RINm5f Cells Express Inactivating BK Channels Whereas HIT Cells Express Noninactivating BK Channels

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Li, Zi-Wei, Jiu Ping Ding, Vani Kalyanaraman, and Christopher J. Lingle. RINm5f cells express inactivating BK channels whereas HIT cells express noninactivating BK channels. J. Neurophysiol. 81: 611–624, 1999. Large-conductance Ca\(^{2+}\)- and voltage-activated BK-type K\(^+\) channels are expressed abundantly in normal rat pancreatic islet cells and in the clonal rat insulinoma tumor (RINm5f) and hamster insulinoma tumor (HIT) beta cell lines. Previous work has suggested that the Ca\(^{2+}\) sensitivity of BK channels in RIN cells is substantially less than that in HIT cells, perhaps contributing to differences between the cell lines in responsiveness to glucose in mediating insulin secretion. In both RIN cells and normal pancreatic beta cells, BK channels are thought to play a limited role in responses of beta cells to secretagogues and in the electrical activity of beta cells. Here we examine in detail the properties of BK channels in RIN and HIT cells using inside-out patches and whole cell recordings. BK channels in RIN cells exhibit rapid inactivation that results in an anomalous steady-state Ca\(^{2+}\) dependence of activation. In contrast, BK channels in HIT cells exhibit the more usual noninactivating behavior. When BK inactivation is taken into account, the Ca\(^{2+}\) and voltage dependence of activation of BK channels in RIN and HIT cells is essentially indistinguishable. The properties of BK channel inactivation in RIN cells are similar to those of inactivating BK channels (termed BK\(_{i}\) channels) previously identified in rat chromaffin cells. Inactivation involves multiple, trypsin-sensitive cytosolic domains and exhibits a dependence on Ca\(^{2+}\) and voltage that appears to arise from coupling to channel activation. In addition, the rates of inactivation onset and recovery are similar to that of BK channels in chromaffin cells. The charybdotoxin (CTX) sensitivity of BK\(_{i}\) currents is somewhat less than that of the noninactivating BK variant. Action potential voltage-clamp waveforms indicate that BK current is activated only weakly by Ca\(^{2+}\) influx in RIN cells but more strongly activated in HIT cells even when Ca\(^{2+}\) current magnitude is comparable. Concentrations of CTX sufficient to block BK\(_{i}\) current in RIN cells have no effect on action potential activity initiated by glucose or DC injection. Despite its abundant expression in RIN cells, BK\(_{i}\) current appears to play little role in action potential activity initiated by glucose or DC injection in RIN cells, but BK current may play an important role in action potential repolarization in HIT cells.

INTRODUCTION

Membrane ion channels play a central role in the regulation of secretion of insulin from the beta cells of the pancreatic islets (Ammala et al. 1997; Ashcroft et al. 1994; Dukes and Philipson 1996). In particular, influx of Ca\(^{2+}\) through voltage-dependent Ca\(^{2+}\) channels appears to be the primary source of Ca\(^{2+}\) necessary for activation of insulin secretion initiated by glucose-induced depolarization of beta cells. Participating with voltage-dependent Ca\(^{2+}\) channels in the regulation of beta cell electrical behavior is a number of distinct K\(^+\) channels the activity of which serves to control beta cell resting potential and terminates either single action potentials or bursts of action potentials. Modulation of various K\(^+\) channels, e.g., inhibition or activation of ATP-sensitive K\(^+\) channels (Dukes and Philipson 1996) or Ca\(^{2+}\) channels (Love et al. 1998) appears to be a major mechanism by which insulin can be regulated.

Several early studies suggested that the Ca\(^{2+}\)\(^{-}\)- and voltage-dependent BK channels might play an important role in the regulation of the electrical activity of beta cells with concomitant effects on secretion (Atwater et al. 1983). Single BK channels commonly are found in beta cell membranes (Bokvist et al. 1990; Smith et al. 1990). BK current is activated rapidly by depolarizing voltage steps particularly when intracellular Ca\(^{2+}\) buffering approximates physiological conditions (Satin et al. 1989). Quinine, a blocker of BK channels (Mancilla and Rojas 1990), influences insulin secretion (Atwater et al. 1979). Furthermore, in rat insulinoma tumor (RIN) and hamster insulinoma tumor (HIT) cells, BK channels were reported to exhibit very different sensitivities to Ca\(^{2+}\), which was proposed to contribute to the differences in the ability of glucose to initiate insulin secretion in the two cell lines (Edddestone et al. 1989). Modulatory effects of glucose on BK channel activity in RIN cells also have been reported (Ribalet et al. 1988). On the basis of this earlier work, some models were proposed suggesting that activation of BK channels might serve to terminate glucose-initiated bursting (e.g., Chay 1990).

Interest in the role of BK channels in beta cell secretion and electrical activity waned with the report that blockade of BK channels by the scorpion toxin, charybdotoxin (CTX), had no effect on beta cell glucose-stimulated electrical activity or action potential repolarization (Kukuljan et al. 1991). The consequences of blockade of BK channels by quinine were discounted because quinine also blocks other K\(^+\) channels (Fatherazi and Cook 1991; Mancilla and Rojas 1990). With the emergence of work demonstrating the central role of ATP-regulated K\(^+\) channels in the response to glucose (rev. by Dukes and Philipson 1996), further work on the possible roles of BK channels in beta cells has diminished except for some interest in the possible role of Ca\(^{2+}\)-dependent K\(^+\) current in acetylcholine-mediated regulation of secretion (e.g., Ammala et al. 1991).

Recently we observed that cells from rat pancreatic islets express both inactivating and noninactivating variants of BK channel (Lingle et al. 1996). Furthermore there are other sug-
gestions in earlier work that inactivation may be a common feature of rat beta cell BK channels. For example, both cell-attached recordings of single BK channels and whole cell Ca$^{2+}$- and voltage-dependent K$^+$ currents exhibit inactivation (Satin et al. 1989; Smith et al. 1990), although the conditions of the earlier results allowed the possibility that the apparent inactivation may have been secondary to changes in submembrane Ca$^{2+}$. In addition, the dependence of BK channel activation on Ca$^{2+}$ in rat beta cells is unusually flat (Tabcharani and Misler 1989), a result expected for inactivating BK channels. Thus the previously described difference in Ca$^{2+}$ sensitivity between BK channels in HIT and RIN cells (Eddlestone et al. 1989) may have resulted, in part, from the presence of inactivating BK channels in RIN cells.

