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Comparative Study of K Channel Behavior in β Cell Lines with Different Secretory Responses to Glucose

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Summary. The patch-clamp technique was used to identify and investigate two K channels in the cell membrane of the HIT cell, an insulin secreting cell line with glucose-sensitive secretion. Channel characteristics were compared with those of glucose-modulated K channels in the RINm5F cell, an insulin secreting cell line in which secretion is largely glucose insensitive. A 65.7 pS channel, identified with the ATP-sensitive K channel was seen in HIT cell-attached patches. Channel activity was dose-dependently inhibited by glucose, by 50 and 100% at 450 μM and 8 mM glucose, respectively, similar to the values previously reported for RIN cells. In inside-out patches channel activity was 50% inhibited by 56 μM ATP and completely blocked between 500 μM and 1 mM, again, similar to the values reported for RIN cells.

As in RIN cells a second, considerably larger (184 pS), K channel was glucose sensitive; the glucose sensitivity was similar to that in RIN cells with 50 and 100% channel inhibition at 7.5 and 25 mM, respectively. After patch excision the mean channel conductance increased from 184 to 215 pS. Under these conditions activity was strongly calcium dependent in the range pCa 5–7, identifying this as a calcium- and voltage-dependent K (K(Ca,V)) channel; the calcium sensitivity was similar to that of the adult rat β cell K(Ca,V) channel. In inside-out RIN cell patches, the large K channel was less abundant but displayed a similar conductance (223 pS). However, its calcium sensitivity was more than 10 times lower than in HIT cells, similar to that of the K(Ca,V) channel in the neonatal rat β cell, which also displays a reduced secretory response to glucose. Based on these observations, it is proposed that the low calcium sensitivity of the K(Ca,V) channel may be causally associated with secretory deficiency in RIN cells and the immature secretory response of the neonatal β cell.

Key Words insulin-secreting cells · glucose modulated K^+ channels · patch clamp · single channel recording

Introduction

Two insulin secreting cell lines have been used extensively in investigations of β cell function, the RINm5F cell line, derived from an X-ray induced rat islet tumor (Gazdar et al., 1980), and the HIT-T15 cell line, produced by SV-40 transformation of hamster islet cells (Santerre et al., 1981). Only the HIT cell maintains a significant secretory response to glucose (Praz et al., 1983; Hill & Boyd, 1985). Secretion during a glucose challenge comprises an initial rise over the first 5 to 10 min followed by a decline to a low but sustained level over the following 10 to 20 min. The initial peak of 5 to 15 times basal release and the sustained level of secretion (2 to 5 times basal release) are glucose dependent over the range 2.8 to 28 mM glucose (Hill & Boyd, 1985). In contrast, glucose-induced insulin release from the RIN cell has been reported to be absent (Praz et al., 1983) or minimal (Leclerq-Meyer et al., 1988) with the greatest release occurring at 3 mM glucose and being only 50% above the basal level observed in the absence of glucose. In a study of the dynamics of RIN cell insulin release 2.8 mM glucose provoked a transient elevation of secretion, reaching a peak value only 20% above basal. High glucose (16.7 mM) induced a sustained release of similar magnitude (Leclerq-Meyer et al., 1988).

Stimulus-secretion coupling in the islet β cell comprises a coordinated series of events which commences with secretagogue recognition by the cell and culminates in the release of insulin. Metabolism of the secretagogue, typically glucose, but including other sugars and certain amino acids, is believed to generate the signals necessary to initiate secretory events (Ashcroft, 1980). It has been hypothesized that control of membrane K permeability may represent an important link between glucose metabolism and insulin secretion, since glucose-induced secretion is accompanied by characteristic changes in islet β cell membrane potential (Dean, Matthews & Sakamoto, 1975; Atwater, Ribalet & Rojas, 1978). The availability of these two insulin secreting cell types, one sensitive to glucose, the other not, affords an opportunity to fur-

Table 1.

*p*Ca	$CaCl_2$(mM)	EGTA (mM)
5	0.01	—
6	4.6	6.0
7	0.55	2.0

ther investigate this hypothesis by determining whether the differences in glucose-induced insulin secretory capability of the HIT and the RIN cell are associated with differences in K channel behavior.

It has been clearly established that a decrease in potassium permeability underlies depolarization of the islet β cell following addition of glucose (Atwater et al., 1978). Several studies have now demonstrated that this is due to the closure of an ATP-sensitive K (K(ATP)) channel (Cook & Hales, 1984). The link between glucose addition and channel closure is believed to be the elevation of intracellular ATP resulting from metabolism of glucose (Ashcroft, Harrison & Ashcroft 1984; Rorsman & Trube, 1985; Misler et al., 1986; Ashcroft, Ashcroft & Harrison, 1988). In three studies, two in islet β cells (Misler et al., 1986; Ashcroft et al., 1988), the third on the RIN cell (Ribalet, Eddlestone & Ciani, 1988), the K(ATP) channel activity has been shown to be 50% reduced by between 450 μM and 1.25 mM glucose and to be close to 100% blocked by 10 mM glucose. The fact that this channel is fully inhibited by concentrations of glucose which represent about 50% of the dose evoking maximal insulin secretion indicates that glucose modulation of a different channel may underlie the changes in the rhythmic burst pattern of β cell electrical activity. In the RIN cell we recently described such a channel, a large (140 pS) nonrectifying voltage-dependent K channel which we termed the maxi-K-(V) channel (Ribalet et al., 1988). This channel is glucose sensitive in the range 2.5 to 25 mM, being 50% inhibited by 4.2 mM glucose. Although excised patch experiments were not performed at that time we postulated that this channel may be the calcium and voltage-dependent K (K(Ca,V) channel, known to be present in the β cell membrane (Cook, Ikeuchi & Fujimoto, 1984; Findlay et al., 1985*c*).

Patch-clamp studies of the glucose-sensitive K permeabilities of the HIT cell were undertaken to define the K channel behavior in the glucose-sensitive insulin secreting HIT cell and to compare the data obtained with those from the glucose-insensitive RINm5F cell. In addition, a comparative study of the large voltage-sensitive K channel in the HIT and the RIN cell inside-out patch was performed to characterize this channel. Data obtained in this investigation indicate that differences in K channel behavior may contribute to the secretory unresponsiveness of the RIN cell.

