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High-Conductance K^+ Channel in Pancreatic Islet Cells can be Activated and Inactivated by Internal Calcium

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Summary. The Ca^{2+}-activated K^+ channel in rat pancreatic islet cells has been studied using patch-clamp single-channel current recording in excised inside-out and outside-out membrane patches. In membrane patches exposed to quasi-physiological cation gradients (Na^+ outside, K^+ inside) large outward current steps were observed when the membrane was depolarized. The single-channel current voltage (I/V) relationship showed outward rectification and the null potential was more negative than −40 mV. In symmetrical K^+-rich solutions the single-channel I/V relationship was linear, the null potential was 0 mV and the single-channel conductance was about 250 pS. Membrane depolarization evoked channel opening also when the inside of the membrane was exposed to a Ca^{2+}-free solution containing 2 mM EGTA, but large positive membrane potentials (70 to 80 mV) were required in order to obtain open-state probabilities (P) above 0.1. Raising the free Ca^{2+} concentration in contact with the membrane inside ($[Ca^{2+}]_i$) to 1.5×10^{-7} M had little effect on the relationship between membrane potential and P. When $[Ca^{2+}]_i$ was increased to 3×10^{-7} M and 6×10^{-7} M smaller potential changes were required to open the channels. Increasing $[Ca^{2+}]_i$ further to 8×10^{-7} M again activated the channels, but the relationship between membrane potential and P was complex. Changing the membrane potential from −50 mV to +20 mV increased P from near 0 to 0.6 but further polarization to +50 mV decreased P to about 0.2. The pattern of voltage activation and inactivation was even more pronounced at $[Ca^{2+}]_i = 1$ and 2 μM. In this situation a membrane potential change from −70 to +20 mV increased P from near 0 to about 0.7 but further polarization to +80 mV reduced P to less than 0.1. The high-conductance K^+ channel in rat pancreatic islet cells is remarkably sensitive to changes in $[Ca^{2+}]_i$ within the range 0.1 to 1 μM which suggests a physiological role for this channel in regulating the membrane potential and Ca^{2+} influx through voltage-activated Ca^{2+} channels.

Key Words pancreatic islet cells · K^+ channel · patch clamp · single-channel recording · Ca^{2+} activation

Introduction

The Ca^{2+}-activated K^+ channel provides an important link between the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and the membrane potential (Meech, 1978; Petersen & Maruyama, 1984). In all cells with voltage-gated Ca^{2+} channels (Reuter, 1983) this link allows regulation of $[Ca^{2+}]_i$ (Petersen & Maruyama, 1984). Indirect evidence suggests that B-cells in the pancreatic islets possess both voltage-gated Ca^{2+} channels (Matthews & Sakamoto, 1975) and Ca^{2+}-activated K^+ channels (Atwater et al., 1979; Henquin, 1979). Recent preliminary patch-clamp studies on pancreatic islet cells have demonstrated directly the presence of large Ca^{2+}- and voltage-activated K^+ channels (Marty & Neher, 1982; Dunne, Findlay & Petersen, 1984) but have not provided any detailed characterization of the channels nor given information about the crucial relationship between $[Ca^{2+}]_i$ and the open-state probability of the channels (P).

We now demonstrate with the help of single-channel current recording from excised inside-out and outside-out membrane patches (Hamill et al., 1981) that the voltage-dependent high-conductance K^+ channel is effectively controlled by a very narrow range of $[Ca^{2+}]_i$. Depolarization can activate the channel in the complete absence of Ca^{2+} and increasing $[Ca^{2+}]_i$ to about 10^{-7} M has little effect. However, within the range 10^{-7} to 10^{-6} M small changes in $[Ca^{2+}]_i$ have marked effects on P. The precise interrelation between membrane potential, $[Ca^{2+}]_i$ and P is complex, but allows fine control of the K^+ conductance within the $[Ca^{2+}]_i$ range of 10^{-7} to 10^{-6} M which includes the recently determined levels of $[Ca^{2+}]_i$ in resting and stimulated insulin-secreting cells (Wollheim & Pozzan, 1984).