Here we have reexamined RIN and HIT cell BK channels to determine whether some fundamental difference in inactivation behavior may account for the apparent difference in Ca$^{2+}$ sensitivity. Our results indicate that RIN cells almost exclusively express the inactivating BK$_k$ channel, whereas only noninactivating BK channels are found in HIT cells. However, the basic Ca$^{2+}$ dependence of activation does not appear to differ among the two variants. The inactivation behavior of BK$_k$ channels in RIN cells appears essentially indistinguishable from the behavior of BK channels in rat chromaffin cells (Lingle et al. 1996; Solaro and Lingle 1992; Solaro et al. 1995, 1997).

METHODS
Preparation of RIN and HIT cell cultures

RIN m5F cells [obtained from the American Type Culture Collection (ATCC) and from the Washington University Tissue Culture Center] were grown in RPMI 1640 medium containing 1% glucose, 1% sodium pyruvate, 1% nonessential amino acids, and 10% fetal calf serum. HIT cells (also obtained from the Washington University Tissue Culture Center) were grown in Ham’s F12 K medium with 2.5% fetal calf serum and 10% dialyzed horse serum. Cells were passaged when confluent using a 0.05% trypsin-0.02% EDTA solution and kept under an atmosphere of 5% CO$_2$ at 37°C. RIN cells were used at a passage number between 60 and 70. For HIT cells, the passage number was between 20 and 30. For HIT cells, no attempts were made to block the activity of K-ATP channels in either inside-out patches or in whole cell recordings. However, as seen in the traces of Figs. 1, 5, and 6, under the conditions of our experiments, such contamination appears to be minor in inside-out patches.

Solution exchange and drug applications were accomplished as described previously (Herrington et al. 1995). Chemicals were from Aldrich or Sigma.

Data analysis

Analysis of whole cell and single-channel currents was done either with Clampfit (Axon Instruments, Foster City, CA) or with our own software. Currents or extracted data were fitted using a Levenberg-Marquardt search algorithm to obtain nonlinear least-squares estimates of function parameters. Estimates of the apparent CTX dissociation constant were made by fitting the complete time course of blockade at one or more drug concentrations to a first-order blocking reaction as described (Saito et al. 1997). Conductance-voltage (G-V) and fractional availability curves were fit with the following form of a Boltzmann equation

$$g(V) = g_{max}/[1 + \exp(-(V - V_{0.5})/k)]$$

where $g_{max}$ is the maximal conductance, $V_{0.5}$ is the voltage at which half the maximal conductance is activated, and $k$ is the voltage dependence of the equilibrium.

G-V curves were generated by measuring the maximal current at any command potential and converting that to a conductance assuming a reversal potential of 0 mV (symmetric K$^+$). This method has limitations but is sufficient for providing a qualitative comparison of
the HIT and RIN cell currents under study here. In general, the use of tail currents measured at a fixed potential after current activation at different potentials would provide a better measure of conductance because the tail current method would correct for any nonlinearities in the single-channel current or errors due to voltage-dependent channel block. However, given the inactivating nature of BK, the time of peak current activation varies considerably at different activation potentials such that repolarization at a fixed time would inherently underestimate the maximal conductance at some potentials. Furthermore, tail currents at negative potentials were not sufficiently well resolved to be reliable for conductance estimates.

**Extraction of total RNA and the reverse transcriptase-polymerase chain reaction (RT-PCR)**

Total RNA was isolated by a modified Chomczynski and Sacchi method (TRIzol Reagent, Life Technologies, Grand Island, NY). A monolayer of cells from a single 35-mm culture dish (~10⁷) was used for RIN cell and HIT cell RNA preparation. Total RNA from chromaffin cells was extracted from freshly isolated adrenal glands (50–100 mg tissue). Comparable amounts of RNA were used to synthesize first strand cDNA (Superscript II, Life Technologies) using oligo(dT) primers. One microoliter of the product of the reverse transcriptase reaction was used in a PCR reaction using primers flanking the splice junction 2 to detect the presence of alternatively spliced variants. Two sets of primer sequences were used in different experiments to examine splice junction 2 variability. The first primer sequences (termed mslo 3 and 4) were TACTGCAAGGCCTGTCATGATGACC (sense) and GGTGTTGGGCGAGTTCCTCATGCC (antisense). The second set of primer sequences (termed mso 14 and 15) was as follows: CATCGCAAGTGATGCCAAAG (sense) and CGACTACTCCGGAAGTCTGGGGAAC (antisense).

**Identification of splice site 2 variants**

Products of the RT-PCR reaction were run on 2% agarose gels to purify the bands of interest. Because Pfu polymerase was used for the RT-PCR, the fragments were blunt-ended and could be subcloned into pZero-1 (Invitrogen, Carlsbad, CA), which had been digested with EcoRV (New England Biolabs, Beverly, MA). PCR screening was carried out using the same primers as in the RT-PCR reaction. Those subclones, which had an insert, were sequenced using the dideoxy method of DNA sequencing (Sequenase Version 2.0, Amer sham, Arlington Heights, IL). Primers used were SP6 and T7, which were vector-specific forward and reverse primers, respectively.

**RESULTS**

**BK channels in RIN cells exhibit inactivation, whereas HIT cell channels are noninactivating**

Previous studies of BK channels in beta cell patches have generally used recordings at a fixed holding potential and submembrane Ca²⁺. This procedure will obscure any rapid time- and voltage-dependent gating behavior. Here, inside-out patches were held at −40 mV with 10 μM Ca²⁺ bathing the cytosolic face of the patch. Figure 1A illustrates the typical behavior of BK channels in a RIN cell patch. After a 100- or 200-ms step to −80 mV, a subsequent step to +80 mV was used to activate BK channels before returning to −40 mV. An interval of 2 or 3 s separated each step to +80 mV. BK channels exhibit rapid activation followed by a slower, but complete, inactivation. The inactivation time constant (τᵢ) from exponential fits to the decay phase of the ensemble current was ~20–30 ms at +80 mV and 10 μM Ca²⁺ (e.g., Fig. 1B). In contrast, when BK channels from patches for HIT cells were studied using identical procedures, no indication of inactivation was observed (Fig. 1C; 139 patches).

In the following sections we examine the properties of inactivation of BK channels in RIN cells to determine to what extent this inactivation process is similar to the previously described BKᵢ channels in rat chromaffin cells (Ding et al. 1998; Solaro and Lingle 1992; Solaro et al. 1995, 1997).

