Inactivation of HIT Cell Ca^{2+} Current by a Simulated Burst of Ca^{2+} Action Potentials

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ABSTRACT A novel voltage-clamp protocol was developed to test whether slow inactivation of Ca^{2+} current occurs during bursting in insulin-secreting cells. Single insulin-secreting HIT cells were patch-clamped and their Ca^{2+} currents were isolated pharmacologically. A computed β-cell burst was used as a voltage-clamp command and the net Ca^{2+} current elicited was determined as a cadmium difference current. Ca^{2+} current rapidly activated during the computed plateau and spike depolarizations and then slowly decayed. Integration of this Ca^{2+} current yielded an estimate of total Ca influx. To further analyze Ca^{2+} current inactivation during a burst, repetitive test pulses to +10 mV were added to the voltage command. Current elicited by these pulses was constant during the interburst, but then slowly and reversibly decreased during the depolarizing plateau. This inactivation was reduced by replacing external Ca^{2+} with Ba^{2+} as a charge carrier, and in some cells inactivation was slower in Ba^{2+}. Experimental results were compared with the predictions of the Keizer-Smolen mathematical model of bursting, after subjecting model equations to identical voltage commands. In this model, bursting is driven by the slow, voltage-dependent inactivation of Ca current during the plateau active phase. The K-S model could account for the slope of the slow decay of spike-elicited Ca current, the waveform of individual Ca current spikes, and the suppression of test pulse-elicited Ca current during a burst command. However, the extent and rate of fast inactivation were underestimated by the model. Although there are significant differences between the data obtained and the predictions of the K-S model, the overall results show that as predicted by the model, Ca current slowly inactivates during a burst of imposed spikes, and inactivation is dependent on both Ca^{2+} influx and membrane depolarization. We thus show that clamping cells to their physiological voltage waveform can be readily accomplished and is a powerful approach for understanding the contribution of individual ion currents to bursting.

INTRODUCTION

In pancreatic islets of Langerhans, raising plasma glucose concentration above 7.25 mM causes the membrane potential of insulin-containing β cells to undergo slow oscillatory plateau depolarizations with superimposed rapid Ca^{2+} spikes (Henquin, 1987). This membrane electrical activity pattern, termed bursting, mediates the Ca^{2+} uptake required for sustained insulin secretion (Meissner and Schmeer, 1974). In recent years, a number of different β cell conductances have been characterized, which has led to the development of several different theoretical models of β cell bursting (reviewed in Ashcroft and Rorsman, 1989). However, the ionic mechanism of bursting is still unknown because some of these models have not withstood experimental scrutiny. An early and widely held hypothesis suggested that cyclic activation of Ca^{2+}-activated K^+ channels produced the rhythmic plateau oscillations (Atwater et al., 1978; 1979), but recent evidence suggests this may not be the case (for review, see Cook et al., 1991). Satin and Cook (1989) found that prolonged voltage-clamp pulse depolarizations (to 10 s in duration) slowly inactivated β cell calcium channels and proposed that Ca^{2+} channel inactivation might underlie bursting (Satin and Cook, 1989). Slow, voltage-dependent Ca^{2+} channel inactivation has been described in neonatal rat (Satin and Cook, 1988), adult mouse β cells (Hopkins et al., 1991; but see Plant, 1988), HIT cells (Satin and Cook, 1989) and human β cells (Kelly et al., 1991). Keizer and Smolen (1991) subsequently showed mathematically that this process, with modified parameters, was a feasible mechanism of bursting. We refer to this model as the "K-S model." In the model, slow, voltage-dependent inactivation of Ca channels (on the order of 10–20%) during the plateau eventually leads to repolarization as diminishing inward Ca current is overtaken by outward current carried by open KATP channels. Subsequent slow recovery from Ca channel inactivation during the hyperpolarized silent phase initiates the next plateau.

In this paper, we develop a new experimental approach for testing different theoretical models of rodent islet bursting. Instead of voltage-clamping the cell membrane using standard square pulse voltage commands, we use a mathematical model of β cell bursting to simulate a single burst of spikes riding on a slow plateau depolarization, and then apply this physiological waveform to voltage-clamped insulin-secreting cells. Physiological voltage waveform commands have been used to study Ca^{2+} channel activity during nerve and cardiac action potentials (McCobb and Beam, 1991; Doerr et al., 1989), but to our knowledge, have not been used to analyze bursting activity in excitable cells. Application of a physiological voltage command is needed to reproduce the physiological extent and time dependence of Ca versus

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voltage-dependent Ca current inactivation, both of which may be significant in determining β cell electrical behavior. It was thus of interest to test whether a set of voltage changes expected to produce changes in the cell membrane potential and intracellular ions similar to those encountered during bursting would rapidly activate and then slowly inactivate Ca channels, as predicted by the K-S and other models incorporating Ca channel inactivation.

