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# Potassium-Selective Ion Channels in a Transformed Insulin-Secreting Cell Line

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**Summary.** $K^+$ channels in inside-out patches from hamster insulin tumor (HIT) cells were studied using the patch-clamp technique. HIT cells provide a convenient system for the study of ion channels and insulin secretion. They are easy to culture, form gigaohm seals readily and secrete insulin in response to glucose. The properties of the cells changed with the passage number. For cell passage numbers 48 to 56, five different $K^+$-selective channels ranging from 15 to 211 pS in symmetrical 140 mM KCl solutions were distinguished. The channels were characterized by the following features: a channel with a conductance (in symmetrical 140 mM KCl solutions) of 210 pS that was activated by noncyclic purine nucleotides and closed by $H^+$ ions (pH = 6.8); a 211 pS channel that was $Ca^{2+}$-activated and voltage dependent; a 185 pS channel that was blocked by TEA but was insensitive to quinine or nucleotides; a 130 pS channel that was activated by membrane hyperpolarization; and a small conductance (15 pS) channel that was not obviously affected by any manipulation. As determined by radioimmunoassay, cells from passage number 56 secreted 917 ± 128 ng/mg cell protein/48 hr of insulin. In contrast, cells from passage number 77 revealed either no channel activity or an occasional nonselective channel, and secreted only 29.4 ± 8.5 ng/mg cell protein/48 hr of insulin. The nonselective channel found in the passage 77 cells had a conductance of 25 pS in symmetrical 140 mM KCl solutions. Thus, there appears to be a correlation between the presence of functional $K^+$ channels and insulin secretion.

**Key Words** $K^+$ channel · patch clamp · single-channel recording · HIT cells · insulin secretion

## Introduction

Glucose is the primary extracellular agent that stimulates insulin secretion in β-cells. Above a threshold level, it induces characteristic oscillating bursts of electrical activity and insulin secretion (Atwater, Ribalet & Rojas, 1978; Ribalet & Beigelman, 1979; Hedeskov, 1980). Glucose must be metabolized by the cell prior to hormone release (Henquin, 1978; Ashcroft, Harrison & Ashcroft, 1984) and this metabolism has been linked to an initial depolarization of the cell via a decrease in the $K^+$ permeability (Sehlin & Taljedal, 1975; Atwater, Goncalves & Rojas, 1982). The drop in $K^+$ flux and subsequent depolarization most likely results from a closing of $K^+$-selective ion channels in the plasma membrane (Ashcroft et al., 1984). The intracellular mediator of this decreased $K^+$ conductance has not been definitely established. Two likely candidates are $H^+$ ions and ATP, both of which are by-products of glucose metabolism and have been reported to close $K^+$ channels in β-cells (Cook & Hales, 1984; Cook, Ikeuchi & Fujimoto, 1984).

In this study, the patch-clamp technique (Hamill et al., 1981) was used to investigate the ion channels present in the plasma membrane of HIT-T15 cells [a simian virus transformed hamster β-cell line that secretes insulin in response to glucose at early passage numbers (Santerre et al., 1981)]. This cell line has a number of experimental advantages: it can be cultured into large populations for biochemical analysis, forms gigaohm seals readily, secretes insulin in response to glucose and can be frozen and stored for later use. Only preliminary single-channel studies have been published using this cell line (Light & Levitt, 1986; Matteson & Matschinsky, 1986). The purpose of this study was to catalog the types of $K^+$ channels in this cell line. Five different $K^+$ channels ranging from 15 to 211 pS in symmetrical 140 mM KCl solutions were distinguished in cells from passage numbers 48 to 56. These cells also secreted insulin. In contrast, no $K^+$ channels were detected in cells from passage number 77 and insulin secretion was markedly reduced.

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## Materials and Methods

### Cell Culture and Radioimmunoassay

HIT-T15 cells at passage 46 were donated by Dr. Robert Santerre, Lilly Research Labs, and were cultured in the department of cell biology and neuroanatomy, University of Minnesota. When needed, each frozen vial of cells was quick-thawed and cultured in: 67.5% Hams F-12 medium, 30% horse serum, 2.5% fetal calf serum, 4 mM glutamine, 10 $\mu$g/ml glutathione, 0.025 mg/ml amphotericin and 0.05 mg/ml gentamicin. The cells were cultured on a 1% gelatin matrix (Folkman, Haudenschild & Zetter, 1979). Every 11 to 13 days the cultured cells were subcultured or "passed." The cells were characterized by their passage number, which ranged from 48 to 77.

Cells from passage numbers 56 and 77 were each cultured for 48 hr and then prepared for radioimmunoassay (RIA). After removing aliquots from the media for insulin assay the cells were centrifuged, washed three times with 0.33 M sucrose and homogenized in 1 M acetic acid with the aid of a Polytron homogenizer. Samples of the extract were serially diluted in the RIA assay buffer. Insulin RIA (Morgan & Lazarow, 1963) of the extract and culture media was carried out using rat insulin standards, with a minimal detectable concentration of 0.04 ng per sample. The total protein in the cell extract homogenates was assayed using a Bio-Rad protein assay kit.