**Onset and recovery of inactivation of RIN cell BK channels**

Ensembles of BK channel openings were generated at a single command potential over a range of submembrane [Ca²⁺] (Fig. 2A) and at a single submembrane [Ca²⁺] ([Ca²⁺]ᵢ) over a range of activation voltages (Fig. 2C). τᵢ decreases both with increases in [Ca²⁺]ᵢ and with stronger depolarizations. When τᵢ is examined as a function of [Ca²⁺]ᵢ, a single activation voltage, a limiting value indicating that any intrinsic Ca²⁺ dependence in the inactivation process is saturated (Fig. 2B). Similarly, when τᵢ is examined at a single [Ca²⁺]ᵢ over a range of activation potentials, τᵢ approaches a limiting value indicating that the underlying inactivation process exhibits little intrinsic voltage dependence (Fig. 2D). The absolute values of τᵢ under the different conditions of voltage and [Ca²⁺]ᵢ are quite comparable with those observed for BKᵢ channels in rat chromaffin cells studied under identical conditions (Soloro and Lingle 1992; Solaro et al. 1995).

The time course of the recovery from inactivation of RIN cell BKᵢ channels was examined in inside-out patches bathed with 10 μM Ca²⁺. After an initial 160-ms step to −140 mV, a 400-ms step to +60 mV was used to first activate and then inactivate BKᵢ channels. After a variable recovery period at −120 mV, the patch was again stepped to +60 mV to deter-
Ca\(^{2+}\), BK\(_i\) channels in RIN cells are half inactivated at \(-67.3 \pm 1.4\) mV, with a voltage dependence of 20.0 \pm 1.2 mV. These values are comparable with those from chromaffin cell BK channels studied in a similar fashion (Ding et al. 1996; Ding and Lingle, unpublished results).

**Inactivation of BK\(_i\) channels in RIN cells involves multiple trypsin-sensitive, cytosolic domains**

Gomez-Lagunas and Armstrong (1995) have used the introduction of trypsin into cells transfected with Shaker K\(_v\) channels to show that the progressive removal of inactivation by trypsin is consistent with there being four inactivation domains per channel. Similarly, inactivation of BK\(_i\) channels in rat chromaffin cells appears to involve multiple, cytosolic trypsin-sensitive domains (Lingle et al. 1996) but with the difference that, on average, BK\(_i\) channels in chromaffin cells contain less than a full four inactivation domains per channel (Ding et al. 1998), perhaps because of heteromultimeric assembly of inactivation-competent and noninactivating subunits.
Able was interspersed with a brief application of trypsin. Figure of peak current that is noninactivating current ($f$ amount of current that does not inactivate at all. Trypsin resulted in an trypsin applications. Trypsin slowed the inactivation rate and increased the 2 step to repeated voltage steps to 140 mV to remove resting inactivation. Each ensemble inactivation process of inactivating BK channels in RIN cells (Fig. 5). If a cytosolic blocker competes with the normal inactivation domain for a site in the channel, this competition can be described by the following kinetic scheme where B is the QX-314 blocked state, O is the open state, and I is the inactivated state

$$A, \quad O \rightarrow I 
\frac{b}{k + f[D]} \quad B$$

where $\lambda_i$ is the channel inactivation rate, $f^\ast$ is the rate of association of drug (D), and $b$ is the rate of dissociation. In accordance with this scheme, if the properties of the blocking reaction are rapid relative to the rate of current inactivation ($\lambda_i$), the slowing of the apparent inactivation rate will exhibit a simple inverse dependence on the fractional reduction in ensemble average current amplitude (Choi et al. 1993; Solaro et al. 1997). Specifically, at a given [QX-314], $\tau_{QX} = 1/(k \cdot \lambda_i)$ and $I_{QX} = k \cdot I_{ctrl}$ where $\tau_{QX}$ is the current decay time constant in the presence of QX-314, $I_{QX}$ and $I_{ctrl}$ are peak ensemble current amplitudes in QX-314 and control salines, and $k$ is the fraction of unblocked channels given by $b/(b + f/QX)$. QX-314 is a relatively bulky cytosolic blocker of BK channels (Oda et al. 1992) and produces a rapid, time-averaged reduction of the BK single-channel current amplitude. As indicated in Fig. 5, an ~60% reduction of the ensemble average current amplitude correlates well with an ~67% reduction in the apparent single-channel current amplitude. This correlation indicates that QX-314 meets the criterion that the kinetics of the blocking reaction are rapid, relative to the onset of inactivation. Therefore, if QX-314 competes with the native inactivation process, any x-fold reduction in the ensemble average current is expected to be associated with an x-fold

Native inactivation process is not slowed by cytosolic blockers of BK channels

Rapid inactivation of the Shaker family of voltage-dependent K$^+$ channels involves an N-terminal domain that is thought to occlude the cytosolic mouth of the ion permeation pathway (Hoshi et al. 1990). Supporting this view, cytosolic blockers of Shaker K$^+$ channels slow the native inactivation process (Choi et al. 1993), presumably by competing with the native inactivation domain for an overlapping binding site. In contrast, inactivation of BK channels in chromaffin cells is not slowed by a number of different cytosolic blockers of BK channels, including tetraethylammonium, QX-314, and the ShakerB ball peptide (Lingle et al. 1996; Solaro et al. 1997). Here, we have examined the ability of QX-314 to influence the inactivation process of inactivating BK channels in RIN cells (Fig. 5). If a cytosolic blocker competes with the normal inactivation domain for a site in the channel, this competition can be described by the following kinetic scheme where B is the QX-314 blocked state, O is the open state, and I is the inactivated state

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prolongation of the inactivation time course. As shown in Fig. 5, QX-314 has no effect on the time course of the inactivation process even at a concentration producing a more than twofold reduction in peak current. We conclude that occupancy of a cytosolic binding site at the internal mouth of the permeation pathway does not hinder the native inactivation process.

Frequency of occurrence of BKs channels in RIN cell patches

In rat adrenal chromaffin cells, although most patches express exclusively inactivating BKs channels, there is some likelihood that noninactivating BKs channels will be observed in patches with BKi channels (Ding et al. 1998). We have proposed that BKi channels in rat chromaffin cells arise from heteromultimeric assembly of two BK subunits, an inactivation-competent bk subunit and an inactivation-null bk subunit (Ding et al. 1998). The overall frequency of occurrence of BKs channels in patches with BKi channels is consistent with a stoichiometry of about two to three bk subunits per channel with a probability of observing a BKs channel being about 1/16 to 1/256. Here we have attempted to place some limits on the possible frequency of occurrence of BKs channels in RIN cell patches.

Because of the large number of BKs channels in most of the patches we have obtained from RIN cell membranes, we only can provide rough estimates of the number of BKs channels in many patches. As the criterion for the presence of a BKs channel, we have required that a channel be open at the end of a 200-ms voltage step to +80 mV in >80% of the voltage steps. By choosing a depolarizing voltage-step duration that is sufficiently long relative to the mean inactivation time constant of BKs channels, one can define conditions where it is unlikely that a BKs channel will be open at the end of any voltage sweep. Having defined those conditions, one then can determine the number of BKs channels in a patch simply from the number of BK channels that remain active at the end of the voltage step in >80% of a set of voltage steps.