In order to develop our experimental technique, we imposed physiological voltage commands on single insulin-secreting HIT cells to study their Ca currents. These cells are widely used as a model of β cells because they release insulin in response to glucose (Santerre et al., 1981), are easy to work with and have Ca currents that qualitatively resemble those of mouse β-cells (Hopkins et al., 1991). Most significantly for our purposes, HIT cell Ca current slowly inactivates in response to prolonged depolarization, and results obtained with HIT cells formed the basis of the original slow-inactivation model of rodent bursting (Cook et al., 1991; Satin and Cook, 1989; Keizer and Smolen, 1991). In addition, single HIT cells show a form of oscillatory spiking activity at room temperature (Keahy et al., 1989) that resembles the burst-like electrical activity of cultured mouse islet cell clusters recorded under similar conditions (Rorsman and Trube, 1986; Satin, unpublished observations). Thus, we decided that they were a reasonable test bed for developing our techniques and a good starting point for our preliminary investigations of inactivation. The burstwave command used to voltage-clamp HIT cells was also used to numerically drive the Ca current equations of the K-S slow-inactivation model of bursting, and the computed currents were compared to those actually observed.

We found that burstwave commands reversibly suppressed the Ca conductance of the HIT cell membrane, with inactivation being partly dependent on Ca influx and partly on depolarization. The K-S model equations for Ca current could account for the slow suppression of Ca current in Ca-containing external solutions and the shape of the fast spike-activated Ca currents. However, using parameters that supported bursting, the model in its present form underestimated the rate and extent of fast, Ca-dependent inactivation, as in previous modeling studies (Keizer and Smolen, 1991), and the inactivation of mean Ba current was faster than predicted, although some cells had appropriate slow inactivation kinetics. These results show that HIT cell Ca current slowly inactivates in response to a burst of action potentials, but that the K-S model cannot fully account for the quantitative aspects of fast Ca channel inactivation. Better curve fits could be obtained using different parameters in the K-S model, but as in previous studies (Keizer and Smolen, 1991), the model equations then failed to show normal bursting. We conclude that clamping cells to their physiological waveforms is a promising new tool for understanding islet electrical activity and testing different ion channel models using this more physiological approach.

Some of these results have appeared recently in preliminary form (Satin et al., 1993).

MATERIALS AND METHODS

Insulin-secreting HIT cells were obtained from Dr. Wil Fujimoto of the Diabetes Research Center of the University of Washington, Seattle. Cells were maintained in T25 flasks and passaged weekly using trypsin-EDTA (Gibco, Grand Island, NY 14072). Aliquots of cell suspension were seeded onto glass coverslips in 35-mm petri plates and kept at 37°C in enriched Ham's F-12 media (Whittaker, Walkersville, MD. 21793) in a humid environment for a week until re-passaging. All cells used were from passages 50–70, and only single isolated cells were selected for study to avoid complications arising from cell-cell coupling.

An Axopatch 1D patch clamp amplifier (Axon Instruments, Foster City, CA. 94404) was used in the tight-seal whole cell mode to measure macroscopic Ca^{2+} currents (Hamill et al., 1981). HIT cells were placed in a small recording chamber on the stage of an inverted phase-contrast microscope (Olympus IM-T2; Olympus Corp., Japan), where cells were patched while being continuously perfused with oxygenated external solutions. The external solution contained (in mM): 120 NaCl, 3 $CaCl_2$, 5 CsCl, 1 $MgCl_2$, 10 TEA-Cl, 5 HEPES, 5E-4 TTX, pH 7.2. The internal pipette solution contained (in mM): 114 Cs-aspartate, 5 CsCl, 2 $MgCl_2$, 2 ATP, 10 HEPES, 10 4-AP, and 1 EGTA; pH 7.2. These solutions had an osmolarity of 267 mOsm. These experimental manipulations were used to completely isolate Ca^{2+} current from competing ion currents (Satin and Cook, 1988). To block Ca^{2+} currents, Cd^{2+} (to 1 mM) was added to the external solution. In experiments where Ba^{2+} was used as the charge carrier, Ba replaced Ca mole for mole.

Patch electrodes were made from standard hematocrit tubing using a programmable patch pipette puller (Model 750B, David Kopf Instruments, Tujunga, CA.). Pipettes were coated with Sylgard resin to reduce tip capacitance and were fire polished using a microforge. Seal resistances ranged from 5–20 Gohms. Series resistance compensation was not used, because the Ca^{2+} currents observed were <100 pA in amplitude (Satin and Cook, 1988; 1989).

Command voltages for making standard I-V and monitoring test pulses were generated on a Mac IIci computer (Apple Computer, Cupertino, CA.) using PulseControl software provided by R. Bookman and J. Herrington of the University of Miami, and an Instrutech 16-bit hardware interface (ITC-16; Instrutech, Great Neck, NY 11021) and Igor graphical/data analysis software (Wavemetrics, Lake Oswego, OR 97035). All pulse-evoked currents were digitized at 5–20 kHz on-line after filtering at one-half the sampling rate using an eight-pole Bessel filter (Frequency Devices, Haverhill, MA 01830). Standard methodology was used to analyze pulse-evoked Ca currents (Satin and Cook, 1988).