Materials and Methods

Cell Isolation

Islets were isolated from rat pancreas by collagenase treatment and islet cells were prepared by the trypsin and EGTA method described by Pipeleers and Pipeleers-Marichal (1981). This islet

Table. The concentrations of EGTA and Ca^{2+} added to K^+-rich solutions to provide the calculated free Ca^{2+} concentration[a]

Total EGTA (mM)	Total Ca^{2+} (mM)	Calculated free Ca^{2+} (M)
1.92	0.01	$<10^{-9}$
1.92	0.97	1.5×10^{-7}
2.30	1.54	3×10^{-7}
1.92	1.54	6×10^{-7}
1.34	1.16	8×10^{-7}
1.73	1.54	1×10^{-6}
2.11	1.93	2×10^{-6}

[a] Total EGTA assumes 96% purity for EGTA supplied by Sigma, U.K. (Miller & Smith, 1984). Total Ca^{2+} is based upon amounts of Ca in standard Ca solutions measured by atomic absorption spectroscopy.

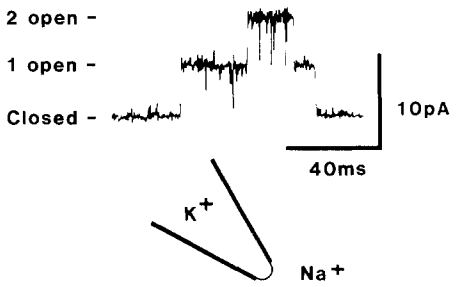

Fig. 1. Single-channel current recording from an excised outside-out membrane patch from a rat pancreatic islet cell. The 'intracellular' pipette solution (in contact with the inside of the plasma membrane) contained K^+-rich solution with no added Ca and 1 mM EGTA. The 'extracellular' bath solution (in contact with the outside of the plasma membrane) contained Na^+-rich solution. The current trace was obtained at 0 mV membrane potential. Filtering: 1 kHz (low pass)

suspension (70% B-cells) is a mixture of single cells and small clumps of several cells. We mainly utilized small clumps of cells in which more than 90% of the cells belong to the B-cell group (Pipeleers & Pipeleers-Marichal, 1981).

Media

The Na^+-rich solution used as the normal 'extracellular' bathing medium contained (mM). 140 NaCl, 4.7 KCl, 1.2 $CaCl_2$, 1.13 $MgCl_2$, 10 HEPES, pH 7.2. The K^+-rich solution used as the 'intracellular' medium contained (mM). 145 KCl, 10 NaCl, 1.13 $MgCl_2$, 10 HEPES, pH 7.2. The pH of the 'intracellular' medium was kept constant at 7.2 since this is the intracellular pH value repeatedly recorded from islets (Pace, Tarvin & Smith, 1983; Lindstrom & Sehlin, 1984).

The free concentration of Ca^{2+} in 'intracellular' media ($[Ca^{2+}]_i$) was determined by the addition of $CaCl_2$ and EGTA buffer mixtures (Table). $[Ca^{2+}]_i$ was calculated using an iterative procedure on a BBC (model B) microcomputer using the stability constants for all of the reactions between Ca, Mg, H and EGTA (Martell & Smith, 1974). In the case of reactions involving H^+ the constants were corrected so as to transform the concentration constants into mixed constants including activity and concentration terms to take account of the fact that pH measurement results in values for activity rather than concentration (Fabiato, 1981). Ca-EGTA buffers were prepared by titration as described by Miller and Smith (1984). pH meters were calibrated using appropriate salt concentrations to guard against errors arising from liquid junction potentials (Illingworth, 1981).

Recording and Analysis

Single-channel current recording from membrane patches excised from islet cells was carried out as described by Hamill et al. (1981) using a List EPC-5 patch-clamp amplifier system and fire-polished glass pipettes with a resistance of 5 to 10 MΩ. Current recordings were stored on tape (Racal 4DS recorder) for subsequent replay and analysis.

Analysis of data to determine channel open-state probability was performed upon 10 or 20 sec continuous stretches of tape record for each situation. Data were digitized at 2 kHz and analyzed by a BBC (model B) microcomputer (Findlay & Furlong, 1984). Multiple channel open states were resolved first by determining the open-state duration for each channel current level in the record (a threshold current level was preselected) and then averaging the results to obtain the average channel open probability.