Figure 6 illustrates this approach for one patch, which contained 14 BKs channels. Three criteria were used to establish the number of channels in this patch. First, the maximal number of channels observed in any individual sweep was 14. Under conditions where the probability of a channel being open is high, this number provides a reasonable estimate of the number of channels in a patch (Horn 1991). Second, the mean number of open channels in a set of 30 sweeps and the variance was used to calculate the number of channels in this patch. This method of moments estimate (Horn 1991) yielded a value of 13.6. Finally, a fit of a binomial function to the binned distribution of number of occurrences of n channels for a set of 30 trials yielded a value for N, the number of channels in the population of 12.3 with a peak average open probability of 0.82. Thus under the conditions of these experiments, i.e., after a hyperpolarizing prepulse to −140 mV to remove most channels from inactivation, this analysis indicates that the maximum number of open channels observed in a set of sweeps provides a reasonable estimate of the number of channels in the patch. Furthermore despite the fact that there are 14 channels present in the patch of Fig. 6, once all channels have inactivated, the percentage of sweeps with openings at the end of the 400-ms voltage step is small. Thus this patch shows no evidence of a channel that would be considered a BKs channel.

To evaluate the relative frequency of occurrence of BKi and BKs channels in RIN cell patches, we have estimated the maximal number of BK channels from 57 patches during 400-ms steps to +80 mV after a 160-ms step to −140 mV. The maximal peak current for this set of patches ranged from 21 to ~332 pA (143.1 ± 91.3 pA, mean ± SD). This is consistent with there being at least 1 to ~15 channels in each patch (6.7 ± 4.7 channels), with a single-channel current amplitude of ~22.8 pA. The actual channel number in patches with large peak currents probably is underestimated. For this set of 57 patches, the total number of BK channels was ~384. In only one of these 57 patches was there any indication of a BK channel that appeared to remain open during the 400-ms depolarizing voltage steps. This channel occurred in a patch with a total of 12 BK channels. In 21 of 100 steps to +80 mV, 1 of
the 12 channels remained active during the entire voltage step. The occurrence of the noninactivating behavior was nonstationary in that 15 of the sweeps with a noninactivating channel were consecutive.

Let us assume that, for this set of 57 patches, 1 of 384 channels was of a BK_i phenotype. This frequency of occurrence of BK_k channels in RIN cell patches is substantially less than what has been observed previously in rat chromaffin cells (Ding et al. 1998). If, as has been proposed for chromaffin cells, BK channels in RIN cells can arise from the random, independent assembly of inactivation-competent (bk_k) and non-
inactivating (bk_i) subunits in which one bk_i subunit is sufficient to confer inactivation on a channel (Ding et al. 1998), the relative occurrence of BK_k channels would provide some information about the relative abundance of bk_k and bk_i subunits in these cells. Even at a ratio of 4 bk_k subunits to 1 bk_i subunit, random, independent assembly into tetrameric channels would predict that only 1 of 500 BK channels would be noninactivating. At a ratio of 3 bk_k subunits to 1 bk_i subunit, this model predicts that 1 of 256 BK channels would be noninactivating. Thus even with only 1 possible BK_k channel of 384 channels, the actual mole-fraction of bk_k subunits still may be appreciable. This would be consistent with the idea that BK channels in RIN cells may contain a reasonable number of bk_k subunits, perhaps consistent with some variability in inactivation rates we have observed for these patches and with the trypsin digestion experiments (Fig. 4). On the other hand, given the uncertainty about the noninactivating nature of the one putative BK_k channel, our estimate of the frequency of occurrence of the putative bk_i subunit may be high.

Calcium dependence of BK channel activation is similar in RIN and HIT

Previous estimates of Ca^{2+} dependence of BK activation in RIN cells used steady-state measurements of channel open probability (Eddlestone et al. 1989). Here, to define the Ca^{2+}-dependence of BK activation in patches from RIN cell membranes, ensemble average currents were generated with 10 and 60 \( \mu \)M Ca^{2+} at different command potentials before and after removal of inactivation by trypsin. For comparison, similar currents were generated for BK channels in patches from HIT cell membranes. Peak current was converted to estimates of normalized membrane conductance assuming a 0-mV reversal potential. In Fig. 7A, G-V curves calculated from the peak current values for BK_k channels in RIN cell patches and BK_i channels in HIT cell patches are plotted for 10 and 60 \( \mu \)M Ca^{2+}. Increases in [Ca^{2+}], result in the typical leftward shift of the G-V curve to more negative potentials. At 10 \( \mu \)M Ca^{2+}, values for \( V_{0.5} \) were 42.9 and 22.7 mV for RIN and HIT cells, respectively. At 60 \( \mu \)M Ca^{2+}, values for \( V_{0.5} \) were ~47.5 and ~37.3 mV for RIN and HIT cells, respectively. The HIT cell G-V curves were somewhat more voltage dependent than the RIN cell curves with values for voltage dependence given in the legend.

Because of the inactivating nature of the BK_i channels, inactivation before the time of peak current may differ at different activation potentials, thereby distorting the shape of the true G-V relationship. Therefore, in a separate set of patches, G-V curves were determined with 10 \( \mu \)M Ca^{2+} both before and after removal of inactivation by trypsin. The \( V_{0.5} \) before trypsin was indistinguishable from the previous set of patches (45.0 mV) and, after removal of inactivation by trypsin, the \( V_{0.5} \) was shifted to 11.8 mV. As indicated by the values given in the figure legend, removal of inactivation by trypsin also resulted in an increase in the steepness of the G-V curves. The G-V curves before and after trypsin are compared with the G-V obtained for the set of HIT cell patches in Fig. 7B. These results do not allow us to determine whether this shift after trypsin application is due simply to removal of inactivation or to some other effect of trypsin on BK gating. In previous work, no clear effect of trypsin on activation of BK_k channels was observed for HIT cell patches.
observed (Solaro et al. 1995). Trypsin also was introduced into three HIT cells to test for nonspecific effects on BK gating. In each case, the \( V_{0.5} \) after trypsin was within 10 mV of the \( V_{0.5} \) before trypsin, with a noticeable increase in leak current in each case.

Overall, the differences seen here between the \( G-V \)s measured for HIT cells and HIT cells are rather minor in comparison with the large differences reported in earlier work (Eddleston et al. 1989). In fact, the \( G-V \)s at 60 \( \mu \)M are essentially indistinguishable, whereas at 10 \( \mu \)M the HIT cell \( G-V \)s measured either with inactivation intact or inactivation removed bracket the \( G-V \) obtained from the HIT cell patches. Thus on balance, any differences in the intrinsic \( Ca^{2+} \) dependence of activation between the \( BK_a \) channels of the HIT cells and the \( BK_a \) channels of the HIT cells seem inconsequential.

