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ATP-Sensitive Inward Rectifier and Voltage- and Calcium-Activated K^+ Channels in Cultured Pancreatic Islet Cells

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Summary. K^+ channels in cultured rat pancreatic islet cells have been studied using patch-clamp single-channel recording techniques in cell-attached and excised inside-out and outside-out membrane patches. Three different K^+-selective channels have been found. Two inward rectifier K^+ channels with slope conductances of about 4 and 17 pS recorded under quasi-physiological cation gradients (Na^+ outside, K^+ inside) and maximal conductances recorded in symmetrical K^+-rich solutions of about 30 and 75 pS, respectively. A voltage- and calcium-activated K^+ channel was recorded with a slope conductance of about 90 pS under the same conditions and a maximal conductance recorded in symmetrical K^+-rich solutions of about 250 pS. Single-channel current recording in the cell-attached conformation revealed a continuous low level of activity in an apparently small number of both the inward rectifier K^+ channels. But when membrane patches were excised from the intact cell a much larger number of inward rectifier K^+ channels became transiently activated before showing an irreversible decline. In excised patches opening and closing of both the inward rectifier K^+ channels were unaffected by voltage, internal Ca^{2+} or externally applied tetraethylammonium (TEA) but the probability of opening of both inward rectifier K^+ channels was reduced by internally applied 1–5 mM adenosine-5′-triphosphate (ATP). The large K^+ channel was not operational in cell-attached membrane patches, but in excised patches it could be activated at negative membrane potentials by 10^{-7} to 10^{-6} M internal Ca^{2+} and blocked by 5–10 mM external TEA.

Key Words islet · K^+ channels · ATP · Ca^{2+} · patch-clamp

Introduction

Insulin secretion from the B-cells of islet of Langerhans in the pancreas is stimulated by glucose which evokes a cyclical pattern of electrical activity in B-cells which consists of slow waves of depolarization with action potential-like spikes (Dean & Matthews, 1970). The slow cyclical changes in membrane potential are most likely due to changes in membrane K^+ permeability (Meissner, 1976; Atwater, Ribalet & Rojas, 1978; Ribalet & Beigelman, 1979) which can now be more precisely defined using the patch-clamp technique for single-channel current recording (Hamill et al., 1981).

K^+ channels which can be closed by ATP acting on the internal surface of the membrane were first described in membrane patches excised from cardiac cells by Noma (1983) and subsequently in membranes from pancreatic B-cells (Cook & Hales, 1984). In cardiac cells the ATP-sensitive K^+ channel is not operational in normal resting cells. This is consistent with the knowledge that the intracellular ATP concentration is about 3–4 mM and that in excised inside-out or open cell-attached membrane patches ATP in a concentration of 2 mM totally inhibits channel opening (Kakei, Noma & Shibasaki, 1985). With regard to pancreatic islet cells even lower ATP concentrations have been reported to abolish K^+ channel openings in excised membrane patches (Cook & Hales, 1984) yet operational K^+ channels with apparently similar conductance properties have been found in intact islet cells (Ashcroft, Harrison & Ashcroft, 1984), which are reported to have the same ATP concentration as that found in cardiac cells (Ashcroft, Weerasinghe & Randle, 1973; Malaisse et al., 1979).

Large unit conductance K^+ channels that can be activated both by membrane depolarization and by increasing internal Ca^{2+} (K^+_{MAXI}) have been described in a wide range of tissues (Latorre & Miller, 1983; Petersen & Maruyama, 1984). In B-cells such channels were first described by Marty and Neher (1982). More recently two detailed studies of K^+_{MAXI} channels in pancreatic islet cells have shown significant differences in the range of internal Ca^{2+} ($[Ca^{2+}]_i$) at which the K^+_{MAXI} channels were activated. Cook, Ikeuchi and Fukimoto (1984) reported that with 1–3 μM internal Ca^{2+} membrane voltages more

