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ATP-Sensitive K^+ Channels in an Insulin-Secreting Cell Line are Inhibited by D-Glyceraldehyde and Activated by Membrane Permeabilization

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Summary. The control of K^+ channels in the insulin-secreting cell line RINm5F has been investigated by patch-clamp single-channel current recording experiments. The unitary current events recorded from cell-attached patches are due to large and small inwardly rectifying ATP-sensitive K^+ channels with conductance properties similar to the two channels previously identified in primary cultured rat islet cells (Findlay, I., Dunne, M.J., & Petersen, O.H. *J. Membrane Biol.* **88**:165–172, 1985). Cell permeabilization through brief exposure to 10 μM digitonin or 0.05% saponin (outside the isolated membrane patch area) results in a dramatic increase in current through the cell-attached patch due to opening of many large and small K^+-selective channels. These channels are inhibited in a dose-dependent manner by ATP applied to the bath (near-complete inhibition by 5 mM ATP). During prolonged ATP exposure (1-5 min) the initial inhibition is followed by partial recovery of channel activity, although further activation does occur when ATP is subsequently removed. From the maximal number of coincident channel openings in the permeabilized cells (in the absence of ATP), it is estimated that there are on average 12 large ATP-sensitive K^+ channels per membrane patch, but in the intact cells less than 5% of the membrane patches exhibited three or more coincident K^+ channel openings, indicating the degree to which the channels are inhibited in the resting condition by endogenous ATP. Stimulation of RINm5F cells to secrete insulin was carried out by challenging intact cells with 10 mM D-glyceraldehyde. D-glyceraldehyde induced depolarization of the membrane from about −70 to −20 mV and evoked a marked reduction in the open-state probability of both the large and small ATP-sensitive channels. D-glyceraldehyde also induced action potentials in a number of cases. All effects of stimulation were largely transient, lasting about 100 sec. The two ATP-sensitive K^+ channels are probably responsible for the resting potential and play a crucial role in coupling metabolism to membrane depolarization.

Key Words K^+ channel · ATP · glyceraldehyde · RINm5F cell

Introduction

Glucose-evoked insulin secretion is associated with membrane depolarization and firing of action potentials (Dean & Matthews, 1968, 1970). The stimulant-evoked depolarization is associated with a reduction in membrane K^+ permeability (Sehlin & Täljedal, 1975; Henquin & Meissner, 1984), and Ashcroft, Harrison and Ashcroft (1984) have with the help of the patch-clamp method identified a K^+-selective channel in insulin-secreting pancreatic beta cells that closes when glucose is metabolized. This glucose-sensitive channel is inhibited directly by intracellular ATP (Rorsman & Trube, 1985) and is identical to the ATP-sensitive K^+ channel in pancreatic beta cells first described by Cook and Hales (1984). However, in the absence of glucose stimulation the ATP concentration of intact beta cells is considerably greater than that required to completely inhibit the channel in excised inside-out membrane patches (Ashcroft, Ashcroft & Harrison, 1985).

Findlay, Dunne and Petersen (1985*b*) have shown that there are in fact two types of ATP-sensitive K^+ channels in pancreatic islet cells, one with a conductance close to that described for the glucose-sensitive channel (Ashcroft et al., 1984; Rorsman & Trube, 1985) and another with a smaller unit conductance. Both these channels have inward rectifier properties. In intact resting islet cells both channel types are operational, although in the majority of cell-attached patches no more than one or two current levels can be observed. After excision of membrane patches into the inside-out conformation and exposure of the membrane inside to the ATP-free bathing solution there is a dramatic increase in the patch current corresponding mostly to between 10 and 20 open K^+ channels, but thereafter run-down of channel activity occurs (Findlay et al., 1985*b*,*c*).

A number of important questions arise from these recent investigations: (1) Are the many channels that are activated in a membrane patch after excision all sensitive to ATP? (2) Does the ATP sensitivity in excised inside-out patches correspond to the situation in cell-attached patches? and (3) Does glucose or glyceraldehyde metabolism also