Results

Figure 1 shows a typical single-channel current recording from an excised outside-out membrane patch exposed to quasi-physiological cation concentration gradients. The trace shows open-channel current noise and several short closings within long openings. The single-channel current voltage (*I/V*) relationship is shown in Fig. 2 (open circles). Although the null potential was not attained it is clear that it is negative and as K^+ is the only ion with a negative equilibrium potential in this experiment (the Cl^- concentration is the same on both sides) the channel must be K^+ selective. Similar results to those shown in Figs. 1 and 2 were obtained in two other outside-out membrane patches.

Figure 3 shows single-channel current recordings obtained from an inside-out membrane patch exposed to K^+-rich solutions on both sides at different membrane potentials. The single-channel *I/V* relationship shown in Fig. 2 (closed symbols) was linear with a slope corresponding to about 250 pS and this was independent of $[Ca^{2+}]_i$ over the range $<10^{-9}$ to 10^{-6} M. The records shown in Fig. 3 were all obtained with a Ca^{2+}-free solution (containing 2 mM EGTA) in contact with the inside of the membrane (bath), but nevertheless clear voltage-dependent channel activation was observed. The proba-

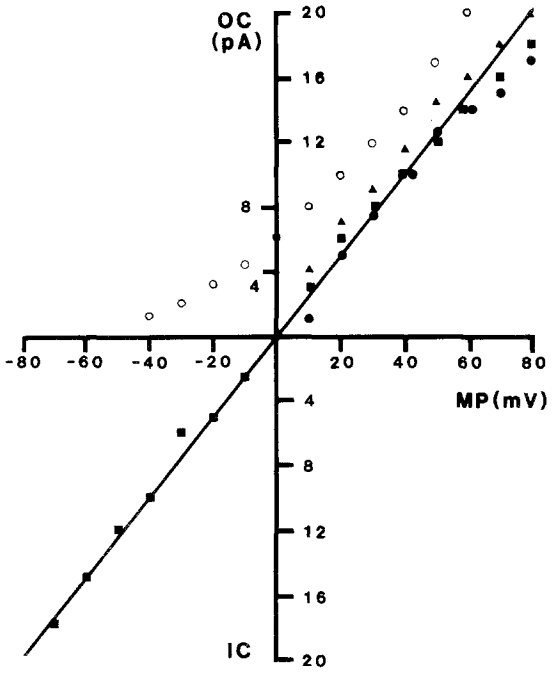

Fig. 2. Single-channel current-voltage relationships from excised patches in the presence of quasi-physiological cation gradients (Na^+/K^+ situation) (data from same experiment as in Fig. 1) (open circles) and in the presence of high-K^+ solutions on both sides (K^+/K^+ situation) (data from same experiment as in Figs. 3–7) (closed symbols). Circles: $[Ca^{2+}]_i < 10^{-9}$ M, triangles: $[Ca^{2+}]_i = 1.5 \times 10^{-7}$ M and squares: $[Ca^{2+}]_i = 10^{-6}$ M