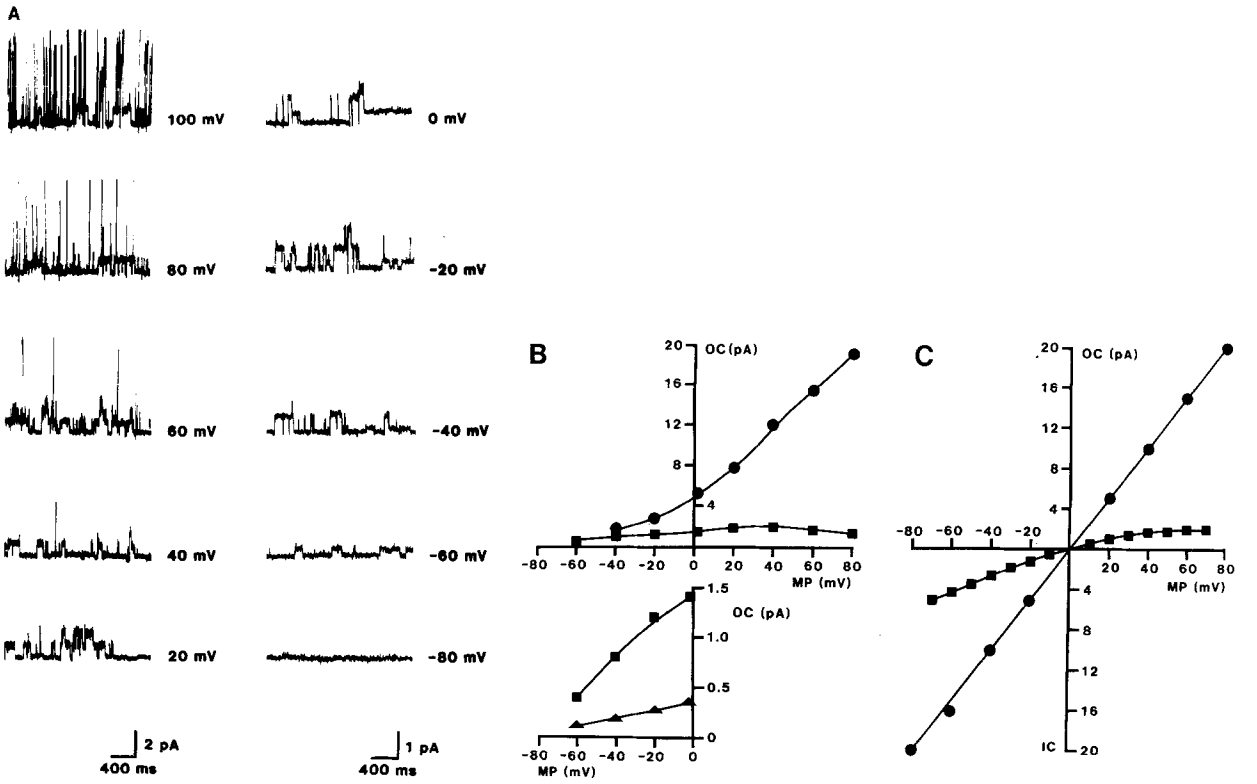

Fig. 1. (*A*) Single-channel current recording from an excised outside-out membrane patch from a cultured rat islet cell. The patch was exposed to quasi-physiological cation gradients (Na^+-rich solution outside; K^+-rich solution inside); the 'intracellular' solution also contained 1 mM EGTA with no added Ca. The patch membrane potential at which each trace was recorded is written next to the individual traces. Records were filtered at 400 Hz low pass for positive membrane voltages, at 200 Hz low pass for negative membrane voltages. (*B* and *C*) Single-channel current-voltage relationships recorded from excised inside-out membrane patches. Circles represent the K^+_{MAXI} channel. Squares represent the K^+_{IR} channel and triangles represent the K^+_{SIR} channel. K^+-rich 'intracellular' solution for K^+_{IR} and K^+_{SIR} channel records also contained 1 mM EGTA and no added Ca. For records of the K^+_{MAXI} channel K^+-rich 'intracellular' solution also contained 5×10^{-7} Ca^{2+}. (*B*) Current-voltage relationships recorded in the presence of quasi-physiological Na^+/K^+ cation gradients. Inset (lower part): Expanded version (vertical scale) of the upper graph to illustrate the K^+_{SIR} channel. (*C*) Current-voltage relationships recorded with K^+-rich solutions on both sides of the patch membrane

positive than +20 mV were required in order to obtain open-state probabilities greater than 0, whereas Findlay, Dunne and Petersen (1985*a*) described open-state probabilities of about 0.5 at negative membrane potentials at such high levels of internal Ca^{2+}.