CTX-sensitivity of \( BK_a \) channels in RIN cells is less than of \( BK_a \) channels in HIT cells

Earlier studies have indicated that noninactivating BK channels in beta cells (Kukuljan et al. 1991) exhibit a CTX sensitivity comparable with that of other systems. Because BK channels in chromaffin cells exhibit a reduced sensitivity to CTX (Ding et al. 1998), inactivating BK channels in RIN cells also may exhibit a reduced sensitivity to CTX that might complicate interpretation of previous results with CTX. To address this issue, BK current was elicited in whole cell recordings by voltage steps to +90 mV with 10 \( \mu \)M pipette \( Ca^{2+} \). This protocol activates a prominent transient outward current and a sustained voltage-dependent K+ current. In the absence of pipette \( Ca^{2+} \), any inactivating voltage-dependent outward current decays with a much slower time course than the inactivating current in the presence of 10 \( \mu \)M pipette \( Ca^{2+} \). Thus the rapidly inactivating current appears to be solely BK current. Application of CTX produces a reduction of the transient outward current with small or negligible effects on the sustained outward current (Fig. 8A). The time course of onset of block by CTX and recovery from block were fit with a simple blocking model (see METHODS) to provide an estimate of the apparent IC\(_{50} \) of block (Fig. 8B). Assuming that the persistent noninactivating current defines the zero BK current level, the example shown results in an IC\(_{50} \) of 17.9 nM. This estimate is largely determined by the time course of block onset and recovery and less influenced by the steady-state level of block. For five RIN cell channels with macroscopic inactivation time constants of ±30 ms, the mean IC\(_{50} \) was 27.9 ± 9.5 nM. Figure 8C illustrates a similar experiment from a HIT cell. No transient outward current is observed with 10 \( \mu \)M pipette \( Ca^{2+} \). CTX produces a more rapid block of outward current. For the HIT cells, the steady-state level of block with 100 nM CTX was used to estimate the zero BK current level. From a fit to the time course of onset and recovery from block by 20 nM CTX, the IC\(_{50} \) for blockade of this HIT cell BK current was ±2.4 nM. For four HIT cells, the IC\(_{50} \) was 2.7 ± 0.2 nM. Thus BK current in HIT cells is more resistant to blockade by CTX than BK current in HIT cells, although the procedure yields less certainty about the exact concentration dependence of the RIN cell BK current.

Activation of BK current and its participation in electrical activity of RIN cells

Comparison of Fig. 8, A and C, suggests that with 10 \( \mu \)M pipette \( Ca^{2+} \), the peak amount of BK current activated in RIN and HIT cells is comparable. However, on average this is not the case. For eight RIN cells studied with the method of Fig. 8, peak BK current in RIN cells at +90 mV was 254.2 ± 124.0 pA/pF. In contrast, for four HIT cells, peak BK current was 640.9 ± 161.8 pA/pF. Given the different properties of activation of BK channels between RIN and HIT cells, we next wished to address two issues: the extent to which \( Ca^{2+} \) influx may trigger BK current activation in the two cell types and the extent to which BK channels may participate in controlling normal electrical activity.

As a first step, we examined the ability of \( Ca^{2+} \) influx to elicit BK current activation in RIN cells. A protocol was used in which \( Ca^{2+} \) influx was first elicited by a voltage step to +10...
Ca	extsuperscript{2+} influx, and currents were activated by a step to +90 mV after a hyperpolarization to (-)140 mV, Cs	extsuperscript{+} (20 mM) was included in the pipette to reduce voltage-dependent K	extsuperscript{+} current and BK current. A: currents are shown before, during, and after application of 100 nM CTX. Note that CTX did not reduce steady-state current at all, indicating that in this cell, only inactivating current is CTX sensitive. With 0 pipette Ca	extsuperscript{2+}, these inactivating currents were not observed. 4-aminopyridine (4-AP) was included in the extracellular saline to block inactivating voltage-dependent K	extsuperscript{+} current. B: time course of current reduction and recovery from CTX block is plotted and fit with a simple first-order blocking scheme (Saito et al. 1997), yielding an effective IC	extsubscript{50} for CTX block of 17 nM. Time constants of block and recovery strongly define the IC	extsubscript{50}. For a set of 5 cells, the IC	extsubscript{50} defined by this method was 27.9 ± 9.5 nM (mean ± SD). Estimates of CTX blockade for noninactivating BK channels from chromaffin cell yields an IC	extsubscript{50} of ~2 nM. These results suggest that BK	extsubscript{i} current in RIN cells is ~5-fold less sensitive to CTX than typical noninactivating BK currents.

A similar experiment for a HIT cell is shown in Fig. 9. In the RIN cells, either removal of Ca	extsuperscript{2+} or addition of CTX produced only small changes in the total outward current activated during normal action potential waveforms. Yet it is clear that there is some Ca	extsuperscript{2+}-sensitive current and CTX-sensitive current that are activated both during the upstroke of the waveform, recorded from a RIN cell, was used as a voltage-clamp command (Fig. 10C) both for RIN cells (Fig. 10A) and for HIT cells (Fig. 10B). It should be noted that we could not elicit directly any action potentials in HIT cells using depolarizing voltage steps, presumably because of the minimal voltage-dependent Na	extsuperscript{+} current present in these cells (see Fig. 9B). During action potential clamp experiments, inward Na	extsuperscript{+} current was blocked with TTX; apamin and 4-AP were used to block different components of outward current. Currents resulting from the action potential clamp waveform were recorded both with and without 1.8 mM extracellular Ca	extsuperscript{2+} (Fig. 10, Aa and Ba) and with and without 100 nM CTX (Fig. 10, Ac and Bc). Despite the resistance of BK	extsubscript{i} current in RIN cells to CTX, 100 nM CTX should block most BK	extsubscript{i} current. Difference currents between control and 0 Ca	extsuperscript{2+} traces provide an approximation of the current resulting solely from Ca	extsuperscript{2+} and BK channels (Fig. 10, Ab and Bb). Difference currents between control and CTX traces provide an approximation of the BK current activated during individual action potentials (Fig. 10, Ad and Bd). Subtraction of the BK current from the traces containing Ca	extsuperscript{2+} and BK currents yields an estimate of the time course of Ca	extsuperscript{2+} current during the action potential waveform (Fig. 10, Ae and Bc).