### Solutions

The pipettes were filled with (mM): 140 KCl, 5 NaCl, 2 EGTA, 1 free $Mg^{2+}$, $10^{-3}$ free $Ca^{2+}$, 5 HEPES, pH 7.4. A system for changing the bathing solution was developed. It consisted of twelve 60-cc syringes connected to a manifold through a series of PE tubing and 3-way stopcocks. The excised inside-out patch was placed in a notch cut into PE tubing that exited from the manifold. The time between the stopcock switch and a change at the patch was about 10 sec. The syringes were filled with various modifications of the pipette solution: different pH or free $Ca^{2+}$ concentration; addition of nucleotides or other pharmacological agents; high $Na^+$ (140 mM), low $K^+$ (5 mM) solution, etc. All solutions were buffered to pH 7.4 unless otherwise stated. All calcium solutions were prepared by adding the amounts of $CaCl_2$, $MgCl_2$ and EGTA necessary to obtain 1 mM free $Mg^{2+}$ and the desired level of free $Ca^{2+}$ determined from the computer program (and stability constants for all the reactions between $Ca^{2+}$, $Mg^{2+}$, $H^+$ and EGTA) designed by Fabiato and Fabiato (1979).

### Recording and Analysis

Single ion channels were studied using the technique of patch clamping described by Hamill et al. (1981). Pipette tips had a diameter of approximately 0.5 $\mu$m (determined by scanning electron microscopy) and were not fire polished prior to use. All patches were of the inside-out configuration, i.e., the cytoplasmic side faced the bathing medium. The data were collected unfiltered on FM tape and played back through a 1 kHz low-pass filter. Distinctions between channels were qualitatively based on differences in their conductance and behavior to pharmacological agents.

**Table 1.** Summary of the radioimmunoassay study on insulin secretion and storage in HIT cells at passages 56 and 77[a]

| Passage | Stored insulin (ng/mg cell protein) | Secreted insulin (ng/mg cell protein/48 hr) |
|---|---|---|
| 56 ($n$ = 3) | 750 ± 105 | 917 ± 128 |
| 77 ($n$ = 3) | <18 | 29.4 ± 8.5 |

[a] Values represent mean ± SEM and were not corrected for any insulin found in the horse serum.

## Results

### Insulin Secretion and Channel Species vs. Passage Number

Cells from early passages (48 to 56) appeared to aggregate together into multi-layered, irregular shaped clumps of cells suggestive of the islet configuration of $\beta$-cells in vivo (Fig. 1*A*, passage 54). The aggregates tended to remain distinct and separate from one another for the duration of the passage. Some of the cells associated with the aggregates tended to be larger and more spherical giving the "islets" a heterogeneous appearance. The morphological growth pattern of the cells after passage 70 was different (Fig. 1*B*, passage 75). By the second day of culture the cells had aggregated into three or four very large, multi-layered amorphous masses. They increased in size and eventually fused with each other before the next passage. These aggregates also appeared more homogeneous and their boundaries were smoother and less defined.

Table 1 compares the stored and secreted insulin values for passages 56 and 77. The stored insulin values were 750 ± 105 ng/mg cell protein for passage 56 and less than 18 ng/mg cell protein for passage 77. The secreted insulin values for these passages were 917 ± 128 ng/mg cell protein/48 hr and 29.4 ± 8.5 ng/mg cell protein/48 hr, respectively. These results are qualitatively similar to those previously reported by Santerre et al. (1981). This drop in insulin secretion was correlated with the type of channel observed at the two passage numbers. More than 90% of the patches on cells from passages 48 to 56 contained $K^+$-selective channels. On the basis of conductance and sensitivity to pharmacological agents five different types were distinguished (Table 2). In contrast, no $K^+$-selective channels were detected in 22 patches from cells of passage number 70 or greater. In 73% of the patches from these cells no channels were detected and in 27% of the patches a 25-pS nonselective channel

