J. Membrane Biol. 112, 213–222 (1989)

The Journal of
Membrane Biology

Expression of a Rapid, Low-Voltage Threshold K Current in Insulin-secreting Cells is Dependent on Intracellular Calcium Buffering

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Summary. Depolarization-activated outward currents ranging in amplitude from 100–1000 pA were studied in cultured, insulin-secreting HIT cells and mouse B-cells using the whole-cell patch clamp. Outward current was identified as a K current since it was blocked by K channel blockers and its tail current reversed near E_K. The K currents of HIT cells dialyzed with internal solutions containing 0.1–10 mM EGTA with no added calcium (Ca), or 10 mM EGTA with 2 mM added Ca, activated rapidly with depolarization. However, the stronger Ca buffer BAPTA (5 mM; no added Ca) blocked the rapidly activating current to reveal an underlying more slowly activating K current. With intracellular EGTA, application of the Ca channel blocker cadmium mimicked the effect of intracellular BAPTA. These data suggest that the rapid K current was mediated by low-voltage threshold, Ca-activated K channels while the slower K current was mediated by high threshold delayed rectifier K channels. Mouse B-cells also had both K current components. Dialyzing these cells with either BAPTA (5 mM, no added Ca) or high EGTA (10 mM with 2 mM Ca) blocked the rapid Ca-activated K current observed when cells were filled with 0.1 to 1 mM EGTA. It is concluded that the extent of Ca-activated K current activation in either HIT or adult mouse B-cells depends on the degree of intracellular Ca buffering.

Key Words calcium-activated potassium channels · delayed rectifier · pancreatic islet cells

Introduction

The outward K current of insulin-secreting cells appears to be mediated by three classes of K-selective channels: (*i*) ATP-sensitive K channels which are insensitive to membrane voltage but are blocked by metabolites of glucose (Ashcroft, Harrison & Ashcroft, 1984; Cook & Hales, 1984; Findlay et al., 1985*c*; Rorsman & Trube, 1985; Misler et al., 1986), (*ii*) large conductance Ca-activated K channels which are activated by both membrane depolarization and intracellular Ca (Cook, Ikeuchi & Fujimoto, 1984; Findlay, Dunne & Petersen, 1985*a*,*b*; Findlay et al., 1985*c*) and (*iii*) depolarization-activated delayed rectifier channels (Rorsman & Trube, 1986; Misler et al., 1988; Zunkler, Trube & Ohno-Shosaku, 1988). While the ATP-sensitive channels appear to mediate the glucose-dependent subthreshold depolarization (Ashcroft et al., 1984; Rorsman & Trube, 1985; Misler et al., 1986) as well as the modulation of suprathreshold electrical activity (Bozem & Henquin, 1988; Cook & Ikeuchi, 1989) the role of the calcium-activated and delayed rectifier currents is less clear.

Although the Ca-activated K channels are well suited to repolarize the B-cell Ca spike (Ribalet & Beigelman, 1980; Bangham, Smith & Croghan, 1986) as in other systems (Brehm, Dunlap & Eckert, 1978; Adams, Brown & Constanti, 1982*a*; Adams et al., 1982*b*, 1985; Lancaster & Nicoll, 1987), recent whole-cell patch-clamp studies suggest that Ca-activated K channels contribute little to the macroscopic K current of B-cells (Rorsman & Trube, 1986; Trube & Rorsman, 1986; Zunkler et al., 1988). However, these studies were carried out by dialyzing the intracellular compartment with a mixture of 10 mM EGTA and 2 mM Ca which might buffer intracellular free Ca so strongly as to uncouple Ca influx from Ca-activated K channel activation.

To test this possibility, we dialyzed two types of cultured insulin-secreting cells, monoclonal HIT cells and adult mouse B-cells, with Ca buffer solutions of various strengths and examined their K currents. When depolarization-dependent intracellular Ca accumulation was prevented by buffering intracellular Ca strongly or by blocking Ca influx with Cd, we blocked a low-threshold, rapidly activating Ca-activated K current to reveal a higher threshold, more slowly activating delayed rectifier current. The relative contribution of each current to the total voltage-dependent K current depended on the degree of intracellular calcium buffering. While EGTA

(to 10 mM) was less effective than 5 mM BAPTA in HIT cells, 10 mM EGTA uncoupled Ca-activated K current activation from Ca influx in mouse cells to the same extent as 5 mM BAPTA.

A preliminary account describing some of these findings has appeared (Satin, 1989).

Materials and Methods

Cell Culturing

HIT cells (Santerre et al., 1981) were obtained from Dr. Robert Santerre of Eli Lilly at passage 47 and maintained in Ham's F12 medium. Cells used were typically from passages 58–80.

Adult mouse islet B-cells were cultured by isolating islets from the pancreases of adult female Swiss-Webster mice using collagenase digestion. Islets were digested in a zero calcium buffer to isolate single cells. Suspensions of single cells were then plated on polylysine-coated glass cover slips and cultured for one week or less. Direct immunostaining for insulin revealed that >80% of these cells were B-cells (for further details, *see* Satin & Cook, 1988).