Materials and Methods

Cell Culture

Cells of the insulin secreting cell lines HIT and RINm5F were kept at 37°C in RPMI 1640 medium supplemented with 10% (vol/vol) heat-inactivated fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine. The cells were divided once a week after treatment with trypsin and plated onto glass coverslips in 4 cm diameter petri dishes to permit transfer to the experimental chamber. Cells were used between 3 and 7 days after plating. One hour before the beginning of an experiment, a petri dish of cells was removed from the incubator and the culture medium was replaced with glucose-free bath solution (described below), and the cells were thereafter maintained at room temperature.

Experimental Media

For preliminary incubation and for studies of the K(ATP) channel, the composition of the bath solution was as follows: 135 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.1 mM $MgCl_2$, 10 mM HEPES with the pH adjusted to 7.2 with 1 M NaOH. For studies of the glucose dependence of the K(Ca,V) channel, KCl was increased to 50 mM with a compensating decrease in NaCl. The pipette contained 140 mM KCl, 1.1 mM $MgCl_2$, 2.5 mM $CaCl_2$ and 10 mM HEPES, pH being adjusted to 7.2 with 1 M KOH. The same solution, less the $CaCl_2$, was used as bath solution in inside-out patch studies of the K(ATP) channel. For inside-out patch experiments on the large K channel, the calcium concentration was controlled by appropriate addition of $CaCl_2$ and EGTA. Calculated according to the method of Fabiato and Fabiato (1979), the values used are shown in Table 1.

Data Handling

The production of patch elecrodes and the circuitry for recording from them have been described in detail (Hamill et al., 1981). Single channel currents were recorded at 2 kHz with an Axopatch 1A, Axon Instruments (Burlingame, CA) amplifier, and data were stored digitally via digital audio processor and video cassette recorder. For analysis, data were acquired at a rate of 5 kHz to the fixed disk of an IBM PC/AT computer via a double memory buffer interface which permits transfer of data points in a continuous mode (Bezanilla, 1985). Amplitude histograms of channel current were compiled to measure the mean channel current and the closed, threshold and open channel current levels for construction of an idealized data record from which the percentage open time of the channel could be determined. For this latter measurement the half amplitude threshold method was employed (Colquhoun & Sigworth, 1983), usually using 1-min data intervals. In patches expressing more than one level of channel activity, the total channel activity was calculated by summing the activity for each different current level. To permit comparison between cell-attached patch experiments in which the effects of secretagogues were investigated (Figs. 2, 4 and

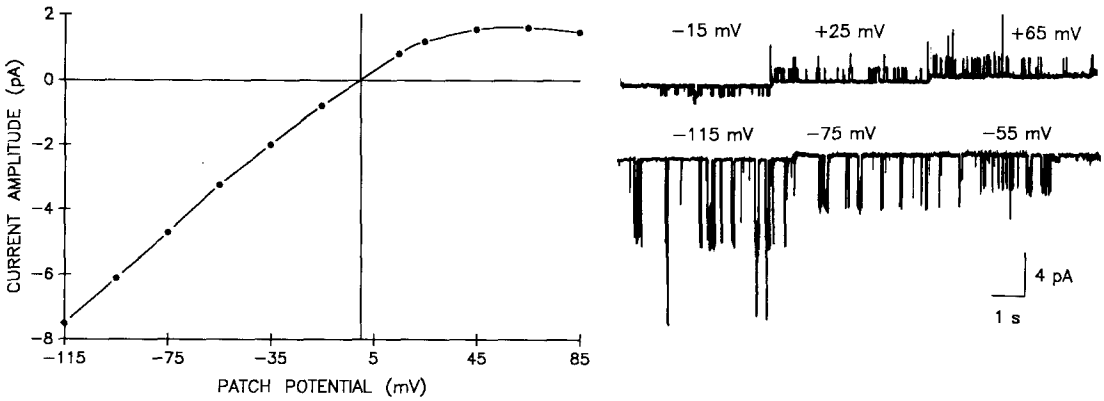

Fig. 1. The ATP-sensitive K channel in the cell-attached patch of the HIT cell. The left panel shows the single-channel current as a function of voltage with 140 mM in the pipette and 5 mM K in the bath. The membrane potential (V_m) was estimated from the pipette potential (V_p) at zero current, assuming intracellular K to be 140 mM and was in this case −69 mV. The patch potential was calculated as $V_m - V_p$. In this example the conductance, calculated from the linear part of the plot, was 69.1 pS. The right panel illustrates single-channel records from the same experiment, with the patch potential shown above the records

Table 2), it was necessary to normalize the data as the absolute levels of channel activity varied widely between individual experiments; the total channel activity in the absence of secretagogue was set to 100%, and the experimental data in the presence of the secretagogue were normalized to this value. For Fig. 5, which depicts the effect of glucose on K(Ca,V) channel activity, the total channel percentage open time during successive 15-sec intervals was normalized to the mean of the five control measurements made prior to glucose addition. For the inside-out patch data illustrating the effects of calcium and potential on K(Ca,V) channel activity (Figs. 7 and 8) absolute values of percentage open time were used and variability expressed using representative error bars to indicate standard error values.

Results

The ATP-Sensitive Potassium Channel

Channel Characteristics in the Cell-Attached Patch

In cell-attached patch experiments, with 140 mM potassium in the pipette and normal extracellular medium, a number of different magnitudes of potassium channel was observed in the HIT cell. Of these the most abundant was an inwardly rectifying, voltage-independent channel which we identified with the ATP-sensitive (K(ATP)) potassium channel originally described in cardiac muscle (Noma, 1983) but also found in the pancreatic β cell (Ashcroft et al., 1984, 1988; Cook & Hales, 1984; Ashcroft, Ashcroft & Harrison, 1985; Findlay, Dunne & Petersen, 1985*a*; Rorsman & Trube, 1985). Figure 1 illustrates the current-voltage relationship of this channel and current records from a representative experiment. The slope conductance in the linear part of the plot in this example was 69.1 pS. In 14 experiments a value of 65.7 ± 1.3 pS was measured (mean ± SEM), with a range of 56–72 pS, the current exhibited pronounced rectification in the outward direction and diminished in magnitude at higher positive membrane potentials. Accepting the assumption that the intracellular K^+ concentration is close to 140 mM, the zero current potential measured in the HIT cell was used as an estimate of the cell resting potential and was in most cases around −70 mV (range −60 to −78 mV). Cells with low estimated resting potentials were occasionally encountered, but these were rejected in cell-attached patch studies.