In view of these two apparent contradictions, the present investigation was undertaken to compare the behavior of the ATP-sensitive K^+ channels in intact cells and excised membrane patches, and to re-examine the Ca^{2+}-sensitivity of K^+_{MAXI} channels in cultured islet cells. In the course of this investigation two important new pieces of information came to light: firstly that there was a third K^+-selective membrane channel in the B-cell membrane, a smaller ATP-sensitive K^+ channel, and secondly that there are many more active K^+ channels in excised membrane patches than apparent in the cell-attached configuration.

Materials and Methods

Cell Isolation and Maintenance

Islets of Langerhans were isolated from adult rat pancreata, and dispersed single cells and small clusters of cells were prepared as described previously (Findlay et al., 1985*a*). Isolated cells were maintained in RPMI 1640 medium (Flow Labs) containing 11 mM glucose (supplemented with 10% vol/vol heat inactivated fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine) on glass coverslips in Falcon-type 3001 plastic petri dishes (35 mm diameter). Petri dishes containing cells were incubated at 37°C in a humidified atmosphere of 95% O_2/5% CO_2 for up to 16 days.

Media

The Na^+-rich 'extracellular' solution contained (mM): 140 NaCl, 4.7 KCl, 2 $CaCl_2$, 1.13 $MgCl_2$, 10 HEPES, 2.5 glucose, pH 7.2. The K^+-rich 'intracellular' solution contained (mM): 140 KCl, 10

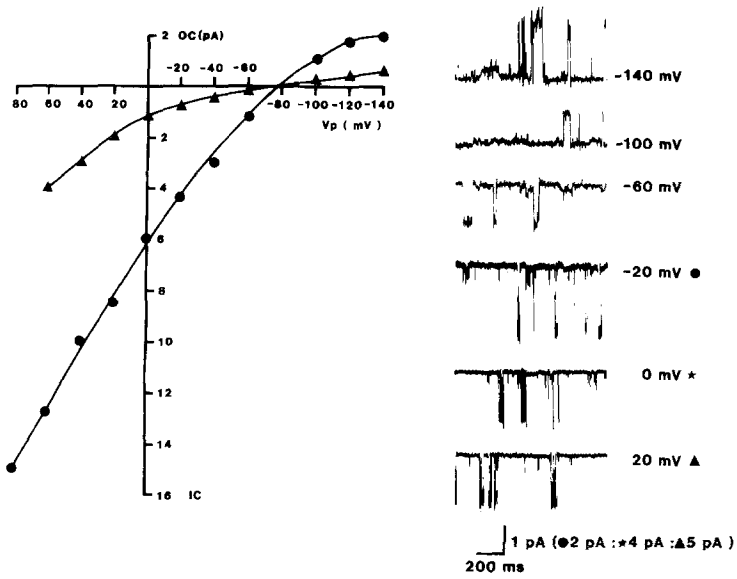

Fig. 2. Single-channel current records and current-voltage relationships obtained from a cell-attached membrane patch. The patch pipette contained K^+-rich solution, and the cell was bathed in Na^+-rich solution. Circles represent the current-voltage relation of the K^+_{IR} channel, triangles the K^+_{SIR} channel. Lines were fitted by eye. Representative single-channel current record traces are shown with the pipette voltage (V_p) potential at which each trace was recorded written next to each trace. Records were filtered at 1 KHz low pass

NaCl, 1.13 $MgCl_2$, 10 HEPES, 2.5 glucose, pH 7.2. The free concentration of Ca^{2+} in K^+-rich solutions was determined by the addition of $CaCl_2$ and EGTA buffer mixtures (Findlay et al., 1985*a*). ATP (Mg^{2+} or Na^+ salts: Sigma, Poole, U.K.) was added directly to K^+-rich solution which also contained 1 mM EGTA and no added Ca; the pH was then re-adjusted to 7.2 with KOH.