Patch-Clamp Techniques

Single cells were visualized using an inverted microscope and whole-cell clamped at room temperature (Hamill et al., 1981; for details, *see* Satin & Cook, 1988). Junction potentials were nulled by an auto search circuit while the pipette was immersed in bathing medium. Since the outward currents were typically >200 pA in amplitude, series resistance compensation was used in most experiments. Following establishment of a giga-seal (typically >10 GΩ) whole-cell mode was commenced by applying negative pressure to the pipette. Voltage pulses used to elicit depolarization-activated K current or to determine current-voltage relations ($I - V$s) lasted 40 msec and were applied at 0.2 Hz, as described previously (Satin & Cook, 1988, 1989). Most of the data were collected within 10 min of establishing the whole-cell configuration to avoid wash out of Ca current in dialyzed cells (Satin & Cook, 1989). In many cells, the total outward current often slowly increased during the course of the experiment. Part of this slow increase is likely to be due to an increase in ATP-inhibited K current resulting from changes in submembrane ATP (Rorsman, Trube & Ohno-Shosaku, 1986) while the appearance of a nonlinear leakage current which grows in amplitude during whole-cell experiments has also been described in insulin-secreting cells (HIT cells: Satin & Cook, 1988, 1989; adult rat B-cells: Hiriart & Matteson, 1988; adult mouse B-cells: Plant, 1988).

Data were filtered at 1–2 kHz using an 8-pole Bessel filter (Frequency Devices, Haverhill, MA), digitized at 1–2 kHz and analyzed as described previously (Satin & Cook, 1988). The fractional block of the K current produced by Cd was determined as the ratio of the peak "difference current" (obtained by digitally subtracting the K current in Cd from control current) to the isochronal control current. Currents were linear leak subtracted as described previously (Satin & Cook, 1988).

Group comparisons were made using Student's t test (2 groups) or Peritz' F-test (>2 groups; Einot & Gabriel, 1975). All values are expressed as means ± SEM.

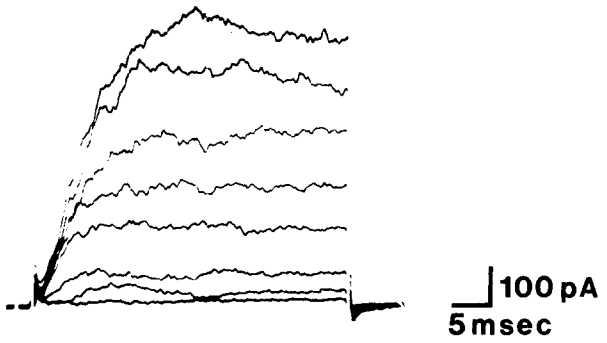

Fig. 1. Macroscopic outward currents of voltage-clamped HIT cells. Currents were elicited by progressively depolarizing a HIT cell from a holding potential of −100 mV to (bottom to top) −20, −10, 0, +10, +20, +30, +40, and +50 mV for 40 msec. Net outward current was apparent at −10 mV and increased in amplitude with further depolarization. The pipette contained 1 mM EGTA and 0 Ca. In this, and all subsequent figures, the data traces shown are uncompensated for linear leakage and capacity currents. The holding potential used in all experiments was −100 mV

Solutions

External solutions had the following composition (in mM): 120 NaCl, 5 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 11.1 glucose, and 10 HEPES; pH 7.2. The presence or absence of TTX did not affect outward currents. Tetraethylammonium (TEA) or cadmium (Cd) were directly dissolved in the external solutions. In the high-K experiments, external K replaced Na. Mg was substituted for Ca in nominally Ca-free saline to keep total divalents constant. Internal solutions contained (in mM): 114 KOH; 114 aspartic acid; 20 KCl; 20 HEPES; pH 7.2; 2 $MgCl_2$; 0–2 adenosine triphosphate (ATP); and 0.1–1 ethylene glycol *bis* (β-amino ethyl-ether)-N,N′ tetraacetic acid (EGTA; Sigma, St. Louis, MO) or 5 K_4-(1,2-*bis*(o-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid (BAPTA; Molecular Bioprobes, Junction Pass, OR). The 10 EGTA/2 $CaCl_2$ solution contained (in mM): 32 KOH, 125 KCl, 5 HEPES, pH 7.2, 4 $MgCl_2$, and 3 ATP (Rorsman & Trube, 1986). The concentration of free Ca in these internal solutions was calculated using the methods of Fabiato and Fabiato, 1979 or Hagiwara and Nakajima, 1966, which yielded similar numbers. With no Ca added, contaminating Ca was assumed to be <30 μM. Thus, in 0.1 EGTA/0 Ca, free [Ca] ≈ 50 nM; in 1 EGTA/0 Ca, free [Ca] ≈ 5 nM; in 10 EGTA/2 Ca, free [Ca] ≈ 50 nM; and in 5 BAPTA/0 Ca, free [Ca] < 1 nM (K′ obtained from Tsien, 1980). The estimated free-EGTA concentrations of solutions containing 0.1 EGTA/0 Ca, 1 EGTA/0 Ca or 10 EGTA/2 Ca were 0.07, 0.90 and 7.40 mM, respectively.

Results

Properties of the Outward Current of HIT Cells

Depolarizing HIT cells for 40 msec from a holding potential of −100 mV to potentials beyond about −20 mV elicited rapidly activating (≤20 msec) outward currents which increased in amplitude with depolarization. In the example shown in Fig. 1, ob-