Channel Response to Glucose in the Cell-Attached Patch

Cell-attached patch experiments were carried out in which HIT cells were exposed to glucose in the range 300 μM to 8 mM for from 7 to 15 min separated by similar intervals without glucose. The steady-state channel activity was measured as described in Materials and Methods and normalized with the open-channel probability values in the absence of glucose taken as 100%. Figure 2 illustrates the effect of glucose on K(ATP) channel activity; data from one experiment are shown to the right and a plot summarizing the data from all experiments (3 to 8 observations at each glucose level) as mean normalized channel activity versus glucose concentration is shown to the left. The effect of glucose on channel activity was already pronounced at 300 μM; a 50% reduction of activity occurred at about 450

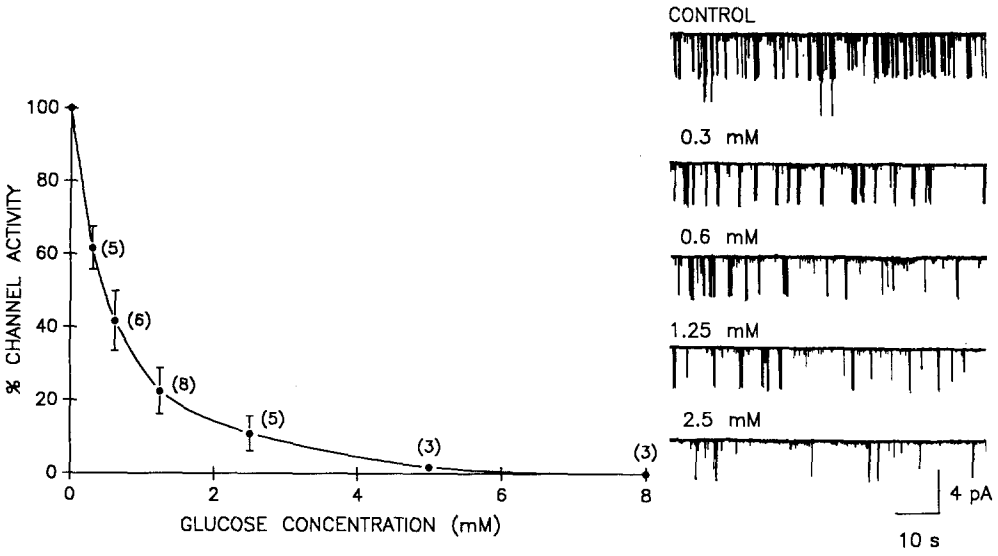

Fig. 2. The glucose dependence of K(ATP) channel activity in the cell-attached patch of the HIT cell. The plot in the left panel summarizes the data from experiments in which cells were exposed to glucose in the range 0.3 to 8 mM; the number of observations at each glucose concentration is given in parentheses. At all glucose concentrations in each experiment, the open channel probability for the individual channels in the patch was computed over a 1-min interval and summed; these values were normalized to the zero glucose value to permit comparison between different patches. The line through the data points was generated using a cubic spline. The right panel illustrates steady-state channel activity at the glucose concentration indicated adjacent to each trace, in a representative experiment. The K concentrations in the pipette and the bath were 140 and 5 mM, respectively; no holding potential was applied to the pipette

μM glucose with activity reduced to zero by 8 mM glucose. In 14 observations the response to glucose was seen to commence within 1 to 3 min of adding the sugar, with a new steady state of activity achieved between 2 and 9 min of glucose addition. The time required to reach steady state did not depend on glucose concentration but rather appeared to vary from cell to cell. Similarly, the response to glucose removal began within 1 to 3 min of glucose wash-out and zero glucose steady-state activity was recorded after 2 to 12 min of glucose removal. In addition to glucose the effect on K(ATP) channel activity of three other initiators of islet insulin secretion was tested. The effects of mannose, leucine and α-ketoisocaproate (KIC) along with the data for glucose illustrated in Fig. 2 are summarized in Table 2. Each of these secretagogues provoked a concentration-dependent reduction in K(ATP) channel activity.

Channel Characteristics in the Inside-Out Patch

To further characterize this channel, inside-out excised patches were prepared and the channel conductance and ATP sensitivity of the channel activity established. The conductance in the inward direction was 67 ± 1.4 (mean ± SEM, n = 4) with pronounced rectification of the outward current evident when 1.1 mM magnesium was present in the bath. Removal of magnesium from the perifusion medium increased the magnitude of the outward currents, though a linear I/V was not achieved. To test the responsiveness of the channel to ATP, the inner face of the membrane was exposed to ATP in the range 10 μM to 1 mM. The mean ATP concentration required to induce a 50% reduction of channel activity (K_i) was 56 μM; the activity was blocked by >95% at 500 μM ATP. The observation that the open channel probability of this channel is sensitive to internally applied ATP is sufficient, in conjunction with the conductance data, to permit unequivocal identification of this channel.

The Large Voltage-Sensitive Potassium Channel

Channel Characteristics in the Cell-Attached Patch

In the absence of glucose a second, voltage-dependent, K channel of considerably larger conductance was occasionally observed. This channel was identified with the maxi-K(V) channel which we recently investigated in the RIN cell (Ribalet et al., 1988). Although this channel was not commonly observed at rest (zero glucose, 5 mM) extracellular