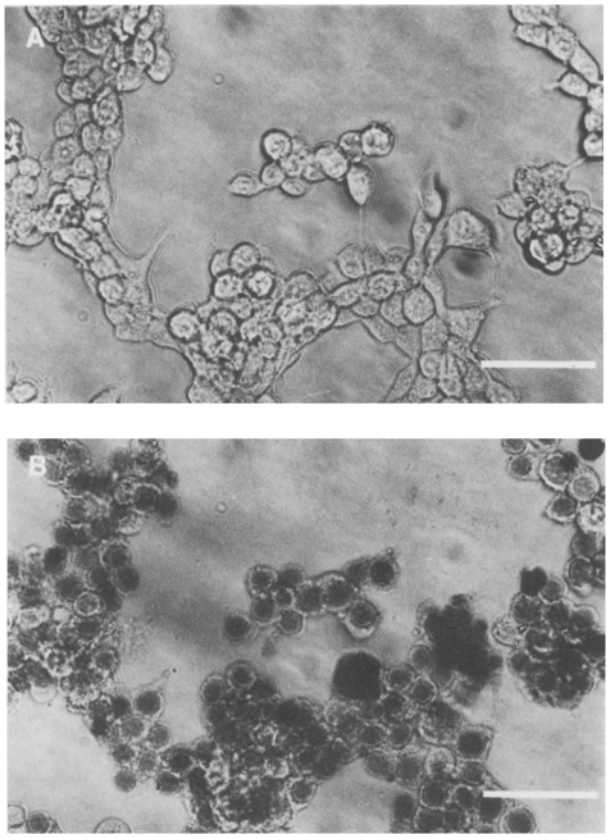

Fig. 1. (*A*) Photomicrograph illustrating intact RINm5F insulinoma clonal cells bathed in the Na^+-rich solution containing 0.2% trypan blue. (*B*) The same dish of cells 1 min after they had been exposed to 0.05% saponin. The bar illustrated in both photomicrographs corresponds to a length of 50 μm

close down the small ATP-sensitive K^+ channels? The study reported here answers these questions and provides new insights into the mechanism by which ATP controls inward rectifier K^+ channels.

Materials and Methods

Cell Culture

Most experiments were carried out on the insulin-secreting cell line RINm5F (Halban, Praz & Wollheim, 1983; Praz et al., 1983). Cells were maintained in RPMI 1640 tissue culture media, containing 11 mM glucose and supplemented with 10% (vol/vol) fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 μg/ml fungizone. Cells were seeded out every two to three days onto Falcon-style 3001 type petri dishes (35 × 10 mm) and kept in a humidified atmosphere of 95% O_2 and 5% CO_2 at a temperature of 37°C. Figure 1 shows a photomicrograph of the RINm5F cells as they present themselves to the investigator in a patch-clamp experiment. Trypan Blue (0.2%) was present in the bathing solution, but as seen in Fig. 1*A* there was no sign of dye uptake into the cells. In contrast, after a brief (20 sec) exposure of the cells to saponin (0.05%) virtually all the cells were markedly stained, indicating extensive permeabilization of the plasma membrane.

A few experiments were carried out using primary cultures of rat pancreatic islet cells prepared as previously described (Findlay et al., 1985*b*).

Media

Patch pipettes were filled with a K^+-rich solution which contained (mM): 140 KCl, 10 NaCl, 1.13 $MgCl_2$, 10 glucose, 10 HEPES and 1 EGTA. No Ca^{2+} was added, and pH was adjusted to 7.2 The cells were bathed in a Na^+-rich solution which contained (mM): 140 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.13 $MgCl_2$, 2.5 glucose and 10 HEPES with the pH set at 7.2. In experiments using the permeabilized "open-cell" patch configurations the cells were bathed in the Ca^{2+}-free K^+-rich solution with 0.5 mM EGTA and the recording pipette was filled with the Na^+-rich solution to which 1 mM EGTA was added and the $CaCl_2$ omitted.

High K^+ solution experiments on intact cells were carried out with the Na^+-rich bathing solution changed to either the K^+-rich solution (containing 2.5 mM glucose) or a solution consisting of (mM): 90 NaCl, 50 KCl, 1.13 $MgCl_2$, 2.5 glucose, 10 HEPES (pH = 7.2). The osmolality of all solutions was 290 ± 5 mOsm/kg.

Upon formation of a giga-seal between cell and pipette the cell was continuously superfused by a stream of control solution from one of a series of piped outlets, with other pipes containing various test solutions. The flow rate of solution was between 50-80 μl/min. Rapid exchange from control to test solution was achieved manually under visual control. Permeabilization of cells was carried out by placing digitonin or saponin in the flow of control solution up-stream from the cell by means of a "blunt" glass micropipette.

Recording and Analysis

Single-channel current recordings were obtained as described by Hamill et al. (1981) using the List EPC-7 patch-clamp amplifier system. Glass patch-pipettes were coated with a sylgard resin (Corning) and fire polished so that they had a final resistance of between 5 and 10 MΩ when filled with the K^+-rich or Na^+-rich solution. Membrane current records (filtered at 1 kHz low pass), were primarily stored on tape (Racal 4DS recorder) for subsequent replay and analysis.

The taped current records were analyzed using a laboratory microcomputer (BBC model B with 6502 second processor) connected to a Winchester Hard Disc System. The data was digitized (3 kHz), and an idealized current record obtained from computerized threshold analysis. The average open-state probability was determined from current records lasting 10-20 sec.

In all single-channel current traces upward deflections represent current flow from the inside to the outside of the isolated membrane patch.