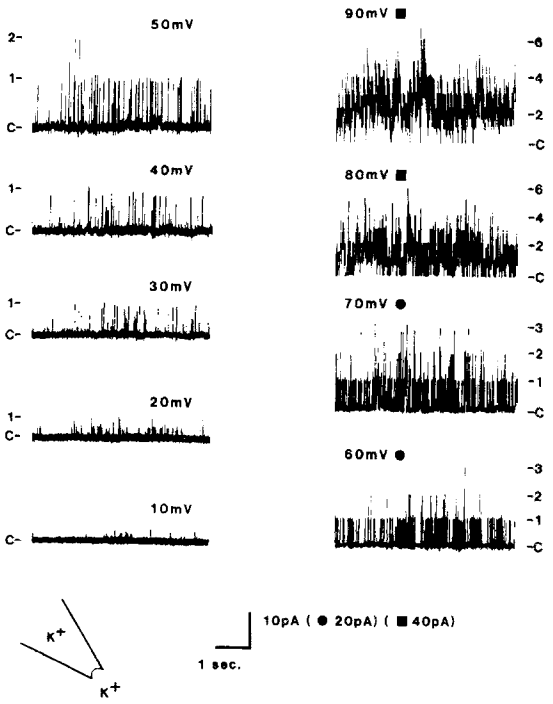

Fig. 3. Single-channel current recordings from one excised inside-out membrane patch. The intracellular solution described in Fig. 1 was used in both the pipette (in contact with outside of cell membrane) and the bath (K^+/K^+ situation). Bath solution contained 2 mM EGTA and no Ca was added. The membrane potential at which each trace was recorded is written immediately above the individual traces. The current levels when all channels were closed (*c*) and 1, 2, 3, 4 and 6 channels, respectively, were open are indicated

bility of channel opening at small depolarizations was, however, very low and this became particularly clear when the large depolarizations (>50 mV) revealed the presence of many apparently identical channels in the same patch. Membrane patches excised from rat islets have invariably been found to contain more than one large K^+ channel and as many as nine discernible current levels have been observed. Similar results were obtained in every one of the 16 preparations tested.

Figure 4 shows a selection of single-channel current recordings all obtained from the same excised inside-out membrane patch at a membrane potential of +60 mV, but with different $[Ca^{2+}]$ in the bathing fluid. It can be seen that changing from a Ca^{2+}-free solution to one with $[Ca^{2+}]_i = 1.5 \times 10^{-7}$ M had little effect upon channel activity but an increase to 3×10^{-7} M and even more so to 6×10^{-7} M evoked marked channel activation. These effects were completely reversible and were repeatedly observed in each of the four preparations tested. Increasing $[Ca^{2+}]_i$ beyond 6×10^{-7} M at a membrane potential of +60 mV, did not evoke further channel opening; indeed at $[Ca^{2+}]_i = 10^{-6}$ M the open-state probability was lower than at 8×10^{-7} M (Fig. 6).

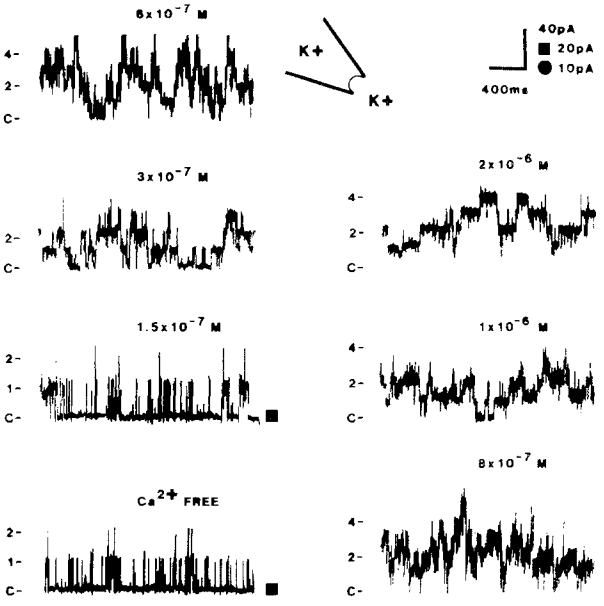

Fig. 4. Single-channel current traces from same patch as in Fig. 3 but all recorded at a membrane potential of +60 mV. Above each trace is written the free calcium ion concentration in the bath solution ($[Ca^{2+}]_i$) at the time of recording

This inhibitory effect of 10^{-6} and also 2×10^{-6} M Ca^{2+} was completely reversible and was repeatedly observed in each of the three preparations tested.

The large K^+ channel can be activated by both membrane depolarization (Fig. 3) and by increasing $[Ca^{2+}]_i$ (Fig. 4) and it is therefore necessary to test a range of membrane potentials for each value of $[Ca^{2+}]_i$. Figure 5 shows single-channel current traces from the same excised inside-out patch that also provided the records shown in Figs. 3 and 4, but now with $[Ca]_i = 3$ and 6×10^{-7} M. Comparing Figs. 3 and 5 it is seen that increasing $[Ca^{2+}]_i$ to 3×10^{-7} M has markedly increased probability of channel opening (P) at the three levels of membrane potential shown and that a further increase to 6×10^{-7} M causes a very marked activation, particularly at +50 mV.