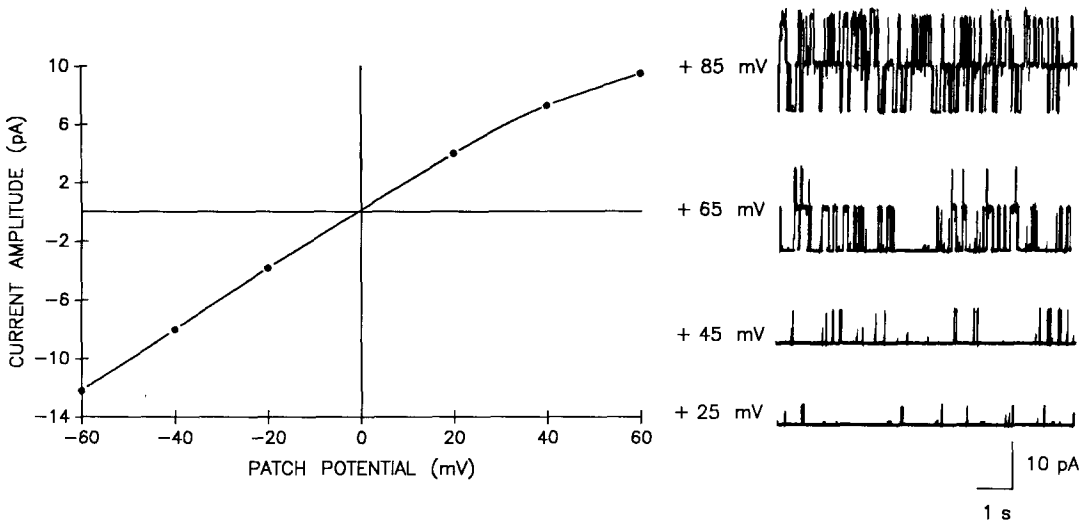

Fig. 3. The large voltage-sensitive K channel in the cell-attached patch of the HIT cell. The left panel shows single-channel current as a function of voltage in a representative experiment, with 140 mM K in the pipette and 50 mM K in the bath. The membrane potential estimated from the zero current potential was −21 mV. In this example the conductance measured in the linear part of the plot was 207 pS. The right panel of the figure illustrates single-channel recordings from the same experiment with the patch potential indicated to the left of each record

potassium) it was far more prevalent than in the RIN cell, in which activity was rarely seen unless the cell was depolarized with a high K extracellular medium and pretreated with dinitrophenol or ouabain. Figure 3, left panel, illustrates the *I/V* relationship of this channel in a HIT cell-attached patch. The slope conductance in the inward direction in this example was 207 pS, with distinct rectification of the outward current at potentials more positive than +20 mV. In 17 cells the conductance was 184.3 ± 4.4 pS (mean ± SEM) with a range of 148 to 210 pS. The rectification of the outward current was a consistent feature of the *I/V* response of this channel in the cell-attached patch (16 of 17 observations) and may result from the blocking effect of intracellular cations as has been demonstrated for a number of different K channels (Yellen, 1984; Findlay, 1987; Vandenberg, 1987; Ciani & Ribalet, 1988). The data traces on the right side of the figure demonstrate the clear voltage dependence of this channel. Inward current data are not shown since the frequency of channel opening was very low. The voltage dependence of the channel in the cell-attached patch will be discussed later.

Table 2. The effects of different insulin secretagogues on K(ATP) channel activity in the HIT cell

Substrate (mM)	Percentage of control activity
Glucose	
0.3125	60.0 ± 5.9 (5)
0.625	41.7 ± 7.1 (6)
1.25	22.5 ± 4.4 (8)
2.5	11.0 ± 3.7 (4)
5	1.8 ± 0.7 (3)
Mannose	
1.25	56.5 ± 4.5 (3)
2.5	19.3 ± 7.1 (3)
5	9.4 ± 3.8 (3)
Leucine	
2.5	27 ± 6.3 (3)
5	0 (1)
KIC	
0.625	71.0 (1)
1.25	37.5 ± 7.1 (3)
2.5	24.2 ± 5.6 (3)
5	12.0 ± 4.4 (3)

For each experiment the channel activity recorded in the absence of the secretagogue was defined as 100% activity, and the data recorded in the presence of the secretagogue were normalized to this value.

Channel Response to Glucose in the Cell-Attached Patch

Since this K channel is voltage sensitive, the membrane was depolarized with a high K^+ extracellular medium so that the effect of glucose on channel activity could be measured at constant membrane potential. For this reason the majority of the studies of glucose sensitivity were performed using a 90 mM Na/50 mM K extracellular medium, which depolarized the membrane to a value close to −20 mV. The cells were depolarized with high K^+ medium and

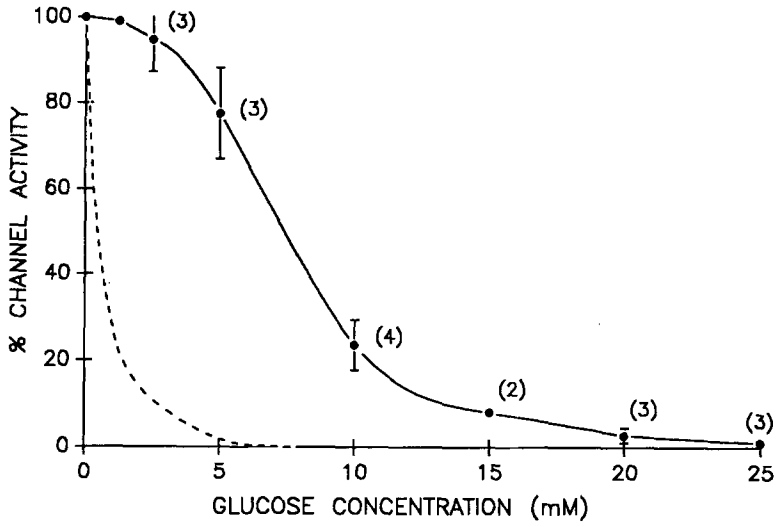

Fig. 4. The glucose dependence of the large K channel in the cell-attached patch of the HIT cell. The plot summarizes the data from experiments in which cells were exposed to glucose in the range 1.25 to 25 mM; the number of observations at each glucose concentration is given in parentheses. In each experiment the open channel probability was normalized to its zero glucose value to permit comparison between different patches. The line through the data points was generated using a cubic spline. The data from Fig. 2 are repeated as a broken line in this figure to permit comparison of the glucose sensitivity of the two channel types. In these experiments the K concentrations in the pipette and the bath were 140 and 50 mM, respectively; no holding potential was applied to the pipette