Results

Single-Channel Currents Recorded from Cell-Attached Membrane Patches

Figures 2 and 3 illustrate single-channel current-voltage (*I/V*) relationships as well as representative single-channel current traces from RINm5F cell-attached membrane patches, with a K^+-rich solution in the pipette and a Na^+-rich solution bathing the

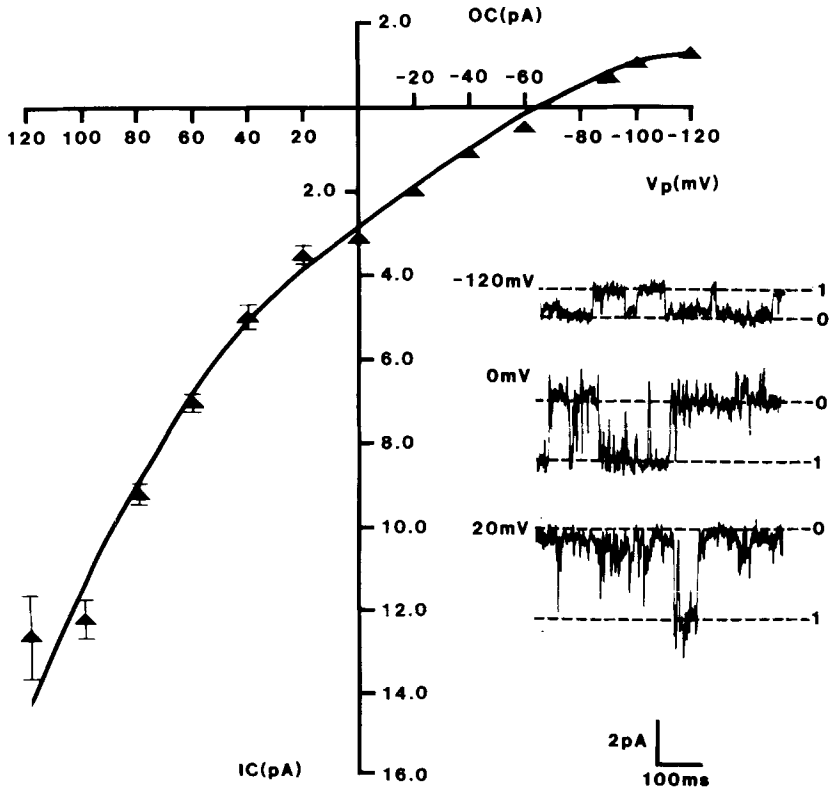

Fig. 2. Single-channel current recordings and the mean current-voltage (*I-V*) relationships for the large ATP-sensitive potassium selective channel (K^+_{IR}). All records were taken from RINm5F cell-attached membrane patches with the Na^+-rich solution bathing the cells and the K^+-rich solution in the pipette. Mean values for the *I/V* plot are taken from 15 experiments. SE bars are shown where SE is greater than the triangles. All single-channel current records were filtered at 1 kHz (low pass) and the pipette potential (V_p) at which the voltage was clamped is next to each trace. The patch membrane current recorded when all channels are closed (*O*) and when one channel is open (*I*) has been indicated. In the case of the recording obtained at a pipette voltage (V_p) of 0 mV, however, the *O* level indicated is the 'all closed' level for the K^+_{IR} channel and not the zero current level through the patch; this is because there is a continuous opening from a smaller K^+ channel (K^+_{SIR}) upon which the *O* line has been set

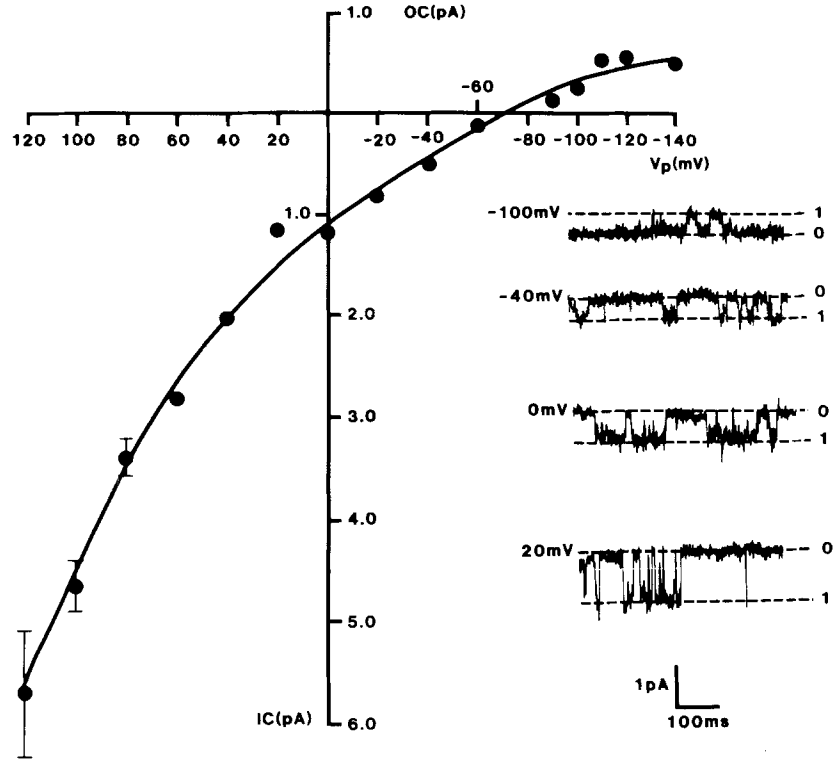

Fig. 3. Single-channel records and the mean current-voltage (*I-V*) relationship for the small ATP-sensitive potassium selective channel (K^+_{SIR}). All records were taken from RINm5F cell-attached membrane patches with the Na^+-rich solution bathing the cells and the K^+-rich solution in the pipette. Mean values have been obtained from 15 experiments, SE bars are shown were SE is greater than the filled circles. All single-channel current records were filtered at 1 kHz (low pass), and the pipette potential (V_p) at which the voltage was clamped is shown next to each trace. The patch membrane current recorded when all channels are closed (*O*) and when one channel is open (*I*) has been indicated