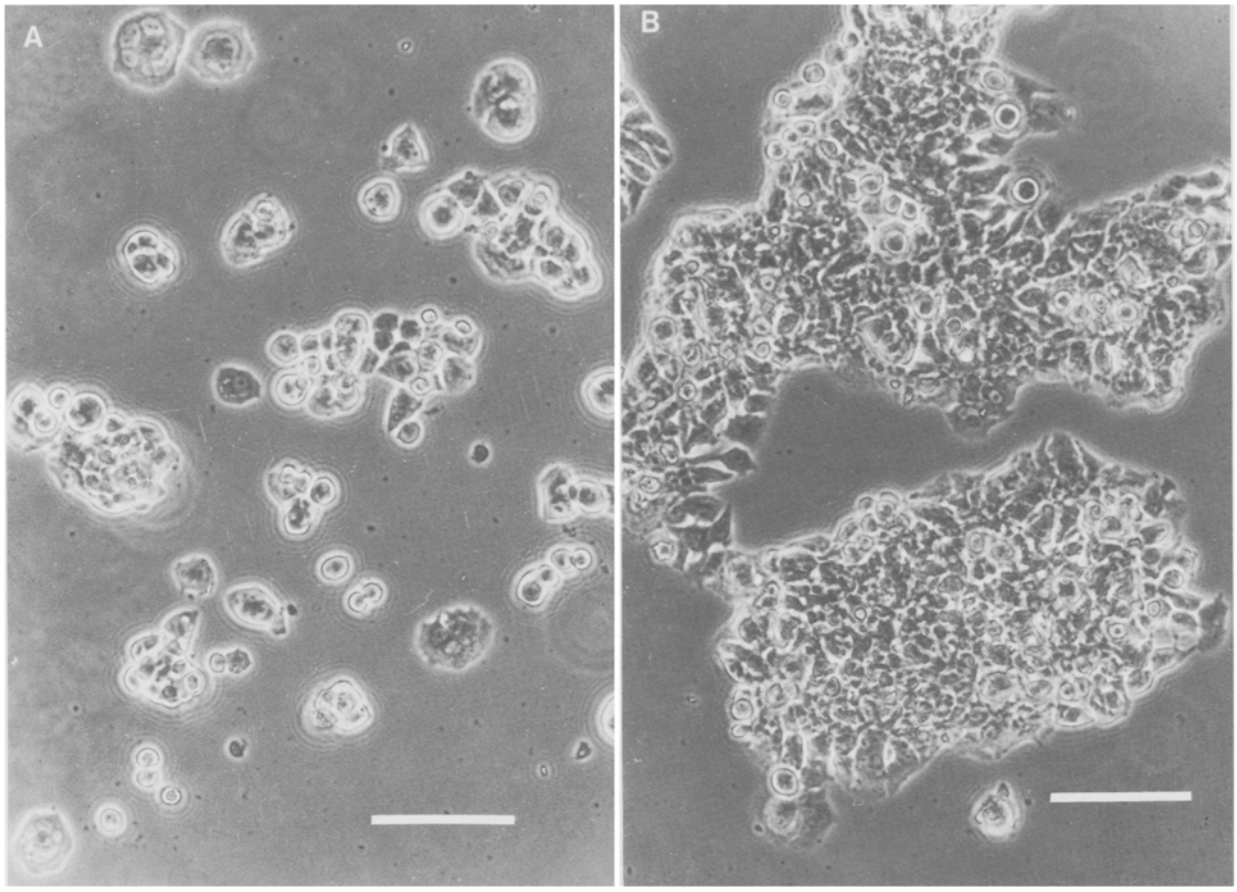


**Fig. 1.** HIT cells as observed by phase-contrast microscopy 3 days after the last passage for (*A*) passage 54 and for (*B*) passage 75. Calibration bar represents 100 $\mu$m

**Table 2.** Summary of the $K^+$ channels in HIT cells from 36 patches containing channels[a]

| | Channel | Conductance | Voltage sensitivity | Calcium sensitivity | Tea sensitivity | Quinine sensitivity | pH sensitivity | ATP sensitivity | Frequency |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ATP-activated $K^+$ channel | 210 pS | yes | no | no | yes | $H^+$ inhibited | ATP stimulated | 2(4.5) |
| 2 | $Ca^+$-activated $K^+$ channel | 211 ± 7 pS | yes | yes | no | no | no | no | 12(2) |
| 3 | TEA-sensitive $K^+$ channel | 185 ± 19 pS | yes | no | yes | no | no | no | 9(2.7) |
| 4 | Inward-rectifying $K^+$ channel | 130 pS | yes | no | no | yes | no | — | 3(1) |
| 5 | Small-conductance $K^+$ channel | 15 ± 2 pS | no | no | no | no | no | no | 28(1) |

[a] The conductance values were measured in symmetrical 140 mM KCl solutions, and where appropriate, are given as mean ± SEM. The frequency refers to the number of patches that contained that channel and the number in the parentheses represents the average number of channels per patch. The dashed lines refer to information that was not obtained.

was observed. Since the selectivity was determined in asymmetrical KCl/NaCl solutions, we cannot rule out the possibility that the channel was actually $Cl^-$ selective.

No obvious factors were found in the early passage cells that were correlated with the different channel types. The cells were trypsinized during each passage in order to remove them from the culture dish. Although gigaohm seals could be obtained during the first 12 hr after trypsinizations, they only rarely contained detectable channels. One day after trypsinization, all the channel types were seen for