In this experiment, we used 2 mM extracellular Ca	extsuperscript{2+} for the RIN cell (Fig. 9A) and for HIT cells (Fig. 10A). A step to +10 mV was used to activate Ca	extsuperscript{2+} influx. A subsequent step to +90 mV then was used to terminate Ca	extsuperscript{2+} influx while strongly favoring BK channel activation. Top: currents in the presence and absence of 2 mM extracellular Ca	extsuperscript{2+} is shown on the bottom. A step to +10 mV was used to activate Ca	extsuperscript{2+} influx. A subsequent step to +90 mV then was used to terminate Ca	extsuperscript{2+} influx while strongly favoring BK channel activation. Top: currents in the presence and absence of 2 mM extracellular Ca	extsuperscript{2+}. Middle: difference currents obtained by subtraction of the 0 Ca	extsuperscript{2+} trace from the trace obtained in 2 mM Ca	extsuperscript{2+}. Thus during the step to +90 mV, a transient Ca	extsuperscript{2+} and voltage-dependent current is activated. However, at +10 mV Ca	extsuperscript{2+}-independent, voltage-dependent K	extsuperscript{+} current remains the primary contributor to outward current in the RIN cells. B: identical voltage-protocol was used to evoke currents in a HIT cell. HIT cells express a robust Ca	extsuperscript{2+} and voltage-dependent current, which in most cells is the predominant outward current, even at +10 mV. In both A and B, perforated-patch clamp recordings were used. Note that for the RIN cell (A) a brief, transient inward current is observed at the onset of the step to +10 mV that is absent in the HIT cell (B). This reflects a difference in Na	extsuperscript{+} current between the 2 cell lines used here.
the action potential and during the action potential repolarization. This is revealed most clearly in the difference currents between the control and test conditions (for removal of Ca$^{2+}$, Fig. 10Ab; for CTX, Fig. 10Ad). This argues that some BK current can be activated during normal RIN cell action potentials, although the BK current is only a minor contributor to the total outward current. However, in marked contrast to the RIN cells, there is a robust activation of BK current in HIT cells using the same action potential clamp waveform. Furthermore in HIT cells BK current appears to be the major contributor to outward current during the action potential. Interestingly, despite the differences in BK current activation between the RIN and HIT cells shown in Fig. 10, the amount and time course of Ca$^{2+}$ current activation in the two cases is remarkably similar (Fig. 10, Ae and Be). Results similar to those shown in Fig. 10 were obtained from two other RIN cells and four other HIT cells. In both cell types, the total amount of Ca$^{2+}$ current activated during action potential waveforms was similar.

To examine the role of BK current activation in normal electrical activity, two procedures were used: first the effect of CTX on glucose-induced action potential firing in RIN cells and second the effect of CTX on depolarization-elicited action potentials. Although previous work has shown that 10 and 20 nM CTX has little effect on such activity (Kukuljan et al. 1991), we wanted to determine whether a more complete block of BK current might reveal some role of BK current. As a consequence, after 20 mM glucose was used to elicit burst activity in RIN cells (Fig. 11A), 200 nM CTX was applied for 5 min. Similar to previous work (Kukuljan et al. 1991), we observed no significant effect of CTX on electrical activity initiated by glucose application. In Fig. 11B, an expanded time base trace of action potentials with glucose alone and after CTX application reveal no clear differences. Furthermore, the peak action potential amplitude and the peak afterhyperpolarization after individual action potentials were unaffected by CTX (Fig. 11C). Similar results were obtained in four cells.
although in two cases slight effects on afterhyperpolarization amplitude were observed.

Action potentials also were elicited by sustained depolarizing current injections (Fig. 10D). Such depolarizations elicited action potentials at frequencies of up to ~5–10 Hz. A 5-min application of 100 nM CTX produced no clear alteration in the electrical activity. Specifically, firing frequency was relatively unchanged, and there was no change in the properties of afterpolarizations following action potentials. In HIT cells, depolarizing current injection failed to elicit any action potentials.

**Slo splice variants in HIT and RIN cells**

BK channels are encoded by the *Slo* gene product (Butler et al. 1993), and RNA from rat chromaffin cells contains an alternative splice *Slo* variant encoding a unique 59-amino-acid cysteine-rich domain (Saito et al. 1997). This alternative splice variant to the *Slo* splice site 2 is similar to a variant also found in human pancreatic islets (Ferrer et al. 1996), although some amino acid differences occur between the two forms. Because of the presence of the 59-amino-acid insert in chromaffin cells, a possible role of this insert in contributing to the inactivating phenotype has been considered (Saito et al. 1997). Because RIN and HIT cells exhibit contrasting BK current phenotypes, we wished to determine whether the presence or absence of the 59-amino-acid insert might correlate with a particular current phenotype.

Here, we therefore have examined splice site 2 variants in RIN and HIT cells. Primer set *Slo* 3,4 flanking the splice site 2 region was used first to generate PCR products from RIN cell, HIT cell, and rat chromaffin cell RNA. Products were separated on agarose gels. From all three RNA sources, bands of ~130 bp were observed; this corresponds to the expected size of the splice site 2 variants containing either no added amino acids or a 3-amino-acid, IYF, insert found in brain and endocrine cells (Adelman et al. 1992; Butler et al. 1993; Tseng-Crank et al. 1994). In addition, in all three cell types, a single or pair of bands at ~310 bp was observed. The 310-bp bands correspond approximately to those expected for the previously observed 59-amino-acid insert. The resulting 310-bp band from RIN cells therefore was subcloned and multiple clones subsequently were sequenced. In 21 of 25 subclones from RIN cells, the resulting subclone encoded an amino acid sequence identical to that of the insert found in rat adrenal chromaffin cells. Furthermore, in 4 of the 25 clones from the RIN cells, a second splice variant of 62 amino acids was observed (Table 1). An identical insert also has been reported in rat adrenal chromaffin cells (Xie and McCobb 1998). The splice site 2 variation also was examined further using a second set of primers (*Slo* 14,15) flanking the splice site. These primers yielded bands of ~222 and 402 bp, corresponding approximately to the sizes expected for the null insert and the 59- or 62-amino-acid insert. The 402-bp band obtained from HIT cells was subcloned further to determine whether multiple variants of similar size might be present in the HIT cells. In eight subclones, all encoded the 59-amino-acid insert, which differed in 3 amino acids from the rat 59-amino-acid insert (Table 2). No variant corresponding to the 62-amino-acid insert was found in the HIT cells. The presence of the 59-amino-acid insert in both RIN and HIT cells suggests that the function of this insert is unlikely to be related to the ability of the BK channel to inactivate.