cells. Under these conditions two different amplitudes of single-channel current events are observed, representing a large (Fig. 2) and a small (Fig. 3) K^+-selective channel. The frequency of opening of both these channels appears to be relatively insensitive to changes in the voltage applied to the pipette (V_p). Depolarizing the membrane patch from a pipette voltage (V_p) of +120 mV

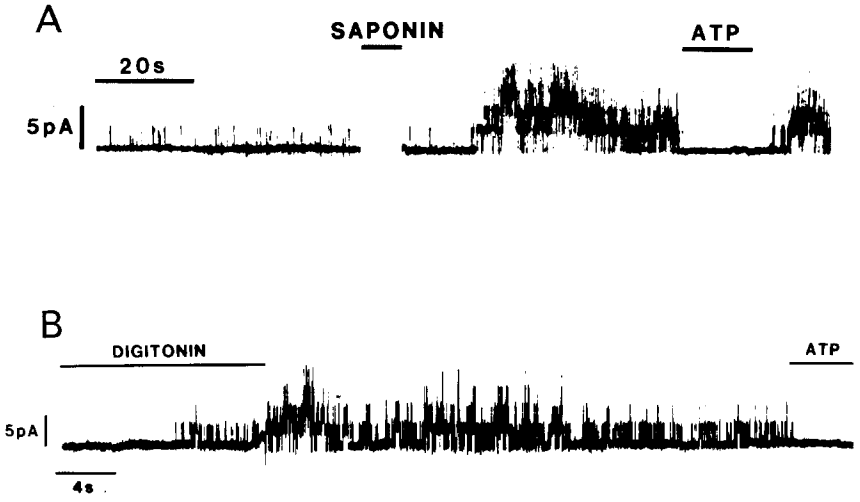

Fig. 4. (*A*) Cell-attached membrane patch recordings obtained from an individual cultured rat islet cell. The pipette contained the Na^+-rich solution, and the cell was bathed in the K^+-rich solution. During the period indicated the cell was permeabilized by gently blowing a 0.05% wt/vol solution of saponin into the stream of the solution bathing the cells. This part of the current trace which lasts approximately 20 sec has been excluded because of artifacts generated by the manipulation of the saponin-containing pipette. During the period indicated by the bar labelled *ATP* the K^+-rich solution bathing the cells was switched to a K^+-rich solution containing 2 mM ATP. The pipette voltage (V_p) was clamped throughout at 0 mV and the pen-recording filtered at 50 Hz. (*B*) A continuous current recording taken from RINm5F cell-attached membrane patch. The pipette contained the Na^+-rich solution, and the cell was bathed in the K^+-rich solution. During the period indicated the cell was exposed to 10 μM digitonin added directly to the solution continually superfusing the cell. The K^+-rich solution bathing the cell was switched as indicated (ATP) to a K^+-rich solution containing 0.5 mM ATP. V_p = 0 mV. The oscilloscope trace was filtered at 400 Hz

through 0 to −40 mV caused a decrease in the amplitude of the single-channel current events. A further increase in the negativity of V_p resulted in no single-channel currents being discernible, followed at high negative potentials (−120 mV in Fig. 2, −100 mV in Fig. 3) by a 'reversal' of the direction of current flow.

The single-channel *I/V* relationship for both the K^+ channels is nonlinear with the inward currents being much larger than the corresponding outward currents. The *I/V* relationships shown in Figs. 2 and 3 are the means from 15 cell-attached patches. At the resting membrane potential of the cell (V_p = 0 mV) the mean amplitude of the large inward-rectifying K^+ channel current (previously termed K^+_{IR} (Findlay et al., 1985*b*)) was 3.2 ± 0.2 pA and the mean amplitude of the small K^+_{IR} channel current (previously termed K^+_{SIR} (Findlay et al., 1985*b*)) was 1.3 ± 0.1 pA. The pipette voltage at which current reversal occurred, which corresponds to the resting membrane potential of the cell (Maruyama, Petersen, Flanagen & Pearson, 1983), was found in both plots (Figs. 2 and 3) to be about −70 mV.

The unit conductance of the K^+_{IR} channel was about 90 pS for inward current in the voltage range (V_p) + 60 to +100 mV, whereas it was only about 20 pS for outward current in the voltage range −80 to −120 mV (Fig. 2). The corresponding conductance values for the K^+_{SIR} channel were 30 and 7 pS, respectively. These values for K^+_{IR} and K^+_{SIR} single-channel conductances in the RINm5F cells are very similar to the values previously reported for the primary cultured rat pancreatic islet cells (Findlay et al., 1985*b*).