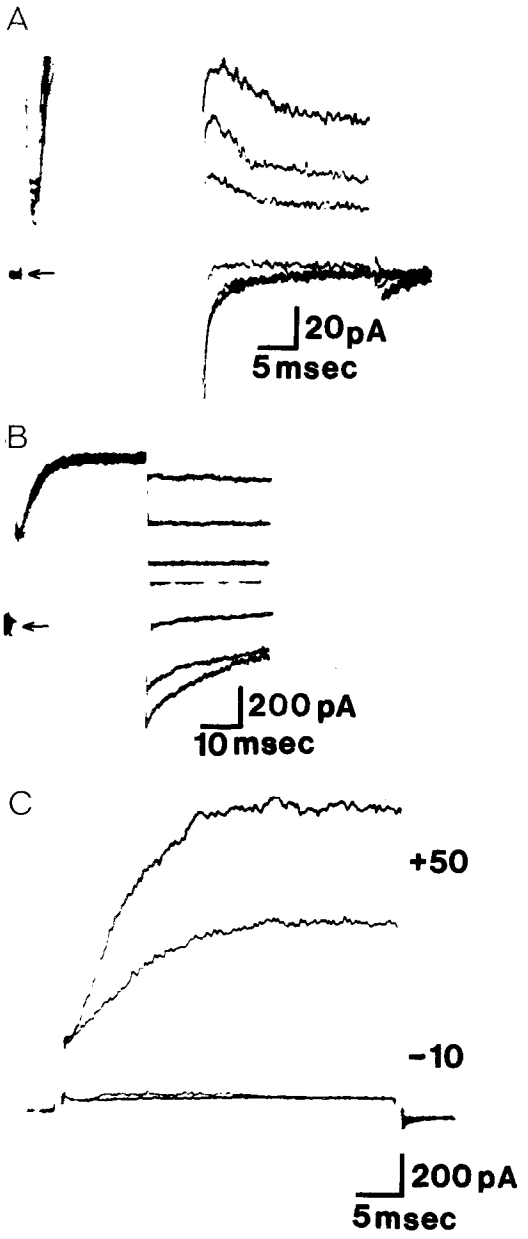

Fig. 2. Outward current in HIT cells is mediated by K channels. (*A*) Tail currents elicited in normal external K (5 mM) reverse near E_K (= −83 mV). Cell was depolarized to +50 mV for 25 msec to activate a large depolarization-activated outward current and was then stepped back to (top to bottom): −10, −20, −30, −60, −90, and −100 mV. The tail current reversed between −60 and −90 mV. The arrows shown here and in (*B*) indicate the baseline holding current at −100 mV. Pipette contained 0.1 mM EGTA. (*B*) Tail currents elicited in high external K (110 mM) also reversed near E_K (= −5 mV). Cell was depolarized to +50 mV for 30 msec and was then stepped back to (top to bottom): +40, +20, 0, −10, −30, −60, and −80 mV. We determined the reversal potential of the tail current (= −10 mV) as the intercept of the tail and leak *I-V*s in order to eliminate the contribution of the typically large leak currents observed in high external K. The leak *I-V* was measured from the amplitudes of the current jump elicited by 40-msec commands to various voltages. Pipette contained 1.0 mM EGTA. The data obtained in (*A*) and (*B*) were from different cells. (*C*) Outward current in HIT cells is sensitive to external TEA (500 μM). TEA application reduced the amplitude of the outward current at +50 mV (top pair of traces) by about 50% while abolishing the small inactivating outward current at −10 mV (bottom pair of traces). TEA also reduced the amplitude of the current noise observed at +50 mV. The large initial current step elicited by the command to +50 mV, which was not affected by TEA, probably resulted from the activation of a nonlinear leakage current which we described previously (*see* Materials and Methods, and Satin & Cook, 1988). Pipette contained no EGTA

tained using a pipette containing 1 mM EGTA, outward current was apparent at −10 mV and partially decayed within 40 msec (*see also* Figs. 2*C* and 4*A*). Stronger depolarizing commands elicited larger outward currents that inactivated to a lesser degree. Outward currents were often very noisy, as expected if large conductance Ca-activated K channels were contributing to the current (Marty & Neher, 1985). Early outward current (measured at 10 msec) at +10 mV averaged 365 ± 61 pA (mean ± SEM, n = 13; cells filled with either 0.1 or 1 mM EGTA).

Evidence That Outward Current Was Carried By K Flux Through K Channels

Brief tail currents, consisting of ionic and capacitative components, were observed on stepping to various membrane potentials following depolarizing commands to +50 mV in normal (5 mM) external K (Fig. 2*A*). Repolarizing to potentials more negative than E_K (i.e., calculated E_K = −83 mV) produced inward tail currents while repolarizing to voltages more positive than E_K (−60 to −10 mV) produced outward tail currents. Furthermore, raising external K to 110 mM shifted E_K to −5 mV and the tail current reversal potential to about −10 mV (Fig. 2*B*), as expected of a K-selective tail current. The tail currents were carried by the same channels activated by depolarization since they increased with outward current amplitude as pulse durations increased from 5 to 40 msec (*data not shown*). Similar results were reported in cultured adult mouse B-cells (Rorsman & Trube, 1986).

Complete replacement of internal and external K with Cs (which is poorly permeable through most K channels; Hille, 1984) together with external application of the K channel blockers tetraethylammonium (or TEA; 10 mM) and internal 4-aminopyridine (4-AP; 5 mM), blocked all outward current to reveal inward Ca currents (Satin & Cook, 1988, 1989). Bath application of a low concentration of TEA (500 μM) reduced a significant fraction of the outward current (Fig. 2*C*). These findings demonstrate that the outward current was due to K current flow through K channels.