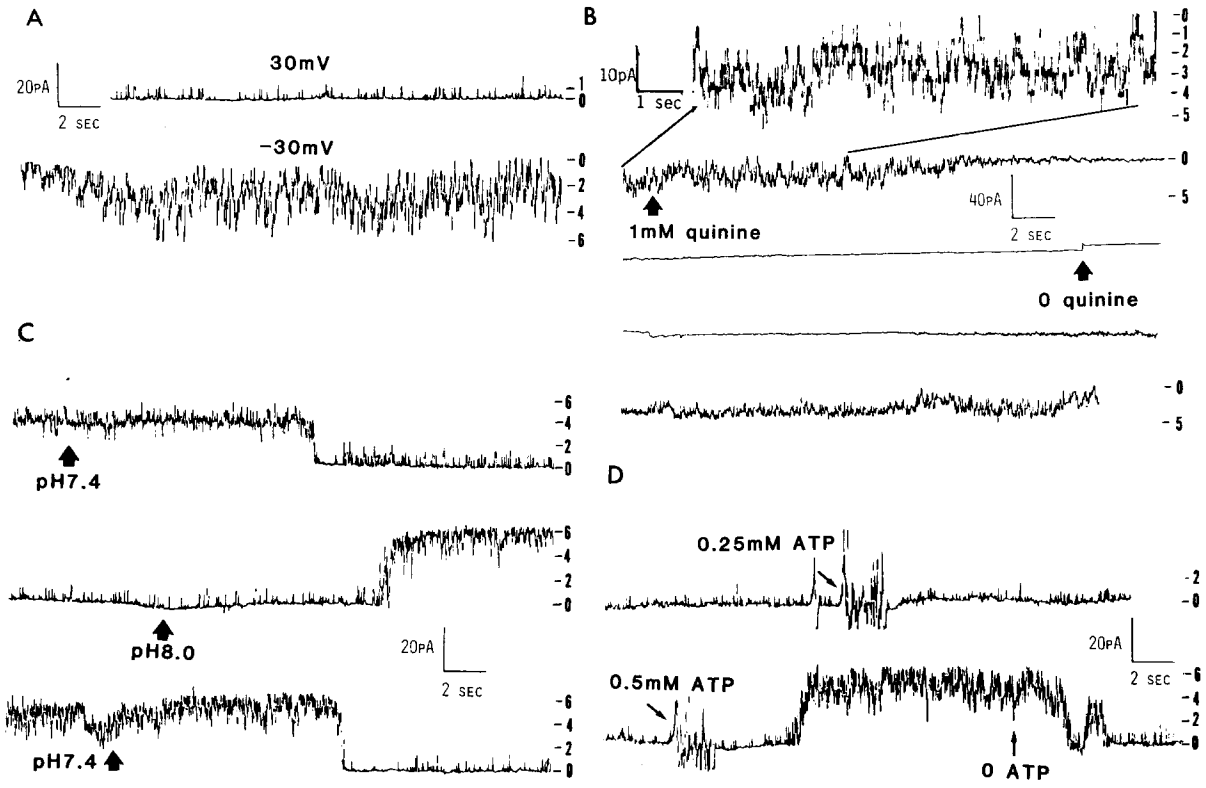


**Fig. 2.** (*A–D*) Single-channel current recordings of the ATP-activated $K^+$ channel in symmetrical 140 mM KCl solutions. The number of open channels is indicated to the right of each tracing. All recordings were filtered through a 1-kHz low-pass filter. (*A*) Effect of membrane potential on channel activity (the upper tracing was held at +30 mV and the lower tracing was held at −30 mV). (*B*) Effect of quinine on channel activity in a patch held at −30 mV. 1 mM quinine was added at the first arrow and washed out at the second arrow (continuous record). (*C*) Effect of pH on channel activity in a patch held at +30 mV. The initial bathing solution was at pH 8.0 and the pH was altered as indicated at each arrow (continuous record). (*D*) Effect of ATP on channel activity. Both recordings were held at +30 mV and ATP was added as indicated by the arrows. The large deflection at the first arrow was mechanical vibration due to pipetting ATP into the bathing solution

cultures up to 15 days, with no obvious correlation between culture time and channel type. The salient features of the different channels from the early passage (48 to 56) cells are described in the following sections. The names used here to describe each was chosen on the basis of a distinguishing physiological property of that channel.

## ATP-Activated $K^+$ Channel

This channel was not obviously sensitive to changes in the free $Ca^{2+}$ concentration between $10^{-8}$ and $10^{-6}$ M, but was strongly voltage sensitive, with an increased open probability when the membrane was depolarized, that is, for a negative pipette potential (Fig. 2*A*). (The potential in the Figures is the potential in the pipette relative to the bath. Thus a potential of −30 mV means that the cytoplasmic (bath) side of the inside-out patch is 30 mV positive (depolarized) relative to the plasma side.) The channel was not significantly inhibited by 5 mM TEA but its activity was completely and reversibly blocked by 1 mM quinine (Fig. 2*B*). As can be seen in Fig. 2*B*, when the quinine was washed out the single-channel conductance continuously increased with no obvious change in gating, indicating the primary effect of quinine is on single-channel conductance. This channel was also reversibly inhibited by $H^+$ ions. Figure 2*C* shows the increased frequency of open channels at pH 8.0 compared to pH 7.4. In contrast, pH 6.8 completely abolished channel activity (*not shown*). Noncyclic purine nucleotides also reversibly affected channel activity. Figure 2*D* shows that 0.5 mM ATP greatly enhanced channel activity at a pipette potential (30 mV) where the channels had a very low probability of being open. Similar concentrations of ADP, AMP, GTP and the nonhydrolyzable ATP analogs AMP-CPP and AMP-PCP also stimulated channel activity. In contrast, 3 mM cAMP, 2.5 mM cGMP and 3.75 mM AMP-PNP had no obvious affect on channel activity.

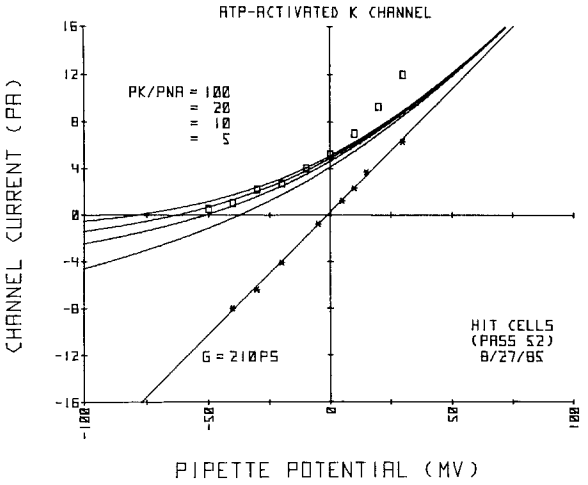


**Fig. 3.** Single-channel current-voltage relationship of the ATP-activated $K^+$ channel from inside-out patches in the absence of ATP. The * represents experimentally obtained values in symmetrical 140 mM KCl solutions. The □ represents experimentally obtained values in asymmetrical solutions (high $K^+$ in the pipette and high $Na^+$ in the bath). The straight line is a least-squares best fit of the values obtained in symmetrical solutions and its slope is the single-channel conductance (210 pS). The curved lines are theoretical plots of the constant field equation using the indicated $P_K/P_{Na}$ values and the conductance from the symmetrical case. All voltages refer to the potential in the pipette relative to the bath