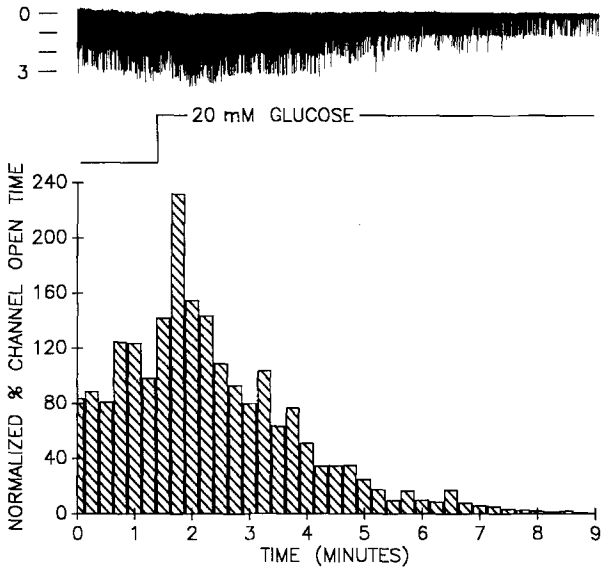

Fig. 5. The time course of the effect of glucose on the activity of the large K channel of the HIT cell. Single-channel data from a cell-attached patch experiment in which the cell was exposed to 20 mM glucose following incubation in the absence of the sugar are shown in the upper part of the figure. The individual channel current levels are indicated to the left of the trace. Addition of glucose is indicated below the data trace and above a summary histogram of the data. The histogram was generated by summing the open channel probability for all of the active channels during successive 15-sec intervals and normalizing the values to the mean of the five measurements taken prior to glucose addition. The pipette contained 140 mM K and the bath 5 mM K; comparable results were obtained when the cell was depolarized with 50 mM K in the bath

left in the absence of glucose for 10–15 min to ensure a steady state of activity was attained. After this time, medium containing glucose at 5–25 mM was rapidly added to the bath for periods of 10–20 min, similar intervals in the absence of glucose separating the glucose exposures. The activity was normalized to the two adjacent periods in the absence of glucose (zero glucose equals 100% activity), and the data from four representative experiments were pooled to illustrate the glucose dose dependence of channel activity (Fig. 4). Interpolation from the figure suggests that a 50% reduction of channel activity would be provoked by about 7.5 mM glucose with activity approaching zero at 25 mM glucose. The broken line in Fig. 4 repeats the data from Fig. 2 and is included to demonstrate the discrete differences in the glucose responsiveness of the two K channels.

Figure 5 illustrates the dynamics of the response of the large K channel to glucose. The upper panel shows that data that are summarized in the lower panel. In this experiment three levels of channel activity were apparent before the addition of glucose. The addition of 20 mM glucose was followed within 30 sec by an augmentation of channel activity then a decline lasting for more than 6 min to reach a steady state of activity representing only 1.7% of the activity prior to glucose addition.

Channel Characteristics in the Inside-Out Patch

Further investigation of this large K channel was undertaken using isolated inside-out membrane patches from HIT cells, and, since similar investigations were not reported in our article on the RIN cell, comparative studies were carried out using RIN cells.

The HIT Cell. In the excised inside-out patch the large channel was extremely active, and, though the range of conductances represented in this patch

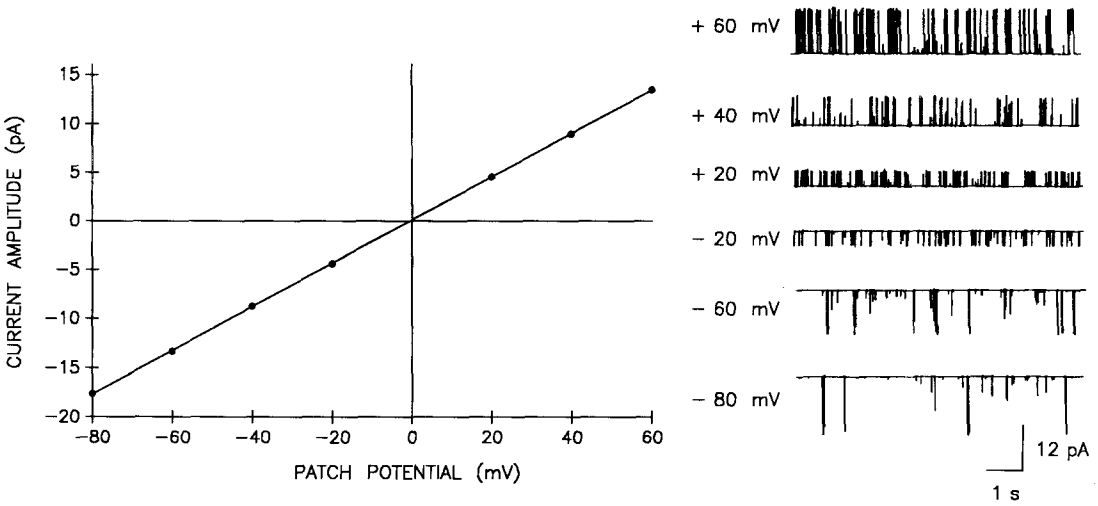

Fig. 6. The large voltage-sensitive K channel in the excised inside-out patch of the HIT cell. The left panel shows the current-voltage relationship of the large K channel in a representative experiment in which the patch was exposed to symmetrical 140 mM K solutions with 1 μM Ca^{2+} in the bath. In this example the conductance measured in the linear part of the plot was 223 pS. The right panel of the figure illustrates single-channel recordings from the same experiment with the patch potential indicated to the left of each record

configuration did not coincide with that measured in the cell-attached patch, the two channels were assumed to represent the same species on the grounds that (i) both were voltage dependent, close to linear and K selective and (ii) in experiments in which data were collected from a patch both before and after excision, channel currents were the same in number before and after excision but were of greater magnitude in the excised patch. Data from a representative HIT cell inside-out patch experiment are shown in Fig. 6. The left panel illustrates the *I/V* characteristics, with experimental data shown in the right panel. The slope conductance is close to linear, being in this example 223 pS. From the data obtained in 16 experiments a conductance of 215.3 ± 8.4 (mean ± SEM) was measured; use of the Fisher-Behrens test verified the significant difference between this value and the mean value obtained in the cell-attached patch.

The mean conductance is in the range reported for the conductance of the calcium- and voltage-dependent K(K(Ca,*V*)) channel in the rat and mouse islet β cell excised patch (Cook et al., 1984; Findlay et al., 1985*c*). To determine whether this large K channel could be identified with the K(Ca,*V*) channel, excised inside-out patch experiments were performed in which channel activity was measured at different bath calcium concentrations, in the range *p*Ca 5 to 7. Calcium sensitivity of the channel was confirmed in nine experiments, and the data from four of these in which complete *I/V* curves were generated at *p*Ca 5, 6 and 7 were averaged and plotted in Fig. 7. This figure clearly illustrates the calcium and voltage sensitivity of the 215 pS channel of the HIT cell. As may be seen from the representative error bars, which illustrate standard error values, the calcium sensitivity of the channel varied considerably between patches.