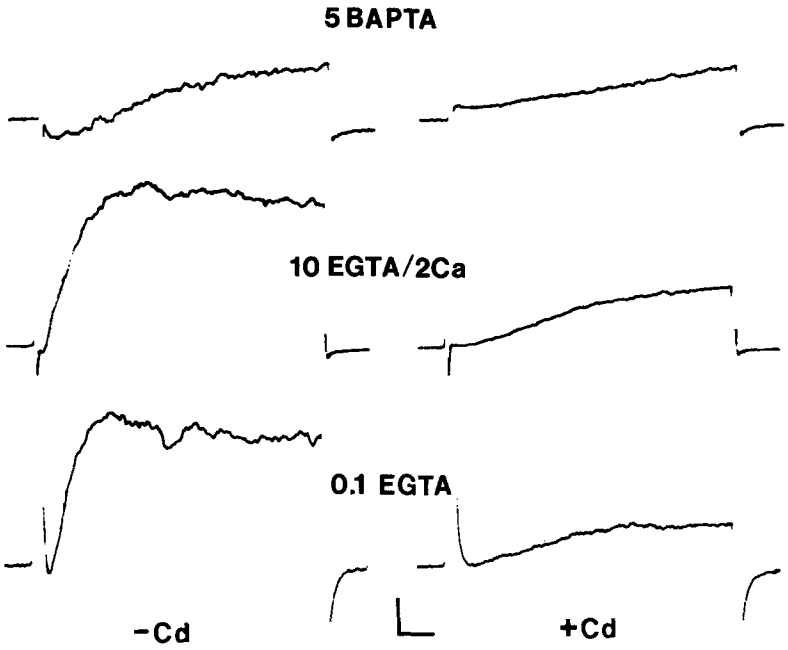

Fig. 3. Effect of varying intracellular Ca buffering on the kinetics and Cd-sensitivity of HIT cell K current. K current was elicited by depolarizing HIT cells from −100 to +10 mV for 40 msec. The K currents shown in this figure were obtained from cells dialyzed with solutions containing 0.1 EGTA/0 Ca (lower), 10 EGTA/2 Ca (middle) and 5 BAPTA/0 Ca (top) under control conditions (left column) and in the presence of 200 μM Cd (right column). Under control conditions, intracellular BAPTA but not EGTA blocked the rapidly activating K current to reveal a more slowly activating K current. Cd application to EGTA-filled cells decreased the amplitude of the Ca and K currents and slowed K current activation, while in BAPTA-filled cells, Cd blocked the Ca current but had less effect on the K current. Cd also reduced macroscopic current noise in each condition. The vertical calibration bar indicates 100, 200, and 50 pA (top to bottom, respectively), while for all traces, the horizontal bar indicates 5 msec

Effect of Limiting $[Ca^{2+}]_i$ Accumulation or Ca Influx on HIT Cell K Current

To determine whether Ca-activated K current contributed to the macroscopic K current, the effect of changing intracellular Ca buffers on HIT cell K current was tested. As shown in the left column of Fig. 3, representative K currents elicited by clamp commands to +10 mV from a holding potential of −100 mV activated rapidly when cells were filled with 0.1 EGTA/0 Ca or with 10 EGTA/2 Ca (Fig. 3, left; lower and middle panels, respectively), while the K currents of cells filled with 5 mM BAPTA activated much more slowly (top panel). Thus, internal BAPTA blocked a rapidly activating K current observed with weaker Ca buffering to reveal a more slowly activating component.

We blocked Ca influx through voltage-dependent Ca channels with Cd to further demonstrate that Ca accumulation activated K current (Adams et al., 1982*b*; Mitra & Morad, 1985). Application of 200 μM external Cd to HIT cells dialyzed with 0.1 EGTA/0 Ca or 10 EGTA/2 Ca (Fig. 3, right; lower and middle panels, respectively) blocked both the net inward Ca current (Satin & Cook, 1988) and rapidly activating K current to reveal a residual slow K current as seen with internal BAPTA. Cd also reduced the amplitude of the K current noise. Subtracting Cd traces from control traces showed that the Ca-activated K current was transient. These results suggest that the fast K current was mediated by Ca-activated K channels while the slow K current was mediated by delayed rectifier K channels.

Neither Cd Nor BAPTA Act By Directly Blocking K Channels

It is unlikely that Cd acted by directly blocking Ca-activated K channels rather than Ca influx, since nominally Ca-free solutions, which also blocked Ca influx, reproduced all of the effects of Cd on K current (*data not shown*). Similarly, it is unlikely Cd blocked delayed rectifier channels, since large delayed rectifier currents were observed even at high doses (up to 500 μM Cd). Internal BAPTA did not block delayed rectifier K channels, since delayed rectifier K currents comparable to those observed with Cd were obtained using BAPTA. BAPTA did not block Ca-activated K current by blocking Ca influx through Ca channels, since large Ca currents were present using BAPTA (e.g., Fig. 3 top left; Fig. 5, top left). These findings support our view that Cd and BAPTA acted by limiting Ca influx or accumulation, respectively.

Characterization of the Ca-Activated and Delayed Rectifier K Currents of HIT Cells

In order to characterize the properties of these two K currents, current-voltage relations (*I-Vs*) were obtained in control and 200 μM Cd solutions in cells dialyzed with 0.1 mM EGTA (Fig. 4*A* and *B*). The raw current traces shown in Fig. 4*A* show that Cd application reduced the amplitude and rate of rise of outward K current at all potentials. Early K currents (measured at 10 msec) were plotted as functions of test voltage in Fig. 4*B*. This time was cho-