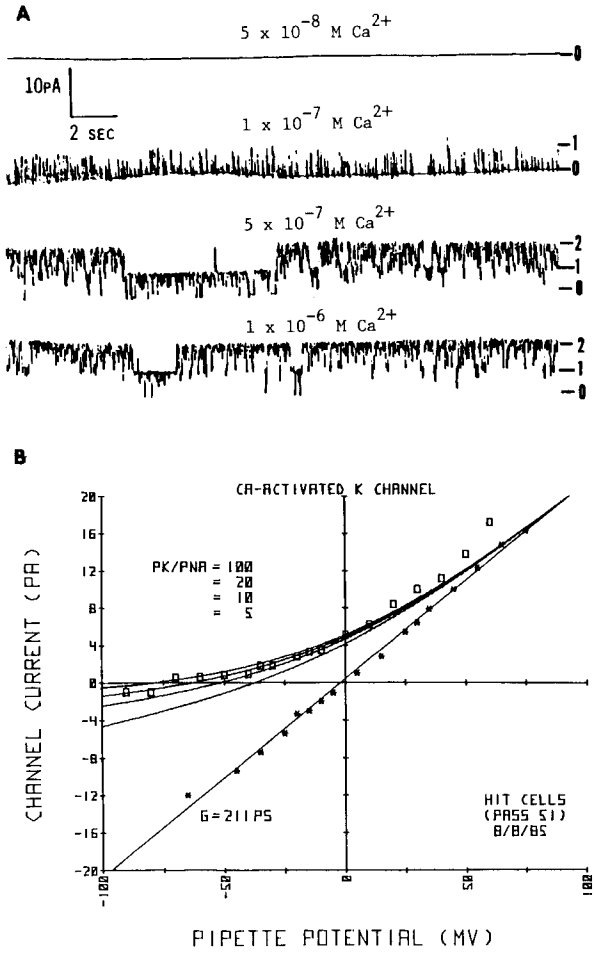


**Fig. 4.** (*A*) Single-channel current recordings of the $Ca^{2+}$-activated $K^+$ channel from an inside-out patch held at +30 mV in symmetrical 140 mM KCl solutions. The four current tracings show the effect of changing the intracellular free $Ca^{2+}$ concentration (free $Ca^{2+}$ levels are indicated above each record). (*B*) Single-channel current-voltage relationship of the same channel in $10^{-6}$ M free $Ca^{2+}$. *See* Fig. 3 for a description of the *I-V* plot

Figure 3 gives the current-voltage relationship of this channel for a patch in symmetrical 140 mM KCl solutions (*) and in asymmetrical high $K^+$/high $Na^+$ solutions (□). The straight line is a least-squares best fit of the symmetrical solution experimental data. It has a slope (conductance) of 210 pS. The curved lines are theoretical fits of the experimental data in asymmetrical solutions using the constant field equation for the indicated $P_K/P_{Na}$ values and using the experimental conductance from the symmetrical case.[1] The channel was $K^+$ selective, following the constant field equation with a $P_K/P_{Na}$ ratio of at least 10 for pipette potentials less than 0 mV. No reversal in the single-channel current was detected.

[1] $$I = \frac{(F^2)}{RT}\psi\left[P_K\left(\frac{[K]_1 e^{F\psi/RT} - [K]_2}{e^{F\psi/RT} - 1}\right) + P_{Na}\left(\frac{[Na]_1 e^{F\psi/RT} - [Na]_2}{e^{F\psi/RT} - 1}\right)\right]$$

where $I$ is the single-channel current, $\psi$ is the applied potential, $F$ is the faraday constant, $R$ is the universal gas constant and the value of $P_K$ is determined from the single-channel conductance ($G_K$) in symmetrical KCl solutions ($P_K = G_K(RT/F^2)/[K]$).

## $Ca^{2+}$-Activated $K^+$ Channel

This channel was both voltage and $Ca^{2+}$ sensitive. It was more likely to be open when the membrane was depolarized (i.e., during a negative pipette potential), and progressively lower concentrations of free $Ca^{2+}$ were required to activate channel opening at more negative pipette potentials. At a pipette potential of 30 mV, the channel spent all of its time in a closed state at $5 \times 10^{-8}$ M free $Ca^{2+}$, most of its time in a closed state at $10^{-7}$ M free $Ca^{2+}$ and most of its time in an open state at $5 \times 10^{-7}$ M free $Ca^{2+}$ (Fig. 4*A*). Figure 4*B* shows that this channel was $K^+$ selective in asymmetrical solutions, with a $P_K/P_{Na}$ ratio of at least 20 and a conductance of 211 pS in symmetrical solutions. This channel was not obvi-