**DISCUSSION**

*Inactivating BK current in beta cells*

The present results establish that rat insulinoma tumor cells express a large-conductance, 
Ca\(^{2+}\) and voltage-dependent BK-type K\(^{-}\) channel, which exhibits rapid inactivation. This inactivating BK channel, termed BK\(_{\text{i}}\), is the predominant BK variant expressed in the RIN cells we have studied. In contrast, HIT cells express exclusively a noninactivating BK\(_{\text{n}}\) variant.

Several aspects of the results are of particular significance. First, the presence of the inactivating BK variant in RIN cells establishes that the BK\(_{\text{i}}\) phenotype may be present in a variety of cell types and have broader functional significance than yet has been appreciated. Second, once inactivation is taken into account, the BK channel in HIT cells appears to be noninactivating because of the presence of a 59-amino-acid insert that confers a new inactivation phenotype.

**Table 1. Splice site 2 variants in RIN and HIT cells**

<table>
<thead>
<tr>
<th>Insert Size</th>
<th>Upstream Residues</th>
<th>Insert</th>
<th>Downstream Residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 aa</td>
<td>... RRL</td>
<td>—</td>
<td>EDE...</td>
</tr>
<tr>
<td>3 aa</td>
<td>... RRL</td>
<td>IYF</td>
<td>EDE...</td>
</tr>
<tr>
<td>59 aa</td>
<td>... RRP</td>
<td>58 aa</td>
<td>EDE...</td>
</tr>
<tr>
<td>62 aa</td>
<td>... RRL</td>
<td>IYS 58 aa</td>
<td>EDE...</td>
</tr>
</tbody>
</table>

Table lists the possible splice site 2 insert sizes in rat insulinoma (RINm5f) tumor and hamster insulinoma tumor (HIT) cells determined in this study.

**Table 2. Amino acid sequence**

<table>
<thead>
<tr>
<th></th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster 59</td>
<td>1</td>
<td>RR—PKMSI</td>
<td>YKRMRRAACCF</td>
<td>DCGRSEDCS</td>
<td>CMSGRVGRGNV</td>
<td>DTLERAFLS</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 59</td>
<td>1</td>
<td>RR—PKMSI</td>
<td>YKRMSRACCF</td>
<td>DCGRSEDCS</td>
<td>CMSGRVGRGNV</td>
<td>DTLERNFPLS</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human 59</td>
<td>1</td>
<td>RR—PKMSI</td>
<td>YKRMRRAACCF</td>
<td>DCGRSEDCS</td>
<td>CMSGRVGRGNV</td>
<td>DTLERNFPLS</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat 62</td>
<td>1</td>
<td>RRRIYSKMSI</td>
<td>YKRMSRACCF</td>
<td>DCGRSEDCS</td>
<td>CMSGRVGRGNV</td>
<td>DTLERNFPLS</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamster 59</td>
<td>51</td>
<td>SVSVNDCSAS</td>
<td>FRAFEDE...</td>
<td>. .</td>
<td>. .</td>
<td>100</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Rat 59</td>
<td>51</td>
<td>SVSVNDCSTS</td>
<td>FRAFEDE...</td>
<td>. .</td>
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<td>100</td>
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</table>

Rat and hamster sequences correspond to inserts identified in RIN and HIT cells in this study. The rat 59-amino-acid-sequence also corresponds to that previously found in rat adrenal chromaffin cells (Saito et al. 1997). The human sequence is from Ferrer et al. (1996).
account, the results indicate that the Ca\(^{2+}\) dependence of activation of HIT and RIN cell BK channels is essentially identical in contrast to the interpretation arising from previous work (Eddlestone et al. 1989). Third, the presence of Slo variants containing a novel 59-amino-acid insert in both RIN and HIT cells indicates that this insert is not strictly associated with the inactivating BK phenotype. Fourth, the results confirm that BK current in HIT cells may play little role in electrical activity initiated by depolarization or by glucose, although a more substantial role of BK current in HIT cells cannot be excluded. Although the present results do not reveal a specific functional role of BK\(_{\text{i}}\) channels, the possibility must be considered that there may be particular physiological conditions that might increase the relative importance of BK\(_{\text{i}}\) current in some aspect of RIN cell or beta cell excitability.

Why have inactivating BK currents been overlooked in previous studies? Several studies of whole cell Ca\(^{2+}\)-dependent currents in both native and clonal beta cell lines, in fact, have suggested the presence of an inactivating component of Ca\(^{2+}\)-dependent current (Satin et al. 1989; Smith et al. 1990), but the significance of such observations in terms of the behavior of single BK channels never has been described explicitly. At the single-channel level, one reason for difficulty in observing BK\(_{\text{i}}\) variant is the following. Studies with excised patches from beta cell membranes typically have used steady-state measurements of BK activity at single potentials (Cook et al. 1984; Eddlestone et al. 1989; Tabcharani and Misler 1989; Kukuljan et al. 1991). Under such conditions, the recordings will be dominated by noninactivating BK\(_{\text{i}}\) channels because BK\(_{\text{i}}\) channels, if present, already will be inactivated. At positive potentials, the time between reopenings of the BK\(_{\text{i}}\) variant may be many seconds. As a consequence, patches with BK\(_{\text{i}}\) channels may appear to have few BK channels at all or, if there are some noninactivating BK channels present, the BK\(_{\text{i}}\) channels would contribute negligibly to the records. For example, the last 200 ms of the traces in Fig. 6 provide an indication of the steady-state activity of 14 BK\(_{\text{i}}\) channels with 10 \(\mu\)M Ca\(^{2+}\) at +60 mV. Yet under normal resting conditions, BK\(_{\text{i}}\) channels would be available for activation and then could contribute substantially to Ca\(^{2+}\)-dependent current during depolarization.

Although our results suggest that the BK\(_{\text{i}}\) variant is the predominant or only BK variant present in the RIN cells we have used, the situation in normal pancreatic beta cells remains unclear. Both BK\(_{\text{i}}\) and BK\(_{\text{s}}\) channels have been observed in normal rat pancreatic beta cells (Lingle et al. 1996). In addition, other work indicates that noninactivating BK\(_{\text{i}}\) variants are, to some extent, present in either RIN cells (Eddlestone et al. 1989) and neonatal rat beta cells (Cook et al. 1984). It seems possible that particular BK channel phenotypes are associated with particular subtypes of beta cells with particular functional roles. In rat chromaffin cells, each phenotypic variant is segregated largely among different cells, such that \(\sim 15\% - 20\%\) of chromaffin cells express predominantly BK\(_{\text{i}}\) currents while a larger fraction (80%) expresses predominantly BK\(_{\text{s}}\) currents (Ding et al. 1998; Solaro et al. 1995). The functional significance of such segregation remains unknown. If a similar segregation occurred in beta cells, such segregation of particular BK current variants among different cell types might contribute to particular functional roles of a given cell subtype.