Recording and Analysis

Single-channel current recordings were carried out using the methods described by Hamill et al. (1981) using either the List EPC-5 or EPC-7 patch-clamp amplifier systems. Glass patch pipettes were coated with Sylgard resin (Corning) and fire-polished. They had a resistance of between 3 and 10 MΩ. Current recordings were stored on tape (Racal 4DS recorder) for subsequent replay and analysis. In all patch-clamp current records upward deflections represent current flow from the inside to the outside of the patch membrane.

Results

Three Separate K^+ Channels

Figure 1*A* shows single-channel current records obtained from an excised outside-out membrane patch which was exposed to quasi-physiological cation gradients. At a membrane potential of −80 mV no discernible channel currents were recorded. Depolarizing the membrane patch revealed two distinct levels of single-channel current which at 0 mV membrane potential had amplitudes of approximately 1 and 0.35 pA. In neither case did the pattern of channel activity appear to be influenced by voltage. At membrane potentials more positive than +20 mV occasional brief currents of a much larger amplitude could be seen (Fig. 1*A*). Changing the membrane potential from +20 to +80 mV increased the frequency of these brief larger single-channel currents. It will be shown below that the large currents seen in Fig. 1*A* represent the high conductance voltage- and Ca^{2+}-activated K^+ channel (K^+_{MAXI}) and that the two voltage-insensitive currents represent two inward rectifier K^+ channels (K^+_{IR} and K^+_{SIR} (small inward rectifier)).

Figure 1*B* shows the single-channel current-voltage relationships for the three channels under quasi-physiological transmembrane cation gradients (Na^+-rich solution outside, K^+-rich solution inside). The K^+_{SIR} channel (Fig. 1*B*, inset) had a slope conductance of about 4 pS at membrane potentials between − 60 and 0 mV. The K^+_{IR} channel had a slope conductance of about 17 pS under the same conditions (Fig. 1*B*). The K^+_{MAXI} channel had a slope conductance of about 90 pS at membrane potentials between −40 and 0 mV (Fig. 1*B*). In excised membrane patches exposed to K^+-rich solutions on both sides of the membrane the single-channel currents for all three channels reversed at 0 mV. The K^+_{SIR} channel currents showed inward rectification with slope conductances for outward membrane current of about 10 pS and for inward membrane currents of about 15 pS. The K^+_{IR} channel showed similar rectification with a conductance of about 30 pS for outward and 75 pS for inward membrane currents (Fig. 1*C*). The K^+_{MAXI} channel had a linear current-voltage relationship with a conductance of 250 pS (Fig. 1*C*).

Single Channel Recording from Cell-Attached Membrane Patches

Figure 2 shows representative single-channel current traces and the corresponding current-voltage

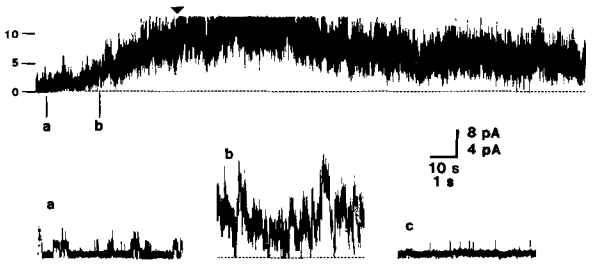

Fig. 3. A continuous record obtained from an inside-out membrane patch after its excision from the intact cell. The patch was exposed to a quasi-physiological Na^+/K^+ cation gradient. The upper trace begins within 15 sec of the patch being excised. The patch was voltage clamped at 0 mV throughout. The dotted line corresponds to the current level recorded when all channels are closed and the numbers to the left of the trace correspond to the equivalent current levels for 0, 5 and 10 simultaneously open K^+_{IR} channels. The arrow indicates the current level at which the tape record of the experiment was saturated. The lower traces (*a* and *b*) represent current records obtained on a faster time base from the indicated portions of the upper trace. Trace *c* was recorded 14 min after patch excision. Records were filtered at 500 Hz low pass