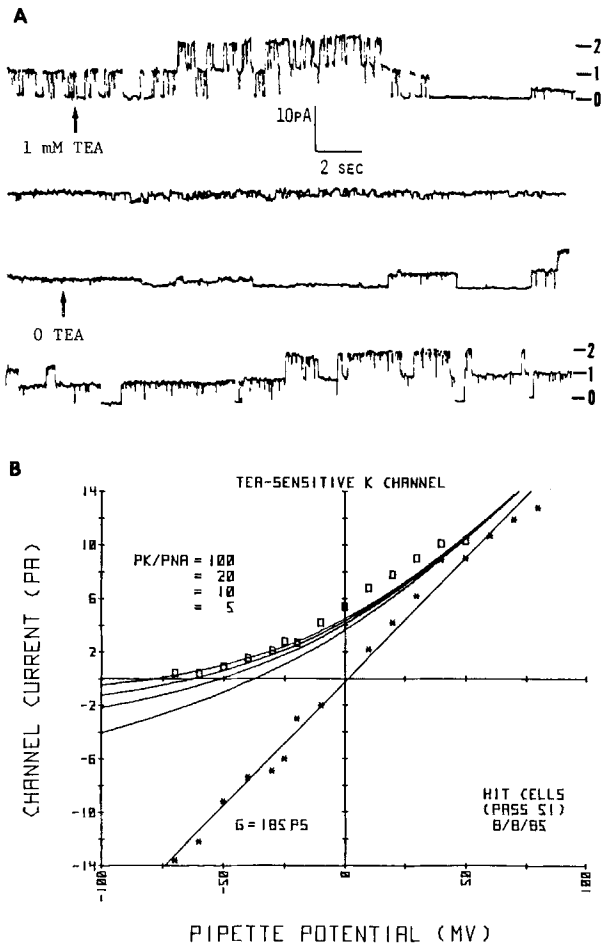


**Fig. 5.** (*A*) Continuous single-channel current recording of the TEA-sensitive $K^+$ channel from an inside-out patch held at +30 mV in symmetrical 140 mM KCl solutions. One mM TEA was added at the first arrow and washed out at the second arrow. (*B*) Single-channel current-voltage relationship of the same channel. *See* Fig. 3 for a description of the *I-V* plot

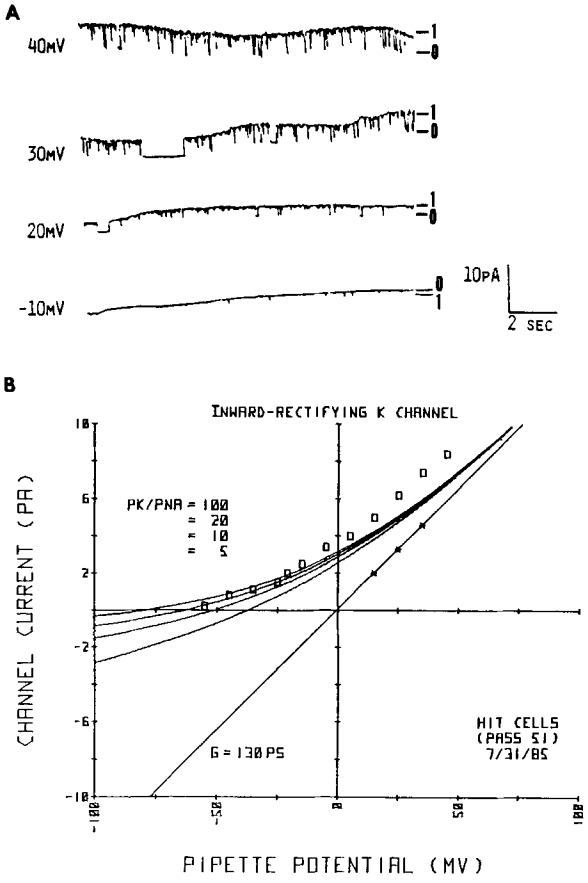


**Fig. 6.** (*A*) Single-channel current recordings of the inward-rectifying $K^+$ channel from an inside-out patch held at various potentials in symmetrical 140 mM KCl solutions. (*B*) Single-channel current-voltage relationship of the same channel. *See* Fig. 3 for a description of the *I-V* curve

ously affected by 2 mM ATP or changes in pH from 6.1 to 8.0, and revealed no detectable block with 10 mM TEA.

## TEA-Sensitive $K^+$ Channel

The single channel conductance for this channel was decreased by approximately 75% in 1 mM TEA (Fig. 5*A*) and was not detectable in 10 mM TEA. Figure 5*B* shows that this channel was $K^+$ selective in asymmetrical solutions, with a $P_K/P_{Na}$ ratio of at least 20 and a conductance of 185 pS in symmetrical solutions. The channel was not obviously sensitive to 1 mM quinine, ATP (up to 5 mM), pH (6.1 to 8.0) or $Ca^{2+}$ ions (~0 to $10^{-16}$ M), but was affected by the membrane potential with an increased open probability when the membrane was depolarized.

## Inward (anomalous) Rectifying $K^+$ Channel

Unlike all other channels that were observed to have a voltage dependence, this channel's open state was enhanced by membrane hyperpolarization (Fig. 6*A*). It was $K^+$ selective in asymmetrical solutions, with a $P_K/P_{Na}$ ratio of at least 20 and a conductance of 130 pS in symmetrical solutions (Fig. 6*B*). In addition, it was insensitive to 10 mM TEA, 2 mM ATP, or to changes in pH (6.8 to 8.0) or the free $Ca^{2+}$ concentration ($10^{-8}$ to $10^{-6}$ M), but was closed by 0.1 mM quinine.