Figure 6 shows recordings from the same patch, but now with $[Ca^{2+}]_i = 8 \times 10^{-7}$ M. At this level of $[Ca^{2+}]_i$ significant channel activation is observed at negative membrane potentials and changing the membrane potential up to +30 mV evokes more channel openings. Changing the membrane potential from +30 to +50 mV, however, reduces P, while polarization to +70 mV again increases P. At the highest $[Ca^{2+}]_i$ tested (2×10^{-6} M) there was a considerable degree of channel opening at a membrane potential of −50 mV (Fig. 7) and further channel activation was observed by reducing the membrane potential and then making it positive up to a level of +20 mV. Further polarization then reduced P and at extreme positive membrane potentials P was as low as at the very negative potentials. It is particularly noteworthy that there were long periods of complete channel closure at +80 mV (Fig. 7).

Figure 8 summarizes all the data from one membrane patch on the relationship between P and membrane potential at the different levels of $[Ca^{2+}]_i$. For the sake of clarity only the ascending part of the curves obtained at $[Ca^{2+}]_i = 6 \times 10^{-7}$, 8×10^{-7}, 1×10^{-6} and 2×10^{-6} M have been displayed in Fig. 8*A* together with the curves representing the lower values of $[Ca^{2+}]_i$, whereas the complete set of data for the high values of $[Ca^{2+}]_i$ are shown alone in Fig. 8*B*. The curves relating P to membrane potential shown in Fig. 8*B* all have several peaks and may therefore appear somewhat irregular. These fluctuations were reproduced several times in individual patches (Fig. 6).

Discussion

The results presented here demonstrate the presence of Ca^{2+}- and voltage-activated high-conduc-

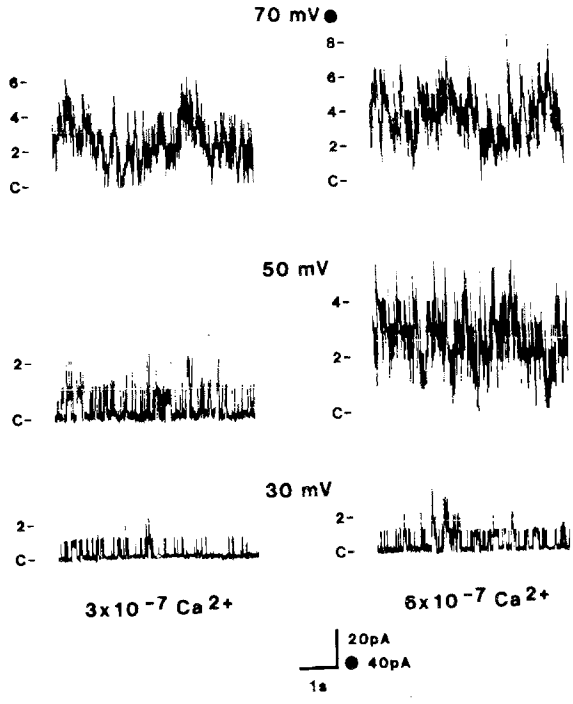

Fig. 5. Traces from same patch as in Figs. 3 and 4. Recordings with two different levels of $[Ca^{2+}]_i$ and at three membrane potentials are shown

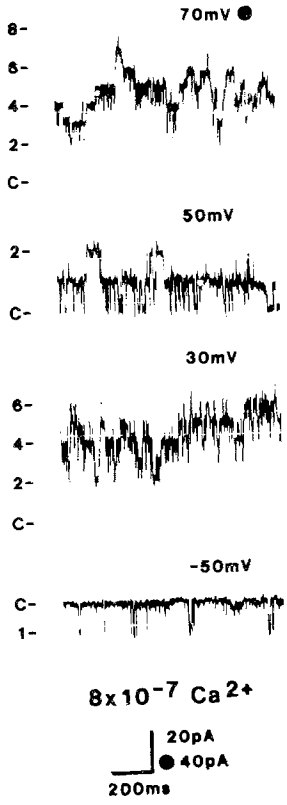

Fig. 6. Traces for same patch as in Figs. 3, 4 and 5 but now with $[Ca^{2+}]_i = 8 \times 10^{-7}$ M at four different membrane potentials