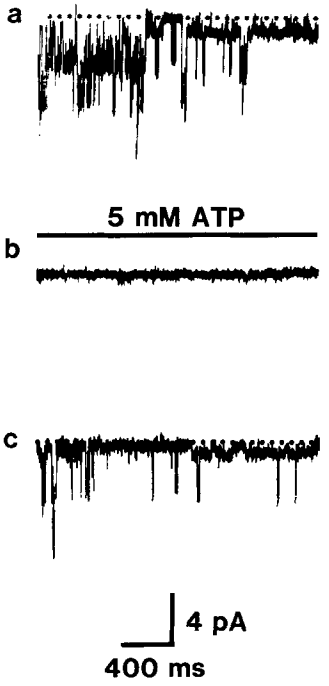

Fig. 4. The effect of ATP upon single-channel currents recorded from K^+_{IR} and K^+_{SIR} channels in an excised inside-out membrane patch. Both the patch pipette and the bath contained K^+-rich solution. The patch was voltage-clamped at a membrane potential of −45 mV. (*a*) Control record in K^+/K^+ situation. (*b*) 5 mM ATP was added to the K^+-rich bath solution. (*c*) Return to control K^+/K^+ situation. The dotted line corresponds to the current level recorded when all channels were closed. Record was filtered at 500 Hz low pass

relationships obtained from a cell-attached membrane patch. Two levels of channel current were observed at all potentials and the activity of the two channels was not clearly affected by voltage. The current-voltage relationships for both channels show inward current rectification and the polarity of the currents reversed when the pipette voltage (V_p) was made more negative than −80 mV. The smaller channel clearly corresponds to the K^+_{SIR} channel with a slope conductance for inward current of about 30 pS, and for outward current of about 10 pS. The larger channel corresponds to the K^+_{IR} channels with slope conductances of about 85 and 20 pS, respectively. This result was typical of the three preparations tested. In recordings obtained from intact cells bathed in K^+-rich solution the two single-channel currents reversed at 0 mV with slope conductances of about 65 and 15 pS for inward current measured between V_p of +20 and +80 mV and slope conductances for outward current of 25 and 5 pS measured between V_p of −20 and −80 mV. Similar results were obtained in both records made from K^+-depolarized intact cells. Single-channel currents corresponding to K^+_{MAXI} channels were not recorded from cell-attached membrane patches.

Inward Rectifier K^+ Channels

Figure 3 is a current record obtained from a membrane patch which begins as the patch was excised from an intact cell and continues for the next 14 min. The upper trace illustrates the 'envelope' of channel behavior over this period.

Immediately after removal of a membrane patch from an intact cell the pattern of K^+_{IR} channel activity was similar to that recorded prior to the excision (Fig. 3*a*). But one minute later the number of active K^+_{IR} channels had increased dramatically (Fig. 3*b*) such that the membrane patch current record began to resemble a 'membrane-noise' record where so many channels were active that individual single-channel currents could no longer be distinguished. Figure 3 shows that outward currents which corresponded to more than 10 simultaneously open K^+_{IR} channels were activated in this particular patch. The number of channels activated upon excision of a patch was variable, outward currents which corresponded to more than 20 simultaneously open K^+_{IR} channels have been recorded from individual membrane patches. By comparison, in records obtained from 60 cell-attached membrane patches 41 showed activity in one or two K^+_{IR} channel current levels, only seven patches showed activity in three or more K^+_{IR} channels, the remainder were silent.

After reaching a peak, K^+_{IR} channel activity then gradually declined. In the example shown in Fig. 3

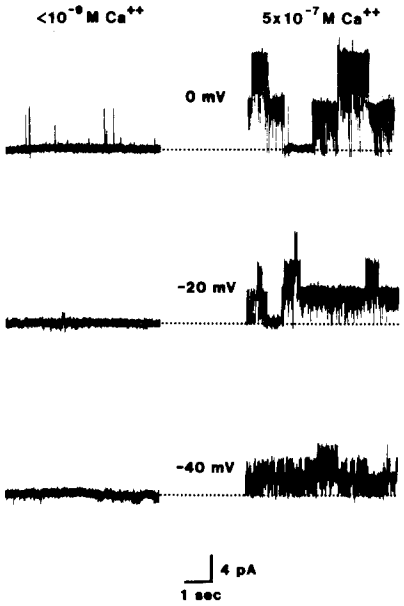

Fig. 5. Single-channel current recordings of the K^+_{MAXI} channel in an excised inside-out membrane patch exposed to quasi-physiological Na^+/K^+ cation gradients. The membrane potential at which each trace was recorded is written next to the individual traces. In the left-hand column the K^+-rich bath solution contained 1 mM EGTA and no added Ca. The right-hand column shows records obtained from the same membrane patch when the K^+-rich bath solution had been exchanged for one that contained 5×10^{-7} M Ca^{2+}. The dotted lines correspond to the current level recorded when all channels are closed. Records were filtered at 500 Hz low pass