## Small-Conductance $K^+$ Channel

This was the most commonly observed channel. It was $K^+$ selective in asymmetrical solutions and had a conductance of 15 pS in symmetrical solutions. There was no detectable influence of voltage, pH (6.1 to 8.0) or the free $Ca^{2+}$ concentration (~0 to


**Fig. 7.** Single-channel recordings of the small conductance channel held at +30 mV in symmetrical 140 mM KCl solutions. The large conductance TEA-sensitive channel is seen in the same record

$10^{-6}$ M) on channel activity. It was also insensitive to 10 mM TEA, 1 mM quinine or 5 mM ATP. Figure 7 shows this channel in a patch with the TEA-sensitive $K^+$ channel.

### Other Possible $K^+$ Channels

In addition to these five $K^+$ channels, three other $K^+$ channels were observed, each of which was found only once.

A pH-sensitive channel was $K^+$ selective in asymmetrical solutions, with a $P_K/P_{Na}$ ratio of at least 10 and a conductance of 116 pS in symmetrical solutions. It was completely and reversibly blocked by 5 mM TEA and was closed by pH 6.1. It was not obviously sensitive to 1 mM quinine, 2 mM ATP or to changes in the free $Ca^{2+}$ concentration (~0 to $10^{-6}$ M).

A quinine-sensitive channel was $K^+$ selective in asymmetrical solutions and had a conductance of 68 pS in symmetrical solutions. It was not obviously sensitive to changes in voltage, pH (6.8 to 8.0) or the free $Ca^{2+}$ concentration ($10^{-8}$ to $10^{-6}$ M). It was also not sensitive to 5 mM TEA or 2 mM ATP but was completely and reversibly blocked by 1 mM quinine.

A low-conductance TEA-sensitive channel was $K^+$ selective in asymmetrical solutions and had a conductance of 40 pS in symmetrical solutions. It was completely and reversibly blocked by 5 mM TEA but was not obviously sensitive to 1 mM quinine, pH (6.1 to 8.0) or changes in the free $Ca^{2+}$ concentration ($10^{-8}$ to $10^{-6}$ M). In addition, it revealed a strong voltage dependence with an increased open probability when the membrane was depolarized.

## Discussion

At least four different types of $K^+$ channels have been reported in $\beta$-cells, two of which (the $Ca^{2+}$-activated and inward-rectifying) are very similar to those found in this study (Table 2). Both Cook et al. (1984) and Findlay, Dunne and Petersen (1985*b*) have described the presence of $Ca^{2+}$-activated $K^+$ channels in $\beta$-cells, with a voltage and $Ca^{2+}$-dependence similar to what we observed in HIT cells. We found that the calcium activation occurred about $10^{-7}$ to $10^{-6}$ M free $Ca^{2+}$, in agreement with the results for $\beta$-cells or for other cell types (Pallotta, Magleby & Barrett, 1981; Maruyama, Gallacher & Petersen, 1983; Schwarz & Passow, 1983; Gallin, 1984). This activation level is consistent with the estimated cytoplasmic-free $Ca^{2+}$ concentration of $10^{-7}$ M reported for an insulin-secreting cell line (Wollheim & Pozzan, 1984). The conductance of the $Ca^{2+}$-activated $K^+$ channel we observed in HIT cells was 211 pS, less than 250 pS reported for $\beta$-cells (Cook et al., 1984; Findlay et al., 1985*b*). One other difference is that the $\beta$-cell channel was pH sensitive (Cook et al., 1984) whereas the HIT channel was not. The $Ca^{2+}$-activated $K^+$ channel was the second most frequently observed channel: 33% of the patches with $K^+$ channels had a $Ca^+$-activated $K^+$ channel, with an average of two such channels per patch.

A second type of channel that was common between the two cell types was an inward-rectifying $K^+$ channel. In the $\beta$-cell, this channel type was observed at three different conductance levels: 30 pS (Findlay, Dunne & Petersen, 1985*a*), close to 50 pS (Ashcroft et al., 1984; Cook & Hales, 1984) and 75 pS (Findlay et al., 1985*a*). The channel we observed in HIT cells had a conductance of 130 pS. Only 8% of the patches with $K^+$ channels had an inward-rectifying $K^+$ channel, with an average of one such channel per patch.

The other six kinds of $K^+$ channels we observed in HIT cells have not been reported in $\beta$-cells. Of these, the most interesting is the ATP-activated $K^+$ channel. This channel is stimulated by ATP, in contrast to the inhibitory effect reported for a number of different cell types (Noma, 1983; Cook & Hales, 1984; Findlay et al., 1985*a*; Spruce, Standen & Stanfield, 1985). Also, this channel had a conductance of 210 pS, much larger than the 25 pS ATP-activated $K^+$ channel observed by Trube and Heschler (1984) in heart cells. It seems unlikely that ATP acts by phosphorylating the channel because ADP, AMP and AMP-PCP were all equally as effective as ATP in stimulating channel activity. Smith, Coronado and Meissner (1985) reported a calcium channel from sarcoplasmic reticulum with a similar sensitivity: it was activated by ATP, AMP or AMP-PCP. The HIT cell channel was also activated by 0.5 mM GTP and was insensitive to cyclic nucleotides. It is possible that the channel responded to the terminal inorganic phosphate that was found in the noncyclic nucleotides but was absent in the cyclic nucleotides or different in AMP-PNP; but this

remains to be elucidated. It is interesting, however, from a physiological standpoint, that 0.5 mM ATP was able to stimulate channel activity at a voltage where the channel was normally quiescent. If similar characteristics occur in vivo, then a rise in the intracellular ATP would help to repolarize the cell by activating this $K^+$-selective channel. How this might relate to insulin secretion or $\beta$-cell physiology is unclear.