Cell-attached patch studies of the voltage dependence of channel activity were carried out in the presence of 50 mM K^+ and in the absence of glucose. As in the inside-out patch the voltage sensitivity of the activity varied significantly between preparations. In the case of the cell-attached patch it was not possible to attribute this variation to an intrinsic property of the channel or to different intracellular calcium levels. The extreme examples recorded under these conditions are included as broken lines in Fig. 7 and are included to illustrate the voltage range in which the channel may be active in the cell-attached patch and to permit a comparison with data obtained in inside-out patches at known calcium concentrations.

The RIN Cell. Excised inside-out patches from the RIN cell were used to determine the channel conductance and to investigate the calcium sensitivity of channel activity. The first noticeable difference in channel behavior between HIT cells and RIN cells was observed at this point, it being quite common to isolate a patch from a RIN cell and observe no large K channel activity at all, irrespective of patch potential or the calcium concentration in the bath. Channel activity was observed in only four of 13 patches and, when present, was much less abundant than in the HIT cell isolated patch. A linear *I/V* relationship was found for the K(Ca,*V*)

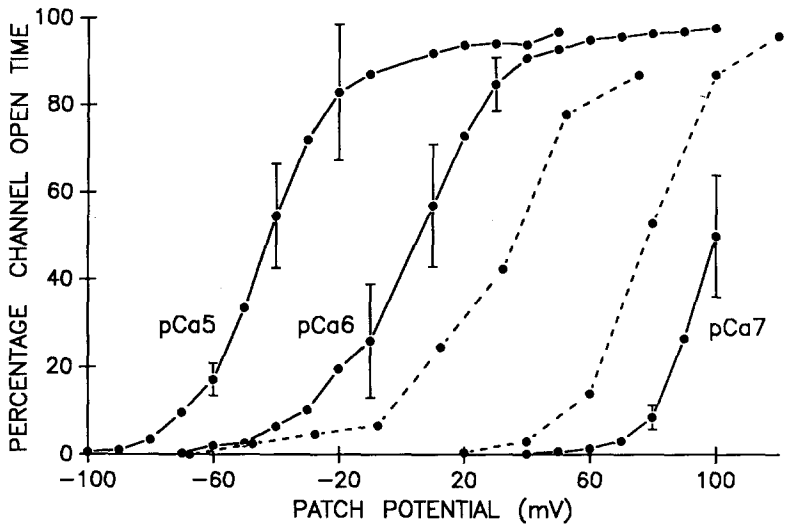

Fig. 7. The calcium sensitivity of large K channel activity in the excised inside-out patch from the HIT cell. The data from four experiments in which patches were exposed to symmetrical 140 mM K solutions and different bath calcium concentrations were used to generate plots of the mean voltage sensitivity of the large K channel at three different bath calcium concentrations. Representative error bars indicate SEM values. In the case of the plot at *p*Ca 7, it was not possible to generate a complete curve since the combination of low calcium and high positive patch potential invariably destroyed the patch. The broken lines in this figure represent the extreme values from nine observations of the voltage sensitivity of this channel recorded in cell-attached patches with 50 mM potassium in the bath

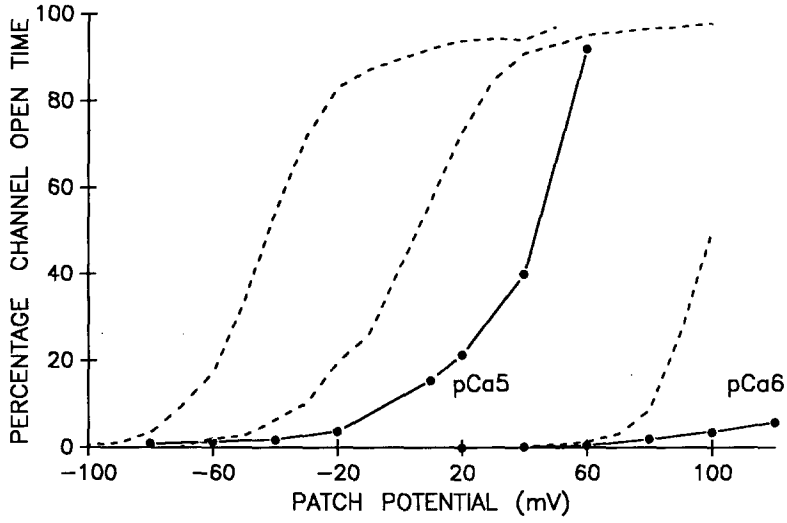

Fig. 8. The calcium sensitivity of large K channel activity in excised inside-out patches from the RINm5F cell. The patches were exposed to symmetrical 140 mM K solutions and the bath calcium was varied in the range *p*Ca 7 to 5. Activity of the large channel was observed in four of 13 patches, and only the data from the two patches displaying the highest calcium sensitivity were used to generate plots of voltage sensitivity of the large K channel versus bath calcium concentrations. The broken lines in this figure repeat the data from Fig. 7 to permit comparison of the calcium sensitivity of channel activity in the two cell types

channel in the RIN cell, with a mean conductance of 229 ± 6.25 pS (mean ± SEM, $n = 4$). No statistical difference was found between this value and the mean conductance for the HIT cell K(Ca,V) channel using the t test. The calcium sensitivity of the channel was investigated in the inside-out patch of the RIN cell using bath calcium at *p*Ca 5, 6 and 7. The solid lines in Fig. 8 illustrate the mean voltage and calcium sensitivity of K(Ca,V) channel activity in the two inside-out patches from the RIN cell which displayed the highest calcium sensitivity; in the other two patches which displayed K(Ca,V) channel activity the calcium sensitivity was shifted to still higher positive potentials; the broken lines repeat the HIT cell data from Fig. 7. It is clear from this figure that the calcium sensitivity of the K(Ca,V) channel in the RIN cell is significantly lower than that of the HIT cell. If the intracellular free calcium in the HIT cell in high K^+ medium is taken to be around 250 nM as measured using Quin-2 and Fura-2 (Boyd et al., 1986; Prentki et al., 1987), and extrapolating this value to the RIN cell, then it would follow that opening of the K(Ca,V) channel in the RIN cell would be rare, and, unless the calcium level in these cells is extremely high, it would at most make only a minor contribution to the control of the membrane potential.