single-channel currents could only be clearly distinguished again 10 min after patch excision. Fourteen minutes after excision this activity had declined further so as to appear only as brief and infrequent channel openings (Fig. 3*c*). The rate of decline of K^+_{IR} channel activity in excised membrane patches was variable. In some cases the pattern of activity had declined to the level of infrequent and brief openings of an apparently single K^+_{IR} channel within a couple of minutes of excision. In other cases the high activity 'noise' pattern typified by the upper trace in Fig. 3 was maintained for more than 20 min.

In excised membrane patches the pattern of K^+_{SIR} and K^+_{IR} channel opening and closure was found to be independent of the voltage applied to the membrane (Fig. 1). K^+_{SIR} and K^+_{IR} channels were also not affected by increasing the concentration of Ca^{2+} applied to the internal surface of the patch membrane from 10^{-9} to 10^{-6} M. Figure 4 shows that when 5 mM Mg^{2+}-ATP was applied to an inside-out patch the otherwise continuous pattern of activity seen in both the K^+_{SIR} and the K^+_{IR} channels (Fig. 4*a*) was rapidly and completely abolished (Fig. 4*b*). This effect was fully reversible (Fig. 4*c*). 1 mM Mg^{2+}-ATP was just as effective as 5 mM Mg^{2+}-ATP in evoking complete inhibition of K^+_{SIR} and K^+_{IR} channels (6 patches). 0.1 mM Mg^{2+}-ATP markedly reduced the frequency of K^+_{SIR} and K^+_{IR} channel openings without affecting the amplitude of single-channel currents (3 patches). The Na^+ salt of ATP was just as effective as the Mg^{2+} salt in evoking inhibition of the K^+_{SIR} and K^+_{IR} channels (3 patches).

The K^+_{MAXI} Channel

Since there is a serious discrepancy between the Ca^{2+}-sensitivity of K^+_{MAXI} channels recorded from cultured neonatal rat pancreatic islet cells (Cook et al., 1984) and those recorded from freshly dissociated adult rat pancreatic islet cells (Findlay et al., 1985*a*), we felt that it was important to re-examine this question in membrane patches excised from primary cultures of adult rat pancreatic islet cells. Figure 1*A* shows that K^+_{MAXI} channels could be activated by membrane depolarization in the virtual absence of Ca^{2+} bathing the internal membrane surface. Figure 5 clearly illustrates that under quasi-physiological transmembrane cation gradients (Na^+-rich solution outside, K^+-rich solution inside) K^+_{MAXI} channels could be markedly activated at negative membrane potentials when the internal membrane surface was exposed to 5×10^{-7} M Ca^{2+}.

The Effect of TEA Upon K^+ Channels

TEA is known to block voltage- and calcium-activated K^+_{MAXI} channels in a variety of tissues (Latorre & Miller, 1983; Vergara, Moczydlowski & Latorre, 1984) but in B-cells it had been suggested that TEA instead selectively blocked a voltage-activated K^+ permeability without affecting a Ca^{2+}-activated K^+ permeability (Atwater, Ribalet & Rojas, 1979; Henquin, Meissner & Preissler, 1979). When 10 mM TEA was applied to the outside of a membrane patch it abolished current flow through the K^+_{MAXI} channel which had been activated by membrane depolarization (Fig. 6*A*). At the same time, however, TEA had no discernible effect upon either the pattern of activity or the single-channel current amplitude of the K^+_{IR} channel (Fig. 6*Ab*). This result was typical of three membrane patches exposed to >2 mM TEA on the external membrane surface. 10 mM TEA applied to the internal membrane surface had no observable effect on K^+_{MAXI} channels.