Of the remaining five channels, three were observed only once out of 36 patches with channels: the pH-sensitive $K^+$ channel, the quinine-sensitive $K^+$ channel and the small conductance TEA-sensitive $K^+$ channel. The large conductance TEA-sensitive channel was observed 25% of the time in patches that had $K^+$ channels, with an average of 2.7 such channels per patch. The small conductance channel was the most frequently observed channel occurring in 78% of the patches that had $K^+$ channels at an average of 1.2 such channels per patch. Unlike the other channels, this one was often observed in patches with the other channel types. In addition, it was not obviously sensitive to voltage or any other manipulation. Therefore, if present in vivo, it would always provide a background potassium leak in the plasma membrane. Perhaps this channel is important for helping to establish a resting membrane potential along with the inward-rectifying potassium channel. Since it is a small conductance channel, its contribution would tend to become less important when the large conductance channels open. Another possibility is that this channel is regulated by some cell factor, other than membrane potential, which is not present in inside-out patches.

On four different occasions it was possible to measure channel activity while the pipette was cell attached (on cell) and then after pulling off the cell. On each occasion, the channel activity either dramatically increased or appeared only when the patch was pulled off, suggesting that the channel becomes modified after removal from contact with the cell contents. The various channels might be associated with different levels of cell modifiers or different degrees of phosphorylation (Ewald, Williams & Levitan, 1985).

In the early passages, the different channels were not associated with the passage number since all five types (Table 2) were found in passage 51 and all eight types were found in passage numbers 51 and 52. Another source of variability is the number of days between the last passage (and trypsin treatment) and the channel observation. With time after passage, the cells may mature and different channels develop, similar to the development of a $Ca^+$ sensitivity in cultured spinal neurons (Blair & Dionne, 1985). However, no obvious correlation between the channel type and days after the last passage was observed.

Although the cell line was originally monoclonal, morphological differences were seen in the cells at any one passage. The different channel types may have been associated with the different cell morphologies. Finally, it cannot be excluded that HIT cells close to passage 50 do indeed each contain eight different $K^+$ channels. This large number of channels may be required to precisely control insulin secretion.

Distinguishing channel types is not as straightforward as it might appear. There are several problems that sometimes occur when patching that make channel identification difficult. For example, determining the patch configuration is not always possible since outside-out patches may occasionally form even when following the procedure for inside-out patches. This can cause a problem because the same channel may reveal different properties to pharmacological agents depending on which side of the membrane they are applied. In addition, the single-channel conductance, which is used to determine channel type, can vary from preparation to preparation.

The calcium-activated potassium channel can be used as a marker for membrane sidedness since the calcium sensitivity only occurs on the cytoplasmic side (Pallotta et al., 1981; Maruyama et al., 1983). Although this channel did not occur with any of the other large conductance channels, patch sidedness was probably not an issue for distinguishing the five channels listed in Table 2. The patch had to be of the inside-out configuration for the calcium-activated channel or it would not have been sensitive to calcium. Its voltage dependence was such that it increased its probability of opening when the pipette interior was made more negative (i.e., during a membrane depolarization).

Two other channels similar in conductance to the calcium-activated channel were the ATP-activated channel and the TEA-sensitive channel. These channels had a similar voltage dependence as the calcium-activated channel; that is, they all increased their probability of opening as the pipette potential was made more negative. If these two channels were merely the calcium-activated channel in an outside-out patch, they would have had the opposite voltage dependence; that is, they would have increased their probability of opening as the pipette was made more positive.

The inward (anomalous) rectifier did have a voltage dependence opposite to that of the calcium-activated channel. However, its conductance was significantly different from the other four channels

so it was not likely to be confused with them. Finally, the small-conductance channel was obviously distinct from the other channels and it frequently appeared in a patch with other channel types (Fig. 7).

There was an apparent correlation between the presence of $K^+$ channels and insulin secretion. Over 90% of the patches from cells of passages 48 to 56 exhibited $K^+$-selective channels, whereas from passage 70 or later no $K^+$ channels were detected and insulin secretion and storage were markedly reduced. One cannot determine from these experiments whether the channels are present and nonfunctional or are completely absent from the plasma membrane of the late passage cells. This cell drift probably results from selection effects that operate at each passage, e.g., the degree of attachment to the dish, resistance to trypsin, cell generation time, etc. (Santerre et al., 1981).

In conclusion, HIT cells provide a suitable model for glucose-induced insulin secretion. Although they differ in the amount of insulin secreted in response to glucose when compared to β-cells (Santerre et al., 1981) they have a number of experimental advantages. They can be cultured into large populations for biochemical analysis, form gigaohm seals readily and can be frozen and stored for later use.

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