Elevation of the bath $[K^+]$ from the normal 4.7 to 50 mM shifted the single-channel *I/V* relationships to the left, and in 12 experiments the mean pipette voltage at which reversal of current polarity occurred was −20 mV. When $[K^+]_o$ was further enhanced to 140 mM (20 experiments) the mean reversal potential was found to be 0 mV.

Single-Channel Current Recording from Cell-Attached Patches of Permeabilized Cells (Open Cell-Attached Recording Configuration)

Figure 4 shows that K^+ channels are activated when insulin-secreting cells are permeabilized and the soluble contents of the cell washed away. In both of these experiments up to four coincident levels of single-channel current were observed in the open cell where only one had been recorded from the intact rat islet cell (Fig. 4*A*) and none from the intact RINm5F cell (Fig. 4*B*). In RINm5F cells an

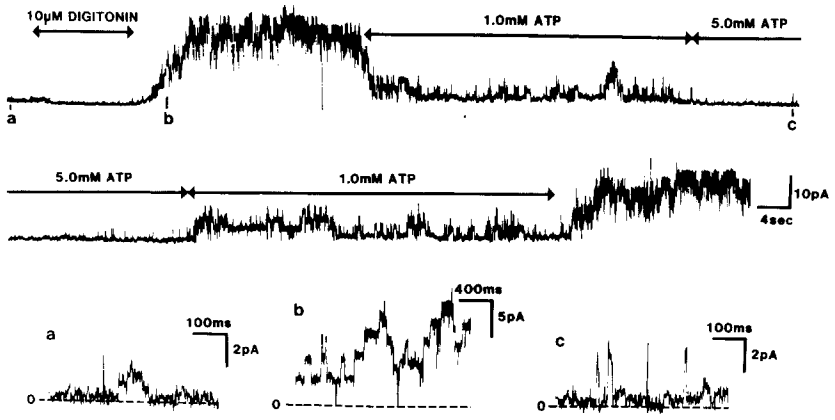

Fig. 5. A continuous recording from a RINm5F cell-attached membrane patch. The pipette contained the Na^+-rich solution, and the cell was continually superfused with the K^+-rich bathing solution. V_p clamped at 0 mV throughout the experiment and the recording filtered at 100 Hz (low pass). At the periods indicated by the bars the cell was first briefly exposed to 10 μM digitonin and later to the K^+-rich solution containing various concentrations of ATP as indicated. *a*, *b* and *c* are consecutive traces which represent records obtained on a faster time-base from the indicated portions of the upper trace. *a* and *c* were filtered at 400 Hz (low pass) and *b* at 200 Hz (low pass)

average of 12 ± 1 (mean ± SE, n = 77) coincident single K^+_{IR} channel currents were recorded when a cell was permeabilized. When the solution perfusing the open cell was exchanged from control to one which contained ATP the K^+ channels were rapidly and reversibly inhibited (Fig. 4). The flow of solution through 'open-cell' preparations was rarely restricted since in most cases K^+ channels reacted to the application of ATP within 1-2 sec.

Figure 5 represents a more elaborate permeabilization experiment typical of 10 such recordings and illustrates the sensitivity of both the K^+_{IR} and K^+_{SIR} channels to ATP applied to the internal surface of the membrane. In the intact cell few channel openings occurred (Fig. 5(*a*)), but within 20 sec of permeabilizing the cell with 10 μM digitonin there is a massive increase in the outward membrane current and what at first appears as noise is, when examined on a faster time base (Fig. 5(*b*)), clearly seen to be composed of single-channel current events representing a number of both large and small K^+_{IR} channels. These channels can be inhibited in a dose-dependent manner when ATP is added to the bathing solution, which is in direct communication with the internal surface of the membrane. In the presence of 5 mM ATP the channel activity (Fig. 5(*c*)) is similar to that observed in the intact cell (Fig. 5(*a*)). Returning to the control solution results in a massive increase in outward current once again brought about by openings from both the K^+_{IR} and K^+_{SIR} channels.

In the experiment represented by Fig. 6*A* the perfusion of a RINm5F open-cell preparation with 5 mM ATP initially caused complete inhibition of K^+ channel activity which lasted for approximately 20

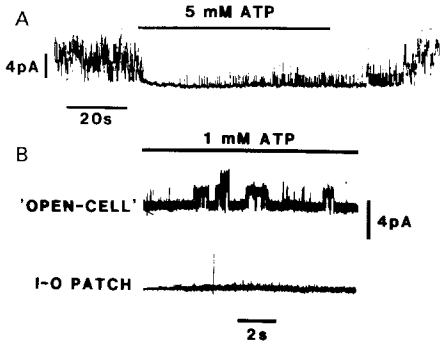

Fig. 6. (*A*) A continuous single-channel current recording obtained from a digitonin permeabilized open RINm5F cell. The open cell was perfused with a K^+-rich solution containing 5 mM ATP for 1 min as indicated by the bar. V_p = 0 mV throughout, and the pen recording was filtered at 50 Hz. (*B*) A comparison of K^+_{IR} channel activity recorded from a cell-attached membrane patch from a digitonin permeabilized cell (upper trace) and subsequently from the same membrane patch after it had been excised from the cell into the inside-out membrane configuration (lower trace). Both records were obtained during short periods (10–20 sec) of superfusion of either the open RINm5F cell or the excised membrane patch by the K^+-rich solution containing 1 mM ATP as indicated. Oscilloscope traces were filtered at 300 Hz. V_p = 0 mV throughout