Whole cell currents were not investigated in this study so it is not possible to make a quantitative observation on the relative abundance of functional K(Ca,V) channels in the HIT and RIN cells. However, in the excised patch the K(Ca,V) channel was identified in more than 75% of HIT cell patches, often with three or four channels per patch, while in the RIN cell this channel was seen in only 30% of patches, in each case only one or two channels be-

ing active. These data suggest that the functional channel is both poorly sensitive to calcium in the RIN cell and also present at low density.

As noted in previous studies of islet β cells and of insulin secreting tumor cells, other membrane K channels coexist with the two described above in the HIT cell. Since the focus of this study was an investigation of channels modulated by glucose, no rigorous attempts were made to catalog these or to investigate their function since it was readily determined that none displayed the glucose sensitivity of the K(ATP) and K(Ca,V) channels. It is worth including that a small, voltage-sensitive inward rectifying K channel, with a conductance of between 25 and 35 pS was regularly observed and probably represents the K_{SIR} channel of Findlay, Dunne and Petersen (1985*a*).

Discussion

In this study it was first attempted to determine whether HIT cells possess glucose-responsive K channels comparable to those recently characterized in the RIN cell (Ribalet et al., 1988) and then to determine whether differences in glucose-induced insulin secretion between the HIT cell and the RIN cell may be associated with differences in the behavior of these K permeabilities. The recent survey of HIT cell K channels which failed to detect the presence of the K(ATP) channel made such an investigation more desirable (Light et al., 1987). Perhaps, then, the first significant observation of this study was the identification of a K channel abundant in both the cell-attached and the inside-out patch, where mean conductances of 65.7 and 67.0 pS were recorded. The channel activity was voltage insensitive and exhibited inward rectification. These properties are characteristic of the ATP-sensitive K (K(ATP)) channel originally described in cardiac tissue (Noma, 1983) and which has been described in the rat pancreatic β cell, both neonatal (Cook & Hales, 1984) and adult (Ashcroft et al., 1984; Misler et al., 1986), the adult mouse β cell (Rorsman & Trube, 1985), the adult human β cell (Misler et al., 1988) and the RINm5F (Ribalet et al., 1988) and CRI-G1 (Sturgess et al., 1986) insulin secreting cell lines.

In cell-attached patch experiments we demonstrated that the addition of glucose to the extracellular medium provoked a dose-dependent reduction of HIT cell K(ATP) channel activity. Figure 2 indicates that as little as 300 μM glucose caused a large decrease in channel activity, with 50% blockage at only 450 μM and almost complete blockage around 5 mM glucose. These results indicate a close similarity of glucose responsiveness of the K(ATP) channel in the HIT cell with that in the RIN cell where 450 μM glucose also blocked the K(ATP) channel by 50% and 5 mM glucose by more than 90% (Ribalet et al., 1988). The observation that the K(ATP) channel is almost completely inhibited by glucose concentrations which are close to the threshold for stimulation of insulin release, reinforces our proposal that the role of this channel in the β cell is confined to the control of the membrane potential between the resting potential and the threshold potential for activation of the secretion-associated cyclic electrical activity (Ribalet et al., 1988).

Comparing our data with those derived in studies of the K(ATP) channel in the adult mouse and rat β cell indicates a similarity of glucose sensitivity. Misler et al. (1986) observed that 1.25 mM glucose caused approximately 50% blockage of K(ATP) channel activity in the adult rat β cell, with activity approaching zero at 10 mM glucose; from the data of Ashcroft et al. (1988) on adult mouse β cells it is estimated that 50% channel inhibition occurs at about 800 μM glucose with 7% of initial activity remaining at 10 mM glucose. The difference of glucose sensitivity of channel activity between tumor cells and islet β cells may reflect the differences in control of glucose metabolism in the two cell types (Ashcroft & Stubbs, 1987).

Mannose, KIC and leucine, which also serve as insulin secretagogues, caused K(ATP) channel blocking in the RIN cell (mannose and KIC) (Ribalet et al., 1988) and the adult rat β cell (mannose and leucine) (Misler et al., 1986). Similar inhibition of K(ATP) channel activity by these secretagogues is also observed in the HIT cell, channel blocking being greatest with glucose, followed by KIC, mannose and leucine. It is clear from these observations that metabolic fuels which provoke insulin secretion in the normal islet also cause blockage of the K(ATP) channel in all of these insulin secreting cells.

In the inside-out excised patch a K_i value of 56 μM ATP was found for the blocking of the K(ATP) channel of the HIT cell. This indicates that the K(ATP) channel of the HIT cell possesses a similar ATP sensitivity to that of the RINm5F cell where a K_i of 50–70 μM has been reported (Ribalet et al., 1988). These K_i values are significantly higher than those found in most studies of K(ATP) channel ATP sensitivity in the rodent β cell where values between 10 and 20 μM have been reported for the neonatal (Cook & Hales, 1984) and adult (Ashcroft et al., 1985; Misler et al., 1986) rat and the adult mouse (Rorsman & Trube, 1985). The possibility that the higher K_i in the RIN and HIT cells represents a generalized defect of tumor cells is negated

by the observations that the K_i for this channel in the CRI-G1 insulin secreting tumor cell line is only 12.6 μM (Sturgess et al., 1986).

It is concluded from this investigation that the HIT cell displays K(ATP) channel activity that is not significantly different, either in its conductance characteristics or in its response to glucose or ATP, from that recently described in the RIN cell. Differences in secretory behavior cannot therefore be attributed to differences in the physical properties or the control of this channel.

The second glucose-sensitive K channel previously described in the RIN cell has a larger conductance (140 pS) and its activity is voltage-dependent; it is, however, rarely seen unless the cells are incubated with drugs that increase the intracellular calcium level (ouabain and 2,4-dinitrophenol) (Ribalet et al., 1988). However, in addition to increasing calcium, these compounds also augment intracellular sodium and magnesium, two cations which at physiological concentrations significantly reduce outward current through several types of K channel including the K(Ca,V) channel (Yellen, 1984). Since the incidence of channel activity is much greater in HIT cells, activity being observed in about 80% of experiments (versus about 15% on the RIN cell), it was not necessary to employ these drugs and it was not therefore unexpected to find a mean conductance of this glucose-sensitive K channel which is considerably larger than its counterpart in the RIN cell (184 versus 140 pS).