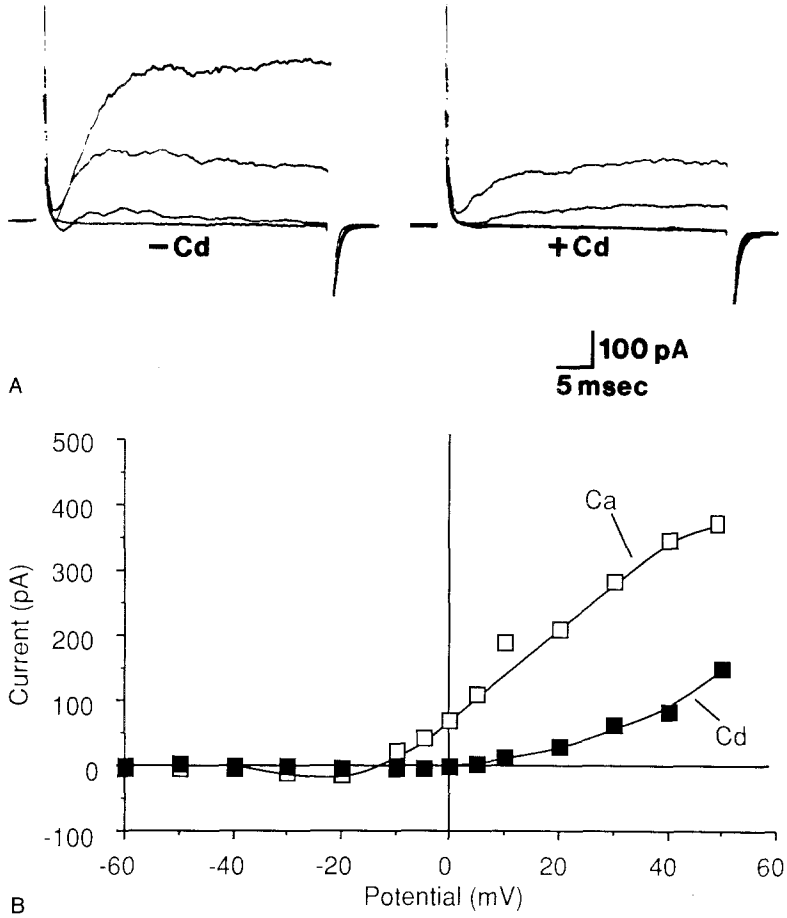

Fig. 4. (*A*) Depolarization-activated outward currents in HIT cells are decreased in amplitude and slowed by Cd. (Left) control ion currents activated by 40-msec depolarizations from a holding potential of −100 mV to (bottom to top) −30, −10, +20, and +50 mV in the absence of Cd. At −30 mV, a steady inward current was elicited while at −10 mV transient inward and outward current components were observed. Depolarization beyond −10 mV elicited outward currents whose amplitude increased with depolarization. Pipette contained 0.1 mM EGTA. (Right) Currents elicited at the same voltages in the presence of 0.2 mM Cd. Cd blocked the inward current at −30 mV and the outward current at −10 mV while reducing the outward currents and their activation rates at +20 and +50 mV. (*B*) Current-voltage relations obtained from HIT cell current amplitudes (measured at 10 msec) for the above data before (open symbols) and after (filled symbols) 200 μM Cd application. Under control conditions, currents were small and inward between −30 and −20 mV and outward at more depolarized potentials. Cd decreased the amplitude of the outward current to reveal a slowly activating K current whose voltage-activation threshold was shifted about 10–20 mV in the depolarized direction

sen because Ca-activated K current is prominent at this time.

The leak-subtracted *I-V* shows that net control current (Fig. 4*B*, open squares) was inward between −50 and −20 mV and outward above −20 mV. The amplitude of the control K current increased nearly linearly as cells were depolarized. Cd (200 μM) reduced inward and outward current amplitudes at between −40 and +50 mV (filled squares). The outward current remaining in Cd activated at more depolarized potentials than under control conditions. Thus, Cd distinguished a fast, relatively low-threshold Ca-activated K current from a slow, relatively high-threshold delayed rectifier K current.

Evidence for Macroscopic Ca-Activated K Currents in Adult Mouse B-Cells

To determine whether strong internal Ca buffering uncouples Ca influx from Ca-activated K current activation in mouse B-cells as in HIT cells, the same solutions and protocols used with HIT cells were used with cultured adult mouse B-cells.

As shown in Figs. 5 and 6, we found that with weak Ca buffering, mouse B-cells had large, voltage-activated K currents which resembled those seen in HIT cells. Early K current (at 10 msec) in mouse cells averaged 249 ± 37 pA (n = 28; combination of all EGTA data). Mouse and HIT cell current amplitudes were not significantly different at the 0.05 level. The left column of Fig. 5 shows representative mouse K currents activated by commands to +10 mV. Like HIT cell K currents, mouse B-cell currents activated rapidly when cells were filled with 0.1 EGTA/0 Ca (lower panel) or 1 mM EGTA/0 Ca (*data not shown*). However, unlike HIT cells, the K currents of mouse cells dialyzed with 10 EGTA/2 Ca (middle panel) activated more slowly and resembled currents observed with 5 mM BAPTA (top panel). Thus, in mouse cells, high EGTA blocked the rapid K current as well as BAPTA.

As in HIT cells, application of 200 μM Cd to cells dialyzed with 0.1 EGTA/0 Ca (Fig. 5 right column, lower panel) blocked rapid, Ca-activated K current to reveal slowly activating K current. However, the current in 10 EGTA/2 Ca or 5 mM BAPTA was slow under control conditions and much less sensitive to Cd blockade (Fig. 5, middle and upper panels), as expected if this current was mediated by delayed rectifier K channels.