sec. But then, in the continued presence of 5 mM ATP, K^+ channel openings reappeared and continued for the remaining 40 sec of perfusion with ATP. The magnitude of the recovery from total inhibition in the continuous presence of 5 mM ATP varied, but it occurred in each of the seven open cells and seven excised inside-out membrane patches which were exposed to 5 mM ATP for between 1-5 min.

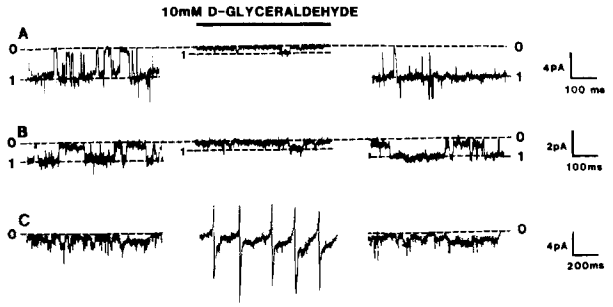

Fig. 7. Single-channel currents obtained from three separate RINm5F cell-attached membrane patches recorded with the K^+-rich solution in the pipette and the Na^+-rich solution bathing the cells. V_p was clamped at 0 mV, and the recordings were filtered at 1 kHz (low pass). Recordings obtained before stimulation, 30 sec after start of stimulation with 10 mM D-glyceraldehyde, and 90 sec after return to control conditions are shown. Trace *A* represents K^+_{IR} single-channel currents, *B* represents K^+_{SIR} single-channel currents and trace *C*, mixed K^+_{IR} and K^+_{SIR} single-channel currents illustrating a more "typical" RINm5F cell-attached patch recording. The patch membrane current recorded when all channels are closed (*O*) and when one channel is open (*1*) has been indicated

Comparison of Single-Channel Currents in Excised Inside-Out and Open Cell-Attached Patches

In excised inside-out membrane patches K^+ channels were extremely sensitive to ATP. In 12 patches 0.5 mM and greater concentrations of ATP were applied for short periods of 10-20 sec and caused complete channel inhibition. 0.1 mM and lesser concentrations of ATP were applied to 14 patches and caused a graded and dose-dependent inhibition similar to that reported previously (Cook & Hales, 1984). We were interested in comparing the ATP sensitivity in the open cell-attached configuration and in the excised inside-out membrane patches. These experiments were performed by first perfusing ATP through an open-cell and then excising the membrane to an inside-out patch and exposing it once again to the solution containing ATP. 1 mM ATP was applied in 18 such experiments. In nine of the open-cell preparations 7.7 ± 2.9% of the control K^+ channel current was retained in the presence of 1 mM ATP (Fig. 6*B*), whereas in the remaining nine preparations channel activity was completely abolished. In the 12 membrane patches which were successfully excised from these open-cell preparations all, without exception, showed that K^+ channel activity was completely abolished in the excised membrane patch by the application of 1 mM ATP (Fig. 6*B*). 0.1 mM ATP was applied to 15 open-cell preparations and 35.5 ± 4.5% of the control K^+ channel current was retained. In seven surviving excised inside-out membrane patches from these same experiments 20.5 ± 7.3% of the control K^+ channel current was recorded during exposure to 0.1 mM ATP.

Effects of 10 mM D-Glyceraldehyde Stimulation on Single-Channel Current in Cell-Attached Membrane Patches

Stimulation of RINm5F cells was carried out by superfusing intact cells with control solutions containing 10 mM D-glyceraldehyde. Membrane currents were recorded from cell-attached patches and D-glyceraldehyde was therefore not in direct contact with the electrically isolated patch membrane.

Figure 7 illustrates single-channel current events from RINm5F cell-attached membrane patches held at a pipette voltage (V_p) of 0 mV. Figure 7*A* is taken from a cell-attached membrane patch where the current trace was dominated by openings from the K^+_{IR} channel. In the control situation the single-channel current amplitude was 3.8 pA. Within 30 sec of challenging the cell with glyceraldehyde the single-channel current amplitude had declined to 0.7 pA and the apparent open-state probability was reduced to 9.6% of the control value. Glyceraldehyde-induced stimulation of the cell was fully reversible, and within 90 sec of returning to the control solution the single-channel current amplitude had increased to 3.5 pA and the open-state probability increased to 110% of the prestimulus level.

Figure 7(*B*) is taken from a cell-attached membrane patch were the K^+-channel current was dominated by the opening of K^+_{SIR} channels. Glyceraldehyde reduced the open-state probability to 8.3% of the preceding control level and the single-channel current from 1.5 to 0.4 pA. Returning to the control solution increased the open-state probability to 90% of the prestimulus level and the single-channel current amplitude to 1.2 pA.