Since TEA selectively blocked voltage-activated K^+_{MAXI} channels it was important to investigate whether it could also block the channels when activated by intracellular Ca^{2+}. Figure 6*B* shows that when external membrane surface was exposed to 5 mM TEA even 10^{-6} M Ca^{2+} applied to the internal membrane surface failed to activate K^+_{MAXI} channels.

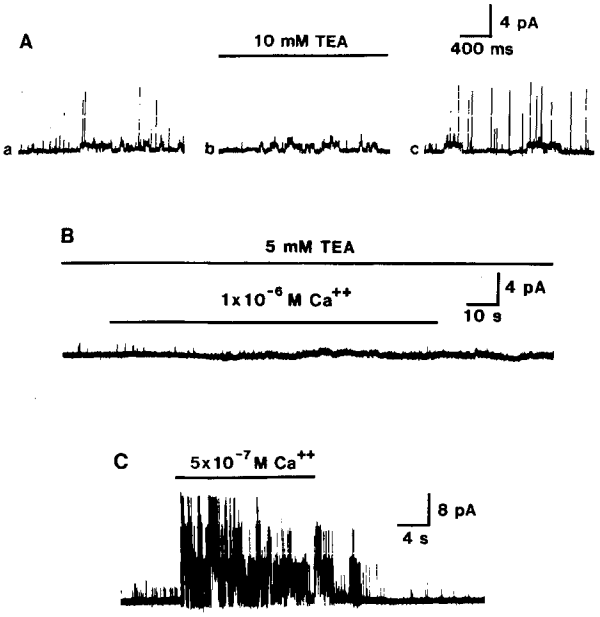

Fig. 6. The effects of TEA upon K^+ channels recorded in excised membrane patches exposed to quasi-physiological Na^+/K^+ cation gradients. (*A*) An outside-out membrane patch which was voltage-clamped at a membrane potential of +60 mV. The pipette was filled with K^+-rich solution, which also contained 1 mM EGTA and no added Ca. *a*, Control trace in Na^+/K^+ situation. *b*, The Na^+-rich bath solution also contained 10 mM TEA. *c*, Return to control solution. (*B*) An inside-out membrane patch which was voltage-clamped at 0 mV throughout. The Na^+-rich solution in the pipette also contained 5 mM TEA. The K^+-rich solution which was superfused past the pipette initially contained 1 mM EGTA and no added Ca. For the period indicated by the bar this was exchanged for K^+-rich solution containing 10^{-6} M Ca^{2+}. (*C*) An inside-out membrane patch which was voltage-clamped at 0 mV throughout. The pipette contained Na^+-rich solution without TEA. The K^+-rich solution which was superfused past the pipette initially contained 1 mM EGTA and no added Ca. For the period indicated by the bar this was exchanged for K^+-rich solution containing 5×10^{-7} M Ca^{2+}. Records were filtered at 1 KHz low pass in *A* and *C*, 200 Hz low pass in *B*

This result was obtained in four patches in which 5 or 10 mM TEA was included in the pipette solution and 5×10^{-7} or 10^{-6} M Ca^{2+} was applied to the internal membrane surface. In the absence of TEA 5×10^{-7} M Ca^{2+} markedly activated K^+_{MAXI} channels (Fig. 6*C*) (nine patches).

Discussion

The results presented here demonstrate that three separate K^+-selective channels coexist in the pancreatic islet cell membranes (Fig. 1*A*). Two of these channels (K^+_{SIR}, K^+_{IR}) have been found to be spontaneously operative in intact, unstimulated islet cells (Fig. 2), both show inward current rectification (Fig. 1*C*) and both can be inhibited at ATP (Fig. 4). The third channel (K^+_{MAXI}) has not been observed in unstimulated intact cells, but can be activated in excised membrane patches by both membrane depolarization (Fig. 1*A*) and raising $[Ca^{2+}]_i$ (Fig. 5).

The larger of the two K^+-selective inward rectifier channels (K^+_{IR}) (Figs. 1 and 2) is very similar to the ATP-sensitive K^+ channel described in cardiac cells (Noma, 1983; Kakei et al., 1985). In contrast to the findings in cardiac cells this channel shows a spontaneous pattern of opening and closure in the majority of cell-attached recordings from our pancreatic islet cells. The smaller, inward rectifier K^+ channel (K^+_{SIR}), which is illustrated here for the first time (Fig. 1*A*), was also recorded in cell-attached membrane patches (Fig. 2). It is clear that the ATP-sensitive inward rectifier K^+ channels dominate the resting cell K^+ permeability and therefore may be primarily responsible for the resting membrane potential.