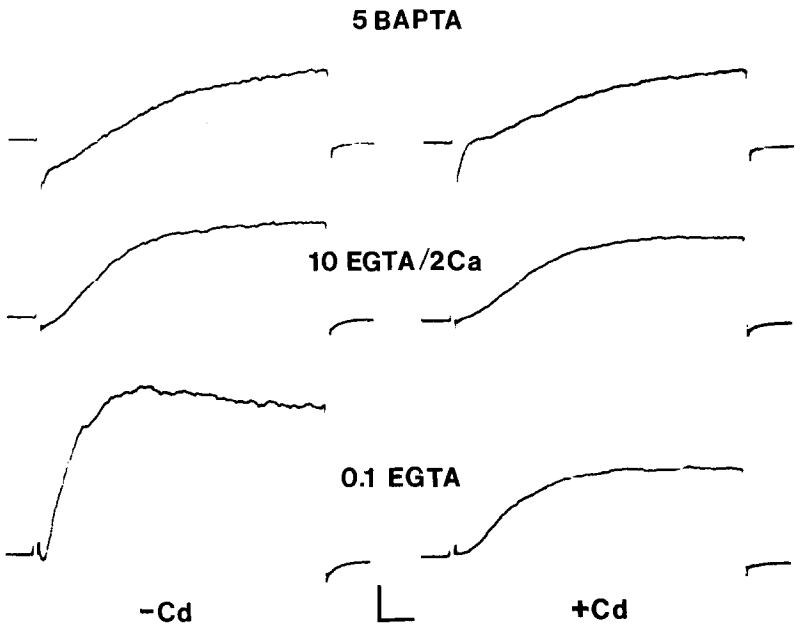

Fig. 5. Effect of varying intracellular Ca buffering on the kinetics and Cd-sensitivity of adult mouse B-cell K current. K current was elicited by depolarizing mouse cells from −100 to +10 mV for 40 msec. The K currents shown in this figure were obtained from cells dialyzed with solutions containing 0.1 EGTA/0 Ca (lower panel), 10 EGTA/2 Ca (middle panel) and 5 BAPTA/0 Ca (top panel) under control conditions (left column) and in the presence of 200 μM Cd (right column). Under control conditions, 5 BAPTA/0 Ca or 10 EGTA/2 Ca blocked a rapidly activating current K current seen when cells were recorded with 0.1 EGTA/0 Ca, to reveal a more slowly activating K current. Cd application to cells filled with 0.1 EGTA/0 Ca decreased the amplitude of the Ca and K currents and slowed K current activation, while in BAPTA or 10 EGTA-filled cells, Cd blocked the Ca current but had much less effect on the K current. The fast inward current which persisted in the presence of Cd was presumably mediated by Na channels (Plant, 1988). As in HIT cells, Cd did not appear to directly block Ca-activated or delayed rectifier K currents. The vertical calibration bar indicates 200, 500, and 200 pA (top to bottom, respectively), while for all traces, the horizontal bar indicates 5 msec

I-V Properties of Mouse Cell K Currents in Control and Cd-Containing Solutions

As in HIT cells, Cd application to mouse cells dialyzed with 0.1 EGTA/0 Ca reduced the amplitude and rate of rise of K current (Fig. 6*A*). The current-voltage relations obtained for early mouse K currents (measured at 10 msec) resembled those obtained using HIT cells under the same experimental conditions (*compare* Fig. 6*A,B* with Fig. 4*A,B*). Thus, under control conditions, early K current (measured at 10 msec) activated above −10 mV and increased with further depolarization. Cd blocked low-threshold K current to reveal high-threshold K current (Fig. 6*A,B*).

Effect of Dialyzing HIT or Mouse B-Cells With Increasingly Strong Ca Buffers

To examine whether increasing Ca buffering decreases the fraction of current mediated by Ca-activated K channels, we plotted the fractional block obtained with Cd as a function of pipette buffer composition (Fig. 7). We found that dialyzing HIT cells with increasing amounts of EGTA (to 10 mM) did not significantly decrease current blockade, even comparing 0 EGTA/0 Ca with 10 EGTA/0 Ca (0.80 ± 0.06, $n = 6$, *vs.* 0.67 ± 0.17, $n = 4$, respectively; data not plotted in Fig. 7). Only solutions with 5 BAPTA/0 Ca significantly reduced the fractional block by Cd ($P < 0.05$ for 5 BAPTA *vs.* all others).

In contrast to HIT cells, dialyzing mouse cells with increasing amounts of EGTA decreased fractional block. The fraction of K current sensitive to Cd ranged from 0.20–0.62. Increasing EGTA from 0.1 to 1 mM (0 Ca added to each) reduced fractional block from 0.62 ± 0.03 ($n = 4$) to 0.41 ± 0.05 ($n = 9$; $P = 0.03$ and NS using stringent Peritz F-test). Using 10 EGTA/2 Ca or 5 BAPTA further reduced fractional block to 0.22 ± 0.04 ($n = 5$) and 0.20 ± 0.07 ($n = 4$), respectively ($P < 0.05$ for either of these *vs.* using <10 mM EGTA; $P > 0.05$ for 10 EGTA/2 Ca *vs.* 5 BAPTA). Thus, strong Ca buffering uncoupled Ca-activated K current from Ca influx in both HIT and mouse B-cells and the latter appeared to be more sensitive to EGTA.