tance K^+ channels in rat pancreatic islet cells. This type of channel was first found in the adrenal chromaffin cells (Marty, 1981) and later in anterior pituitary cells (Wong, Lecar & Adler, 1982), but is also present in muscle, nerve and exocrine gland cells (Latorre & Miller, 1983; Petersen & Maruyama, 1984). The single-channel conductance in the islet cells is about 250 pS in a situation with K^+-rich solutions on both sides of the membrane and this is similar to the values found in most other tissues (Petersen & Maruyama, 1984). The magnitude of the single-channel outward currents in the presence of quasi-physiological transmembrane cation gradients in the islet cells (Figs. 1 & 2) are also in agreement with recent findings in exocrine acinar cells from the salivary glands (Maruyama, Gallacher & Petersen, 1983*a*), the pancreas (Maruyama et al., 1983*b*; Maruyama & Petersen, 1984) and the lacrimal gland (Findlay, 1984; Trautmann & Marty, 1984).

The high-conductance K^+ channel is often referred to as a Ca^{2+}-dependent channel; however, in many tissues it has been shown that depolarization can evoke channel opening in the absence of internal Ca^{2+} (Petersen & Maruyama, 1984) and this is also the case for the islet cells (Fig. 3). Increasing $[Ca^{2+}]_i$ within the range 10^{-7} to 10^{-6} M shifts the relation between P and membrane potential to the left (Fig. 8*A*) so that smaller membrane potential changes are required to achieve the same degree of channel opening. This is a well-known phenomenon (Barrett, Magleby & Pallotta, 1982; Maruyama et al., 1983*b*; Findlay, 1984; Maruyama & Petersen, 1984), but the curves shown in Fig. 8*A* are nevertheless markedly different from those previously obtained from studies on the muscle cell K^+ channel (Barrett et al., 1982) or the exocrine acinar cell K^+ channel (Maruyama et al., 1983*b*; Findlay, 1984). In mammalian exocrine glands the maximal sensitivity to change in $[Ca^{2+}]_i$ occurs within the range 10^{-8} to

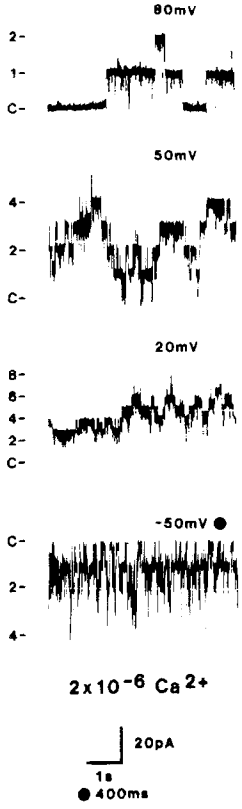

Fig. 7. Traces from same patch as in Figs. 3–6 but now with $[Ca^{2+}]_i = 2 \times 10^{-6}$ M at four different membrane potentials