The inhibitory effect of 1 mM or greater concentration of ATP upon K^+_{SIR} and K^+_{IR} channel activity in excised membrane patches was clear (Fig. 4) and confirmed the result obtained by Cook and Hales (1984). But both K^+_{IR} and K^+_{SIR} channels could be seen to be active in intact cells (Fig. 2) where the ATP concentration has been estimated to be between 3–4 mM (Ashcroft et al., 1973; Malaisse et al., 1979). Both the level of activity and the number of visible channels in intact cells (Fig. 2) were, however, clearly much less than those observed upon excision of the membrane patch (Fig. 3). Since currents that correspond to between 10–20 simultaneously open K^+_{IR} channels could be recorded from individual membrane patches the figure of 150 K^+_{IR} channels per B-cell (Ashcroft et al., 1984) is probably a considerable underestimate. It could be suggested that excision was in some way activating the channels, but it seems more likely than an *in situ* inhibitory influence was lost when the internal surface of the membrane patch was exposed to the ionic environment of the experimental bathing solution. Since estimates of B-cell ATP content (Ashcroft et al., 1973; Malaisse et al., 1979) are based upon the total ATP in the cell the concentration of ATP free in the cell cytoplasm could be much less, perhaps sufficiently small to allow a low level of activity in K^+_{IR} channels in intact unstimulated cells. It is also possible that the ATP concentration required to inhibit K^+-channel opening is higher in cells than in excised membrane patches, as has been recently shown in experiments on open-cell attached patches in cardiac cells (Kakei et al., 1985). Stimulation of B-cells by the metabolism of glucose could locally increase the ATP concentration via a mem-

brane-bound phosphoglycerate kinase (Dean, Matthews & Sakamoto, 1975) and completely inhibit K^+_{IR} and K^+_{SIR} activity and explain glucose-evoked depolarization of islet cells. Such a scheme of events would be consistent with the finding that glucose added to the bath solution can inhibit opening of K^+ channels in cell-attached membrane patches (Ashcroft et al., 1984).

At the present time we have no explanation for the irreversible decline in the activity of ATP-sensitive K^+ channels in excised membrane patches (Fig. 3). A similar phenomenon has been noted in membrane patches excised from cardiac cells (Kakei & Noma, 1984; Trube & Hescheler, 1984) and an insulin-secreting cell line (Findlay et al., 1985*b*). ATP-sensitive K^+ channels in cardiac cells did not show this loss of activity when they were recorded from membrane patches in the open cell-attached configuration (Kakei et al., 1985).

The Ca^{2+}-sensitivity of K^+_{MAXI} channels varies widely between tissues (Latorre & Miller, 1983; Petersen & Maruyama, 1984). In both adult pancreatic islet B-cells and an insulin-secreting cell line the intracellular concentration of Ca^{2+} in unstimulated cells has been found to be approximately 10^{-7} M, which increases two- to threefold upon stimulation (Rorsman, Abrahamsson, Gylfe & Hellman, 1984; Wollheim & Pozzan, 1984; Deleers, Mahy & Malaisse, 1985). Figure 5 confirms our previous experience (Findlay et al., 1985*a*) and shows that the K^+_{MAXI} channels in cultured pancreatic islet cells can be activated within this range of $[Ca^{2+}]_i$ which has also been shown to directly evoke insulin secretion (Yaseen, Pedley & Howell, 1982).

TEA blocks K^+_{MAXI} channels in a number of tissues (Latorre & Miller, 1983; Vergara et al., 1984) and Cook and Hales (1984) reported that it inhibited both K^+_{MAXI} and K^+_{IR} channel types in pancreatic B-cells. In this investigation, however, as in a study of K^+ channels in an insulin-secreting cell line (Findlay et al., 1985*b*), TEA instead selectively blocked both voltage- and calcium-activation of the K^+_{MAXI} channel without affecting the K^+_{IR} channel (Fig. 6*A* and *B*).

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