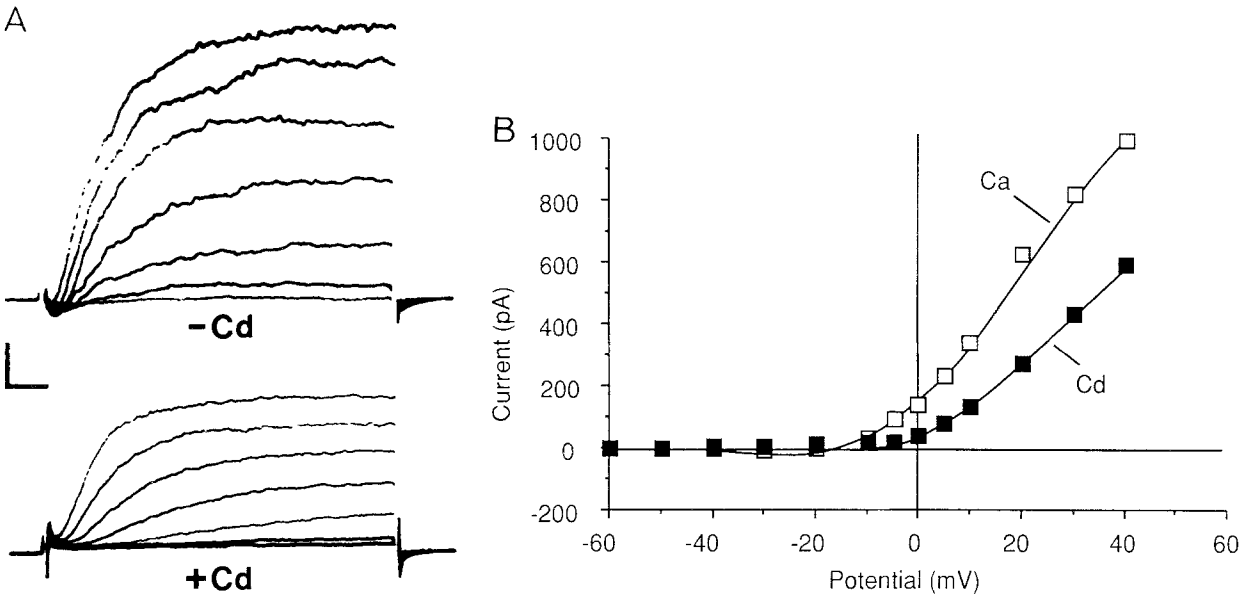

Fig. 6. Macroscopic K currents of voltage-clamped adult mouse B-cells and their sensitivity to Cd under conditions of weak Ca buffering. (*A*) Under control conditions (upper), progressively depolarizing a mouse cell from a holding potential of −100 mV to (bottom to top traces) −20, −10, 0, +10, +20, +30, and +40 mV for 40 msec elicited a K current which activated rapidly and increased in amplitude with depolarization, as in HIT cells. Application of 200 μM Cd (lower) reduced the amplitude and slowed the activation of the K current. Pipette contained 0.1 mM EGTA and 0 added Ca. (*B*) Current-voltage relations obtained from early current amplitudes (measured at 10 msec) for the above mouse B-cell data before (open symbols) and after (filled symbols) 200 μM Cd application. Under control conditions, a small inward current was observed between −30 and −20 mV while the current was net outward at more depolarized potentials. Cd decreased the amplitude of the outward current to reveal a slowly activating K current whose voltage-activation threshold was shifted about 10–15 mV in the depolarized direction, as we found for HIT cells

Discussion

Two Voltage-Gated Outward Current Components in HIT and Mouse B-Cells: Ca-Activated and Delayed Rectifier K Currents

We have shown that HIT cells have large, voltage- and Ca-activated outward currents in addition to the much smaller inward Ca currents we have previously characterized (Satin & Cook, 1988, 1989). Total outward current was carried by K flux through K channels since its deactivation tail current reversed near E_K in normal and elevated external [K] and outward current was blocked by K channel blockers. Dialyzing HIT or cultured adult mouse B-cells with strong Ca buffers to limit intracellular Ca accumulation, or blocking Ca influx with Cd blocked a rapidly activating, low-threshold K current component to reveal an underlying more slowly activating component. Thus, the rapid and slow components of both preparations were mediated by the opening of Ca-activated and delayed rectifier K channels, respectively. Our data cannot tell which of the two recently described subtypes of single, delayed recti-

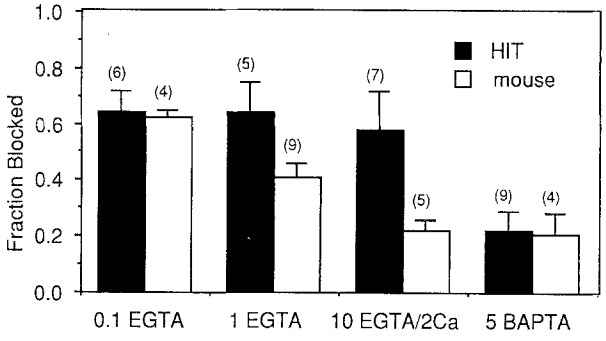

Fig. 7. The mean fractional blockade of early K current by supramaximal Cd (200–500 μM) plotted as a function of intracellular Ca-buffering conditions. K currents were elicited by depolarizing HIT or mouse cells to +10 mV for 40 msec in the presence and absence of Cd. Raising intracellular EGTA had little effect on the amount of Cd-blockable K current in HIT cells. Outward current was blocked by 0.64, 0.64 or 0.58 (1.00 = complete block) when 0.1 EGTA/0 Ca, 1 EGTA/0 Ca or 10 EGTA/2 Ca were used, respectively. The fractional block was reduced to 0.22 by using 5 BAPTA/0 Ca. Increasing EGTA decreased the amount of Ca-activated K current in mouse cells. Fractional blockade was 0.62, 0.41, and 0.21 using 0.1 EGTA/0 Ca, 1 EGTA/0 Ca, 10 EGTA/2 Ca, respectively. Use of 5 mM BAPTA resulted in a block of 0.22. Error bars indicate standard errors of the mean for each group and the numbers in parentheses are the number of cells in each group

fier K channels mediated the macroscopic delayed rectifier current (Zunkler et al., 1988).