In the two cell types the large K channels displayed closely similar glucose sensitivities, activity being inhibited by glucose in the range 1.25 and 25 mM glucose. In the HIT cell 50% channel inhibition was achieved with about 7 mM glucose and 100% channel inhibition at about 25 mM glucose. In the RIN cell these levels of blocking were achieved with 5 and 20 mM glucose, respectively (Ribalet et al., 1988). In both of these tumor cell lines this channel is progressively blocked by glucose across the range in which the sugar provokes dose-dependent modulation of islet insulin secretion and the secretion-associated β cell membrane electrical activity. Furthermore, Fig. 5 shows that the interval between addition of 20 mM glucose and the establishment of steady-state channel activity is similar to the interval between addition of 19.7 mM glucose and the attainment of peak insulin secretion from perfused HIT cells (Hill & Boyd, 1985). These observations support the proposal that modulation of this K channel is causally related to the glucose dose dependence of the burst pattern of electrical activity recorded from the islet β cell and therefore to the glucose dose dependence of insulin release (Ribalet et al., 1988).

Excision of the HIT cell membrane patch was accompanied by an increase in the measured value of the conductance of the large K channel as well as by the loss of the inward rectification observed in the cell-attached patch. The mean conductance increased by about 16% in the excised patch to a value of 215 pS. These changes are likely to reflect the different ionic environment to which the inside of the membrane was exposed following patch excision.

The 215 pS conductance value measured in symmetrical 140 mM K^+ in the excised patch is in good agreement with previously published values for the calcium- and voltage-dependent K (K(Ca,V) channel in many tissues including the neonatal (244 pS) (Cook et al., 1984) and adult (250 pS) rat pancreatic β cell (Findlay, Dunne & Petersen, 1985*b*). The observation that the channel activity is sensitive to "intracellular" calcium concentration permits unequivocal identification of this channel with the K(Ca,V) channel. The mean value of the percentage open time of the HIT cell K(Ca,V) channel displays a similar calcium sensitivity to that of the K(Ca,V) channel in the adult rat β cell (Findlay et al., 1985*b*). The range of calcium sensitivities displayed by the K(Ca,V) channels, as evidenced by the error bars in Fig. 7 prevents an accurate estimate of intracellular Ca^{2+} from the voltage versus channel activity plots for the channel in the cell-attached patch; however, measurement of Quin-2 (Boyd et al., 1986) and Fura-2 (Prentki et al., 1987) fluorescence in the HIT cell in the presence of high K^+ medium has given estimated intracellular calcium values of 238 and 251 nM, respectively, concentrations consistent with these channel data.

Two significant differences of K(Ca,V) channel behavior were observed when excised inside-out patches were prepared from RIN cells. First, the incidence of functional large K channels was considerably lower than in HIT cell inside-out patches (30% versus >75%) and second, the calcium sensitivity of the RIN cell K(Ca,V) channel activity was more than 10 times lower than that of the HIT cell. Whether the former observation indicates a low channel density or that a large fraction of the channels were in a quiescent state cannot be determined from these studies. However, since the excised patches were exposed to both high calcium concentrations and high positive membrane potentials in attempts to activate the channels, it may be concluded that the number of activatable channels in the RIN cell is considerably lower than in the HIT cell and that this channel type would make a commensurately small contribution to the K permeability of the cell. That the functional K(Ca,V) channels displayed a very low sensitivity to calcium in the

RIN cell may be of considerable significance, since this indicates a pronounced difference in K channel behavior between the HIT cell and the RIN cell, a difference which may be associated with the differences in the glucose-induced insulin secretory responses of the two cell types. In support of this proposal a parallel may be drawn between the function of the K(Ca,V) channel in the RIN cell and that in the neonatal rat β cell in which a similar low calcium sensitivity has been demonstrated (Cook et al., 1984). Since the glucose sensitivity of β cell insulin release develops during the perinatal period (Hole, Pian-Smith & Sharp, 1988) it is possible that the reduced calcium sensitivity of the channel observed in the neonatal rat β cell is also associated with a secretory state characterized by a poor response to glucose.

Several investigators have used the drug N-bromoacetamide to illustrate that the calcium sensitivity of the K(Ca,V) channel can be reduced or removed without eliminating the voltage sensitivity of the channel (Pallota, 1985; Cornejo, Guggino & Guggino, 1987). These data indicate that the two sensing mechanisms of the channel may be independent and that the calcium sensing site may be modified. From this viewpoint the results of a study of the K(Ca,V) channel in spinal neurons cultured from the *Xenopus* embryo are significant (Blair & Dionne, 1985). In this study it was shown that after 7 to 11 hr in culture no calcium sensitivity of channel activity could be demonstrated while after 22 to 43 hr in culture the calcium sensitivity of the channel was clear over the range 0.5 to 100 μM. Whether the change from calcium insensitive to calcium sensitive is an all-or-none event or whether calcium sensitivity increases gradually was not investigated. However, these data indicate that the development of calcium sensitivity of the K(Ca,V) channel is a phenomenon associated with cell maturation and may, in the case of the rat β cell, accompany the change from the immature to the mature secretory response to glucose. By analogy it is suggested that in the case of the RIN cell this process may be impaired and the resultant disruption of the normal control of membrane K permeability by calcium and therefore of the secretion-associated membrane potential electrical activity may be instrumental in the poor glucose responsiveness of insulin secretion of these cells.

In summary, it has been demonstrated that the HIT cell membrane contains both the ATP-sensitive K channel and the calcium- and voltage-sensitive K channel, as does the RIN cell. Comparison of the conductance and modulation of the K(ATP) channel in the RIN cell and the HIT cell indicates no significant difference in either property. In the case of the K(Ca,V) channel, the glucose sensitivity of the channel activity is very similar in the HIT and the RIN cell though channel density may be reduced in the RIN cell. The major difference in K permeability behavior is the discrepancy of calcium sensitivity of the channel, it being more than 10 times less calcium sensitive in the RIN cell. In this regard the RIN cell is similar to the neonatal rat β cell, a cell in which glucose-induced insulin secretion may also be obtunded. It is proposed that the impaired secretory response of both of these cell types may be associated with the reduced calcium sensitivity of the K(Ca,V) channel.

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