The current records illustrated in Fig. 7*A* and *B* are not typical of RINm5F cell-attached patch recordings. The vast majority, 20 out of 23 experiments, of cell-attached current recordings display openings from both K^+_{IR} and K^+_{SIR} channels. The current record illustrated in Fig. 7*C* is a more typical RINm5F cell-attached recording. Glyceraldehyde stimulation evoked action potentials (Fig. 7*C*) in about 40% (9 out of 23 experiments) of the cell-attached membrane patches beginning on average 19 sec after the start of stimulation.

The change in membrane potential during stimulation with glyceraldehyde was estimated from the change in the reversal potential obtained from a series of single-channel *I/V* relationships for both the

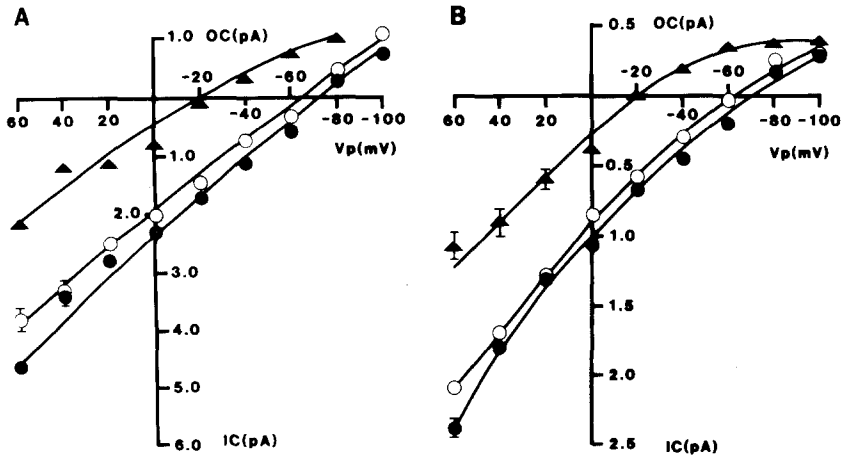

Fig. 8. Single-channel current-voltage relationships for both the K^+_{IR} (*A*) and the K^+_{SIR} (*B*) channels, obtained from RINm5F cell-attached membrane patches with the K^+-rich solution in the pipette and the Na^+-rich solution bathing the cells. In each plot solid circles represent initial control values and open circles values after returning to control conditions; solid triangles represent the *I/V* relationship in the presence of 10 mM D-glyceraldehyde. Mean values were obtained from 23 experiments, SE values have been added where the SE was greater than the symbol used. All lines were fitted by eye

K^+_{IR} and K^+_{SIR} channels (Fig. 8). In the control situation the reversal potential was found to be −72 mV (*A*) and −71 mV (*B*), whereas in the presence of D-glyceraldehyde the *I/V* relationships were found to shift leftwards by approximately 50 mV, with new reversal potentials at −21 and −20 mV, respectively, indicating that glyceraldehyde had depolarized the cells by about 50 mV. The D-glyceraldehyde-induced depolarization was reversible since the cells were able to re-establish a high negative resting membrane potential of approximately −65 mV (reversal potentials of −66 and −64 mV recorded from *A* and *B*, respectively), within 90-120 sec of returning to control solution.

The effects of D-glyceraldehyde stimulation were transient. In the experiment represented in Fig. 9 action potentials were seen within 10 sec of stimulation, but after 100 sec of continued stimulation with D-glyceraldehyde, the action potentials had disappeared. On average ($n = 9$) action potentials were initiated 19 ± 5 sec after stimulation and the firing period lasted 73 ± 10 sec.

The transient depolarization of the cells as observed at the single-channel current level is also shown in Fig. 9 illustrating single-channel current records from the K^+_{SIR} channel before, during, and after stimulation. Prior to D-glyceraldehyde exposure the single-channel current amplitude was 0.8 pA (trace *a*), 33 sec after stimulation action potentials had developed and the channel amplitude was reduced to 0.4 pA, with a clear reduction in the open-state probability (trace *b*). 97 sec after stimulation and still in the presence of 10 mM D-glyceraldehyde the action potentials had disappeared, the single-channel current amplitude had increased to 0.5 pA, and the open-state probability had increased (trace *c*). After return to the control solution the single-channel current amplitude was restored to 0.8 pA and the frequency of channel openings was similar to the prestimulus level (trace *d*).