The Relative Contribution of the Two Currents to Total K Current Depends On Endogenous Ca Buffering

Dialyzing cells with increasing EGTA concentrations progressively decreased the fraction of Cd-blockable outward current in mouse but not HIT cells, while both HIT and mouse B-cells K currents were uncoupled from Ca influx by BAPTA. BAPTA is thought to be more effective than EGTA in controlling intracellular $[Ca^{2+}]_i$ because BAPTA chelates Ca more rapidly (Tsien, 1980; Thomas, 1982; Marty & Neher, 1985). The increased sensitivity of mouse cells to EGTA might result if submembrane [Ca] accumulated more slowly in these cells than in HIT cells and was therefore more vulnerable to a slow Ca buffer like EGTA. Only BAPTA may act quickly enough to damp rapidly accumulating HIT cell free-Ca concentration. Different Ca-buffering kinetics may explain why Ca accumulation-dependent Ca channel inactivation is an order of magnitude slower in mouse cells than in HIT cells (Hopkins, Satin & Cook, 1989; Satin & Cook, 1989).

In any case, 60–80% of the macroscopic K current of both cell types was carried by rapid, low-threshold Ca activated under conditions of weak Ca buffering, while Cd-block of Ca influx or strong Ca buffering revealed residual higher-threshold, more slowly activating (at physiological potentials; e.g., $<+10$ mV) delayed rectifier K current. These results strongly suggest that the amount of Ca-activated K current elicited by rapid depolarizations will depend on the extent of Ca buffering. The extent of expression of Ca-activated K current in mouse cells appeared to depend on free EGTA and not free Ca (*see* Materials and Methods), since mouse cells filled with 0.1 EGTA/0 Ca had more Ca-activated K current than cells filled with 10 EGTA/2 Ca, even though the estimated free Ca was identical in the two solutions (Fig. 7).

Relationship to Previous Studies of Insulin-Secreting Cells

Although single Ca-activated K channels have been characterized in insulin-secreting cells (Cook et al., 1984, Findlay et al., 1985*a–c*), their contribution to macroscopic outward current has been unclear. In adult mouse B-cells dialyzed with 10 mM EGTA/2 Ca, a minor fraction of voltage-activated outward current was reported to be due to Ca-activated K current (Rorsman & Trube, 1986; Zunkler et al., 1988) while RINm5F cells dialyzed with 1 mM EGTA/0 Ca showed prominent Ca-dependent K current (Findlay & Dunne, 1986). Our results show that the strong Ca buffering used in some previous studies (10 mM EGTA/2 Ca added) probably uncoupled Ca influx from K channel activation. This would account for the apparent insensitivity of K current to changes of intracellular (Rorsman & Trube, 1986) and extracellular calcium concentration (Zunkler et al., 1988). However, K currents recorded with pipettes containing 1 mM EGTA and no added Ca still had substantial Ca-activated K current, suggesting that EGTA may not always buffer free-Ca transients effectively (Tsien, 1980; Thomas, 1982; Eckert & Chad, 1984; Marty & Neher, 1985; Carbone & Lux, 1987). These results suggest that the expression of Ca-activated K current under whole-cell conditions depends on the particular Ca buffer used, its concentration and the cellular preparation itself.

Possible Role of the Two K Currents in B-Cells

We recently described a rapidly activating Ca current which is likely to mediate the upstroke of the B-cell Ca spike. The rapid, Ca-dependent inactivation of this current might contribute to spike repolarization although activation of either of these K currents may also contribute. The lower threshold and more rapid activation of the Ca-activated K current suggests that it, rather than the delayed rectifier current, may be more important. The sensitivity of the low-threshold, Cd-sensitive K current to millimolar TEA in the present study, and in RINm5F cells (Findlay & Dunne, 1986), is consistent with such a role since 1 mM external TEA increased the amplitude and duration of mouse B-cell (Atwater, Ribalet & Rojas, 1979) and RINm5F cell (Findlay & Dunne, 1986) Ca spikes. Thus, the Ca-activated K current may activate rapidly enough to control the amplitude as well as the duration of the Ca spike. This does not rule out a role for the delayed rectifier K current since high doses of TEA (20 mM) also abolish this current in cultured adult mouse B-cells (Rorsman & Trube, 1986). However, the slower activation kinetics of the delayed rectifier at physiological voltages (i.e., less than +10 mV) argues against a prominent role for this channel in repolarization of spikes which last much less than 40 msec near their peaks. Since the present studies have used the invasive whole-cell patch-clamp technique, the results cannot easily be extended to intact cells with their native Ca-buffering

mechanisms. Ultimately, the physiological contribution of the two K currents will require studies using specific blockers of these channels or better understanding of B-cell Ca buffering.

We thank Dr. Marta Perez-Armendariz for her advice on culturing mouse B-cells and Mr. Louis Stamps for his expert tissue culturing. Dr. Wil Fujimoto and Ms. Jeanette Teague generously supplied us with HIT cells obtained from Lilly. Support was provided by the NIH and the Veterans Administration.

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Received 31 January 1989; revised 9 August 1989