Our results also raise the possibility that, even within different subclones or passages of an insulinoma tumor line, BK variants may differ. The earlier work on the RINm5f line (Eddlestone et al. 1989) indicated that the occurrence of BK channels in patches was rare, perhaps consistent with the presence of BK\(_{\text{i}}\) channels in such patches. However, the increase in BK channel open probability versus pCa in the same study using steady-state measurements of channel activity seems more consistent with the presence of at least some BK\(_{\text{i}}\) channels. Thus it probably cannot be excluded that changes in expression of particular proteins may occur in different subclones, leading to some of the differences observed between our work and earlier studies.

Inactivation mechanisms and structural components of BK\(_{\text{i}}\) channels

The properties of inactivation of the BK\(_{\text{i}}\) variant in RIN cells appear indistinguishable from that of the BK\(_{\text{i}}\) variant in rat chromaffin cells. The rates of inactivation and dependence of those rates on voltage and Ca\(^{2+}\) are similar to those from BK\(_{\text{i}}\) channels in the two cell types (Solaro and Lingle 1992; Solaro et al. 1995). The rates of recovery from inactivation and the steady-state availability of BK\(_{\text{i}}\) current for activation at 10 \(\mu\)M Ca\(^{2+}\) are comparable (Ding et al. 1996; Ding and Lingle, unpublished data). The slowing of inactivation by trypsin and the lack of effect of the cystolic channel blocker, QX-314, are also identical for BK\(_{\text{i}}\) currents in the two cell types (Solaro et al. 1997). Thus functionally and mechanistically, the channels in the two cell types seem identical. The present results add no new information about the nature of the underlying inactivation mechanism. Although inactivation appears to involve multiple, trypsin-sensitive cystolic inactivation domains, unlike the inactivating Shaker channel (Choi et al. 1993) no evidence yet supports the idea that the inactivation domains directly occlude the ion permeation pathway.

In addition to the functional similarities of the beta cell and chromaffin cell BK\(_{\text{i}}\) variants, both beta cells and chromaffin cells share expression of an interesting alternatively spliced variant of the Slo subunit that encodes BK channels (Ferrer et al. 1996; Saito et al. 1997). The splice variant found in human islets (Ferrer et al. 1996) differs in two amino acids from that found in rat chromaffin cells (Saito et al. 1997). Our results indicate that the variant found in rat beta cells is, in fact, identical to that found in rat chromaffin cells. Despite the presence of this unique alternative splice variant in RIN cells and chromaffin cells, this variant per se does not result in formation of inactivating BK channels when expressed in Xenopus oocytes (Saito et al. 1997). Furthermore its presence in HIT cells in which no inactivating BK channels have been observed also argues that its function is unrelated to BK channel inactivation.

Significance of BK channels in beta cell function

Secretion of insulin from beta cells can be regulated by modulation of any of a number of physiological targets. For example, reduction of Ca\(^{2+}\) current (Satijn et al. 1994), pharmacological activation of K-ATP channels (Dukes and Phillipson 1996; Ribalet et al. 1988), or alteration of nucleotide metabolism (Nichols et al. 1996) are just three of many ways that the secretion of insulin can be regulated. Earlier work has failed to define a critical physiological role of BK current in
beta cell function (Kukuljan et al. 1991). The present results raise the possibility that the roles of BK channels may have been overlooked because a key functional property of beta cell BK channels, namely inactivation, has not been considered.

The present results do, in fact, show that BK current is, to some extent, activated during action potential clamp waveforms. However, in RIN cells BK current remains only a minor contributor to total outward current activated during action potentials and action potential repolarization. Thus our results are generally consistent with previous results from RIN cells, which suggest that the role of BK current is minor. The relatively minor role of BK current in RIN cell excitability also is underscored by our results with CTX. Despite the relatively weaker sensitivity of BK current in RIN cells to CTX, in agreement with earlier work (Kukuljan et al. 1991) our results indicate that blockade of BK current by 100 nM CTX, a concentration sufficient to block >90% of either inactivating or noninactivating BK current, has only minor effects on depolarization-elicited repetitive firing in RIN cells. Similarly, 100 nM CTX has no clear effect on action potential firing initiated by glucose.

In contrast to RIN cells, a robust BK current can be activated by action potential waveforms in HIT cells. Although we did not observe either spontaneous action potentials or depolarization-elicited action potentials in the HIT cells we have examined, spontaneous action potentials in HIT cells have been observed by others (Kcahey et al. 1989). Our results would suggest that BK current could be the primary repolarizing current in such cells. The differences in BK current activation by action potential waveforms between HIT and RIN cells seem somewhat surprising given that the BK current densities do not seem profoundly different. Furthermore although we have not examined this point in detail, the relative amounts of Ca$^{2+}$ currents in the two cells are similar when activated under identical conditions (e.g., Fig. 10). The difference between HIT and RIN cells in the ability of comparable amounts of Ca$^{2+}$ influx to activate BK current raises the possibility that there may be some difference in how Ca$^{2+}$ influx is coupled to BK current activation. One possible explanation might be that the resting inactivation of BK current in RIN cells under perforated-patch conditions is severe, such that the BK current available for activation in RIN cells is quite small. However, this seems an unlikely explanation, because at normal resting Ca$^{2+}$ levels BK current inactivation is minor. Another possible explanation is that some differences may exist between the two cell lines in how tightly coupled BK channels are to sites of Ca$^{2+}$ influx. For example in rat adrenal chromaffin cells, activation of BK current during action potential repolarization appears to require selective coupling of BK channels to particular Ca$^{2+}$ channel variants, specifically L-type Ca$^{2+}$ channels (Prakriya et al. 1996; M. Prakriya and C. J. Lingle, unpublished results). Thus a difference in the ability of particular Ca$^{2+}$ channels to colocalize with BK channels between HIT and RIN cells might underlie the large differences in the amount of Ca$^{2+}$- and voltage-dependent K$^{+}$ current activated by action potential waveforms.

There is some precedence from work on beta cells for the idea that Ca$^{2+}$ influx through particular Ca$^{2+}$ channel subtypes may be linked to particular Ca$^{2+}$-dependent targets. Specifically, Ca$^{2+}$ influx through L-type Ca$^{2+}$ channel variants appears to be tightly coupled to the secretory machinery in mouse pancreatic beta cells (Bokvist et al. 1995). Thus selective coupling of particular Ca$^{2+}$ channel subtypes to particular Ca$^{2+}$-dependent targets may be a critical factor contributing to normal secretory cell function. In beta cells, such coupling may play an important role in maintenance of the normal stimulus-secretion process.

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