ca^{2+} added to pipette solution, which contained 0.5 mM EGTA. External solution contained 2.5 mM Ca except in *d* where Ca^{2+} was omitted from the solution. Holding potential was −85 mV throughout. (*a*) Control before CCK application. Voltage steps: ±40, 60, 80, 100 and 120 mV. (*b*) 3 min after start of exposure to CCK (2×10^{-10} M). Voltage steps: ±40, 60, 80, 100 and 120 mV. (*c*) 5 min after start of CCK stimulation. Voltage steps: ±30, 40, 50, 60, 70, 80, 90, 100 and 110 mV. (*d*) 10 sec after replacing 2.5 mM Ca^{2+} solution with nominally Ca^{2+}-free solution, but still with CCK (2×10^{-10} M). Voltage steps: ±40, 60, 80, 100 and 120 mV. (*e*) 1 min after return to control (without CCK). Voltage steps: ±40, 60, 80, 100 and 120 mV. (*B*): Plots of the steady-state whole-cell currents as a function of membrane potential

long delay for the action of CCK may seem surprising since microelectrode data show CCK-evoked hyperpolarization which reaches maximum within a few seconds after start of stimulation [11]. In the present experiments a Ca^{2+}-chelator (EGTA) was present in the cell interior and the CCK-evoked increase in $[Ca^{2+}]_i$ [15] may therefore have been markedly delayed. The rather acute effect of external Ca removal on the sustained CCK-evoked increase in current may indicate a requirement for continuous Ca^{2+} influx in order to maintain an elevated $[Ca^{2+}]_i$ [7].

With the help of single-channel current recordings on excised inside-out patches of pig pancreatic acinar plasma membranes, we have previously shown that the open state probability (p) of the large K^+ channel, which is totally dominating the conductance of the whole cell, is regulated by $[Ca^{2+}]_i$ [11]. CCK would therefore, by increasing $[Ca^{2+}]_i$, be expected to increase p and this would explain (at least qualitatively) the evoked increase in current. However, the effects may seem too large to be explained solely in terms of an increase in p.

If we use the equation:

$$I = Npi$$

where I is the whole cell K^+ current, N the number of channels per cell, p the open-state probability, and i the single-channel K^+ current we can, as previously shown [11], calculate the product Np as a function of the membrane potential. If this is done using for example the I/V curves from Fig. 6 in combination with the i/V curves from Fig. 7 it would appear that application of CCK evoked a marked increase in Np which can best be explained by an increase in both p and N. The same type of result was obtained by analyzing the effect of changing $[Ca^{2+}]_i$. This cannot, however, be regarded as clear

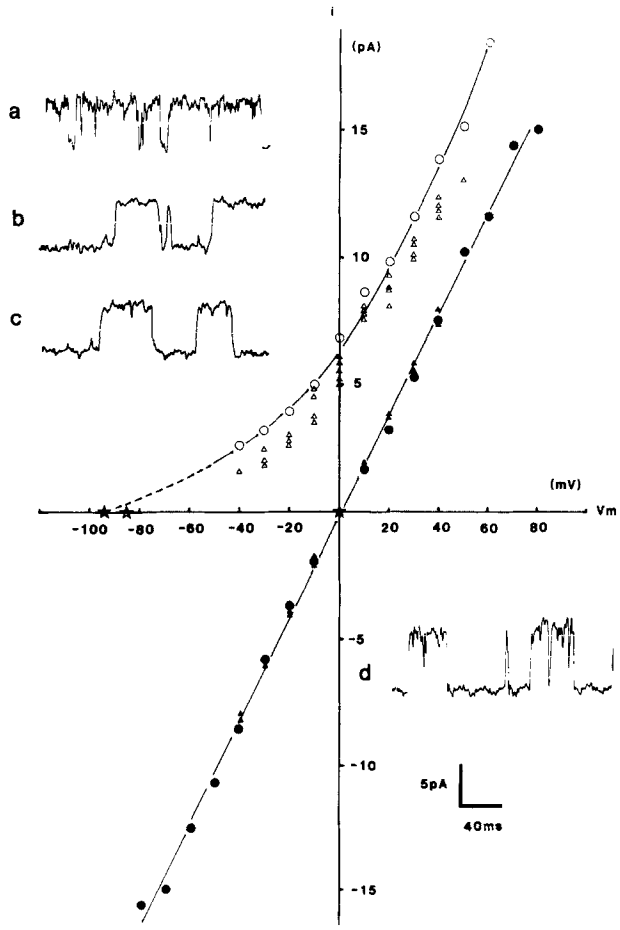

Fig. 7. Single-channel current-voltage relationships under different ionic conditions. ○, data obtained with the standard sulphate solutions (Na_2SO_4 in pipette, K_2SO_4 in bath) described in the methods section. No calcium was added to the 'intracellular' (bath) solution, which contained 1 mM EGTA. ●, data obtained with symmetrical intracellular K_2SO_4 solution; no Ca^{2+} was added to the bath solution. △, measurements carried out with Cl containing solutions (NaCl in pipette, KCl in bath). The internal (bath) ionized Ca^{2+} concentration ($[Ca^{2+}]_i$) was adjusted using Ca^{2+}-EGTA buffers and ranged between 10^{-8} and 10^{-7} M. ▲, data obtained in the presence of symmetrical KCl solutions, $[Ca^{2+}]_i$ was 10^{-7} M. Insets *a–d* show traces of single-channel current recordings all obtained at a membrane potential of 0 mV. There were 'normal' Na/K gradients across the membrane in all four cases. *a–c* were obtained in presence of Cl solutions, *d* in presence of sulphate solutions. (*a*): $[Ca^{2+}]_i = 5 \times 10^{-7}$ M; (*b*): $[Ca^{2+}]_i = 10^{-7}$ M; (*c*): $[Ca^{2+}]_i = 10^{-8}$ M; and (*d*): no Ca^{2+} added and 1 mM EGTA present. (Note that the pancreatic K^+ channel, unlike some others, can be activated by depolarization in the absence of Ca. It is Ca^{2+}-activated, but not Ca^{2+}-dependent [14])

evidence for an increase in N, and further work will be required before this question is settled.

It would appear therefore that the main controller of K^+ conductance in the pig pancreatic acinar cells is $[Ca^{2+}]_i$ and that an elevation of $[Ca^{2+}]_i$ increases the open-state probability of the large K^+ channel and probably also the number of active channels. The effect of CCK seems to be mediated entirely by an increase in $[Ca^{2+}]_i$.

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