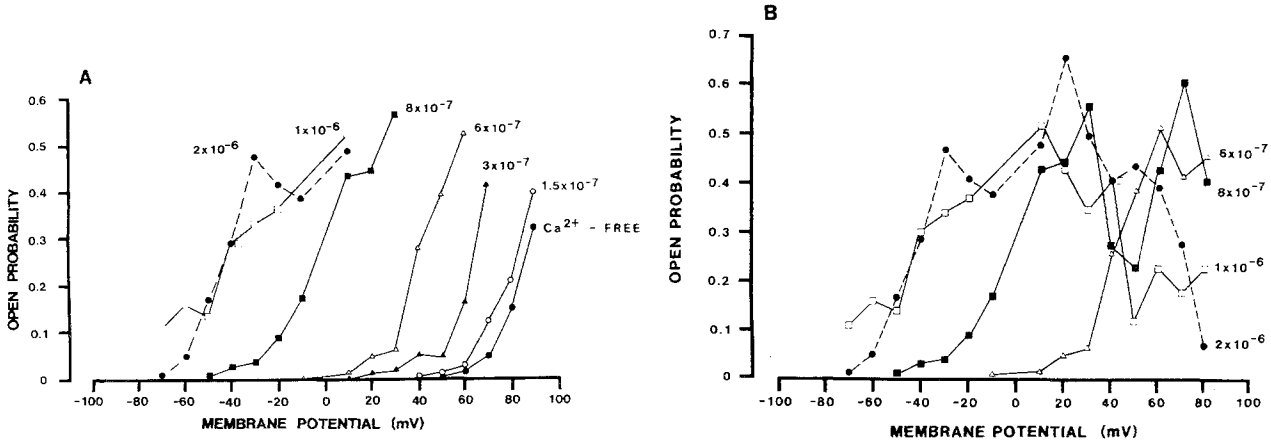

Fig. 8. Relationship between membrane potential and open-state probability at different levels of $[Ca^{2+}]_i$ from the patch represented by the results shown in Figs. 3–7. *A* shows only the ascending part of the curves for the sake of clarity, whereas *B* shows the complete curves for $[Ca^{2+}]_i$ from 6×10^{-7} M to 2×10^{-6} M

10^{-7} M whereas in rat muscle cells the corresponding range is 10^{-6} to 10^{-4} M. In clonal anterior pituitary cells the relationship between membrane potential and P has only been partially explored at different levels of $[Ca^{2+}]_i$, but it would appear that the maximum sensitivity to changes in $[Ca^{2+}]_i$ does occur within the range 10^{-7} to 10^{-6} M (Wong et al., 1982) which is similar to our data shown in Fig. 8*A*. Figure 8*A*, however, does not show the complete set of data obtained. As seen in Fig. 8*B* higher levels of $[Ca^{2+}]_i$ will reduce P at very positive membrane potentials. Ca^{2+} blockade of Ca^{2+}-activated K^+ channels from rabbit muscle incorporated into lipid bilayers at $[Ca^{2+}]_i$ above 10^{-4} M has recently been the subject of a detailed and mechanistic analysis (Latorre, Vergara & Moczydlowski, 1983; Vergara & Latorre, 1983). In the pig pancreatic acinar cells it was noted that large depolarizations at $[Ca^{2+}]_i$ of 10^{-7} M and above sometimes led to a state of prolonged channel closure (Maruyama et al., 1983*b*). Figure 8*B* demonstrates that the phenomenon is quantitatively of great importance at $[Ca^{2+}]_i = 1$ or 2 μM where depolarization activates the channel up to potentials of about 0 to 20 mV but further changes of the membrane potential to more positive values are associated with a marked decrease in P. This phenomenon could be observed also at $[Ca^{2+}]_i = 8 \times 10^{-7}$ M, but was only just noticeable at the extreme positive potentials (+70 and 80 mV) in 6×10^{-7} M Ca^{2+}.

Ca^{2+}-activated K^+ channels are regarded as crucial for the function of the pancreatic B-cells (Atwater, Rosario & Rojas, 1983). However, in order to assess their contribution it is essential to have precise information about the relationship between channel opening, membrane potential and $[Ca^{2+}]_i$. Such information has now for the first time been provided (Fig. 8) and the results show a very complex pattern of activation and inactivation not previously described for this type of channel. In a recent quin-2 study of $[Ca^{2+}]_i$ in an insulin-secreting cell line the resting level was found to be about 10^{-7} M and a variety of stimulants were able to increase $[Ca^{2+}]_i$ by a factor of 2 to 3 (Wollheim & Pozzan, 1984). The range of $[Ca^{2+}]_i$ which we demonstrate controlling the large K^+ channel in the islet cells includes the levels of $[Ca^{2+}]_i$ measured during rest and maximal insulin secretion by Wollheim and Pozzan (1984) and the levels of $[Ca^{2+}]$ which evoke half and maximal insulin release from islets made permeable by electric discharge (Yaseen, Pedley & Howell, 1982). It is therefore possible to assign a physiological role to the Ca^{2+}-activated K^+ channel in providing feed-back control of Ca^{2+} uptake into islet cells. Whether cellular factors other than voltage and $[Ca^{2+}]_i$ can further modulate *in situ* activity of the large K^+ channel remains to be determined.

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