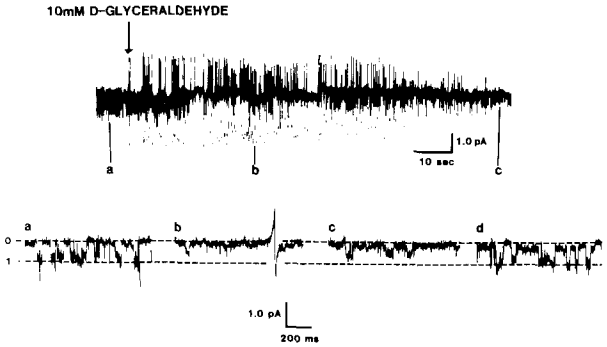

Fig. 9. A continuous recording taken from a RINm5F cell-attached membrane patch with the K^+-rich solution in the pipette and the Na^+-rich solution bathing the cells. The pipette voltage was clamped at 0 mV throughout and the record filtered at 300 Hz (low pass). At the point indicated 10 mM D-glyceraldehyde was added to the bathing solution and was present throughout the length of the recording. *a-d* are consecutive traces representing the currents recorded on a faster time-base, taken at the indicated times with the exception of trace *d*, which was obtained 1½ min after returning to the control solution. All expanded time-base records (*a-d*) were filtered at 300 Hz (low pass). The patch membrane-current recorded when all channels are closed (*O*) and when a single K^+_{SIR} channel (*1*) is open have been indicate

Discussion

The results presented here demonstrate a relatively high density of two types of ATP-sensitive inward rectifier K^+ channels in the plasma membrane of insulin-secreting cells. In the intact resting cells the channels are largely inhibited by the normal concentration of intracellular ATP. Nevertheless, the remaining activity of these two channels dominates the current recordings and it is therefore likely that the normal resting potential of about −70 mV (determined in these single-channel current recording experiments from the reversal potential for current

through the inward rectifier channels when there is no K^+ gradient across the patch membrane) is due to these channels. The only other type of K^+ channel that has been described in insulin-secreting cells is the Ca^{2+}- and voltage-activated high-conductance channel (Cook, Ikeuchi & Fujimoto, 1984; Findlay, Dunne & Petersen, 1985*a*; Findlay et al., 1985*c*), but this channel, which has similar properties to those described for many other cell types (Latorre & Miller, 1983; Petersen & Maruyama, 1984), is not operational in resting intact islet cells (Findlay et al., 1985*b*).

The results shown in Figs. 4 and 5 demonstrate that all the channels that are activated by permeabilization are ATP-sensitive. Our results indicate that the inward rectifier K^+ channels are less sensitive to ATP in the open cell-attached patches than in the excised inside-out patches, in agreement with data recently obtained in cardiac cells by Kakei, Noma and Shibasaki (1985). Furthermore, our data indicate that the degree of inhibition initially obtained by exposing the membrane inside to a particular concentration of ATP is not maintained so that the complete shut-down seen immediately after introducing 5 mM ATP gradually gives way to increasing channel activity in spite of sustained ATP exposure (Fig. 6).

The results shown in Figs. 4 and 5 taken together with the data presented by Findlay et al. (1985*b*) indicate clearly that the K^+ channels in the resting pancreatic islet cells have a very low probability of opening and that this is most likely due to the ATP normally present in the cells. Because of the marked run-down of ATP-sensitive channels in excised patches (Findlay et al., 1985*b*,*c*) many single-channel recordings obtained from such preparations look not dissimilar to those obtained from intact cells, and this has resulted in the notion that the degree of channel activation in intact cells is far too high in relation to the known intracellular ATP concentration (Rorsmann & Trube, 1985; Ashcroft et al., 1985). The postulated discrepancy, however, does not really exist as the channel activity in the resting intact cells represents a very severely inhibited state and is entirely compatible with a high cellular ATP concentration.

In normal pancreatic islet cells it has been shown that glucose and glyceraldehyde evoke similar electrical effects (Dean, Matthews & Sakamoto, 1975). Since the RINm5F cells do not respond to glucose stimulation (Halban, Praz & Wollheim, 1983), we have used glyceraldehyde which has been shown to markedly reduce the open-state probability of both the ATP-sensitive inward rectifier K^+ channels (Fig. 7). There was also a marked reduction in the amplitude of the single-channel currents during stimulation (Fig. 7) which corresponded to a membrane depolarization of about 50 mV (Fig. 8). Ashcroft et al. (1984) showed that glucose inhibited an inward rectifier channel, but when channel openings did occur during glucose-evoked inhibition the full control amplitude was observed. As far as the RINm5F cells are concerned it has been shown that stimulation with 10 mM D-glyceraldehyde evokes a marked but transient depolarization, increase in the intracellular ionized Ca^{2+} concentration ($[Ca^{2+}]_i$) and insulin secretion (Wollheim & Pozzan, 1984; Wollheim, Ullrich & Pozzan, 1984). These results are in good agreement with our patch-clamp data as the effect of D-glyceraldehyde on both the open-state probability and the single-channel current amplitude are transient as is the induction of action potentials (Fig. 9). The membrane depolarization can be explained by the inhibition of both the inward rectifier K^+ channels if the cells are permeable to another ion, Na^+ for example. The action potential generation is a consequence of the depolarization and can be adequately explained by the voltage-activated Ca^{2+} currents which have been characterized in the RINm5F cells (Findlay & Dunne, 1985). The stimulant-evoked reduction in K^+ channel opening could be due to the elevation of cellular ATP content (Ashcroft et al., 1985), and/or to a change in the sensitivity of the channels to ATP brought about by changes in intracellular metabolite concentrations.

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