Physiological burstwave commands were calculated by integrating the Sherman, Rinzel and Keizer (or SRK) mathematical model of islet bursting (Sherman et al., 1988). Bursting in this model closely resembles standard microelectrode recordings of bursting in intact mouse islets (Ribalet and Beigelman, 1979). A single islet burst cycle was computed numerically using the methodology described in Keizer and Smolen (1991). Burstwave commands were then sent to the patch clamp command input as 10K point voltage arrays. These were converted using Igor software into a physiological voltage clamp command that was sent out in real time through the D/A converter to the patch clamp command input. Burst data were subsequently acquired and stored on a PCM-based VCR recorder (DR 8900, Neurodata Corp., NY, 10010) at 20 kHz for off-line analysis. Taped burst currents were filtered at 1 kHz, and then digitized at 2 kHz (spike currents) or filtered at 2.5 kHz and sampled at 5 kHz (test pulse currents). Prior to determining difference currents for spike-current analysis, each burst current wave had its baseline offset and leak current subtracted. Difference currents (control - Cd^{2+} current) were obtained by a 16-bit digital subtraction after synchronization of the wave pair. Only cells showing stable baselines, leak currents and fully blocked Ca^{2+} channels in Cd^{2+} were selected for analysis.

All experiments were conducted at room temperature.

Theoretical predictions for Ca^{2+} current

Experimental results obtained using the novel clamp command were compared with the predictions of the K-S model of islet bursting by numerically

subjecting the model to the same clamp command. This model is based on patch clamp data from HIT cells (Satin and Cook, 1989; Keizer and Smolen, 1991), depends on slow Ca^{2+} channel inactivation for bursting, and was used with parameters that simulate bursting (Keizer and Smolen, 1991). The differential equation for membrane potential in this model is given by

$$C_m\,(dV/dt) = -(I_{Ca,fast} + I_{Ca,slow} + I_{K,delayed\ rectifier} + I_{K\text{-}ATP})$$

where Ca^{2+} current has Goldman-Hodgkin-Katz rectification Φ:

$$\Phi(V) = V/[1 - \exp(V/13.35)]$$

Fast and slowly inactivating components are described as

$$I_{Ca,fast}\,(V, I) = g_{max,Ca^{2+},f}\,m_{f,\infty}\,(V)(1 - I)\Phi(V)$$

$$I_{Ca,slow}\,(V, J) = g_{max,Ca^{2+},s}\,m_{s,\infty}\,(V)(1 - J)\Phi(V)$$

Here $m_{f,\infty}$ and $m_{s,\infty}$ represent activation and I and J inactivation, where the inactivation variables satisfy first-order kinetics:

$$dI/dt = [I_\infty(V) - I]/\tau_I$$

$$dJ/dt = [J_\infty(V) - J]/\tau_J$$

I represents Ca^{2+}-dependent inactivation of Ca^{2+} channels. Fast, Ca^{2+}-dependent inactivation is modeled by making channel opening probability sensitive to increases in domain $[Ca^{2+}]$ which are very near (≈100 angstroms) the mouth of the channel and equilibrate rapidly (<1 μs). τ_I and τ_J are hundreds and thousands of ms, respectively (see Keizer and Smolen (1991) for additional details).

Delayed-rectifier K^+ current is given by

$$I_{K,\ delayed\ rectifier}\,(V, n) = g_{K,max}\,n(V - V_K)$$

with activation parameter n being rapid, and satisfying

$$dn/dt = [n_\infty(V) - n]/\tau_n$$

ATP-inhibited K^+ current is defined by

$$I_{K\text{-}ATP}\,(V) = g_{K\text{-}ATP}(V - V_K).$$

Global changes in intracellular $[Ca^{2+}]$, such as would release insulin-granules, are determined according to

$$d[Ca^{2+}]/dt = f\{-[3/(2Fr)][I_{Ca,fast} + I_{Ca,slow}] - k_{Ca}[Ca^{2+}]\}.$$

where F is Faraday's constant and r is the cell radius.

Integration of the SRK and KS mathematical models was done using Heun's method (a predictor-corrector form of the trapezoidal rule) with a time step of 0.05 ms; occasionally 0.01 ms was used to check accuracy (Keizer and Smolen, 1991). Computations were done on an IBM RS/6000 workstation or a CRAY YMP computer.

RESULTS

Fig. 1 shows the response of a single HIT cell to a computed burst waveform that closely resembles physiological bursting (Ribalet and Beigelman, 1979). The burstwave (Fig. 1, *lower*) started from a holding potential of −65 mV, corresponding to the silent or interburst potential, slowly depolarized in a ramp and then more rapidly depolarized to the peak of the plateau potential (~−47 mV) from which rapid spikes to near −22 mV commenced. The frequency of the simulated spikes decreased from 7 Hz to 3 Hz during the plateau, and their amplitude decreased by 3.1 mV. Net Ca^{2+} current (minus leak and capacity current) during the burst was obtained by digitally subtracting the burstwave-evoked current measured in cells bathed in control solution from current obtained after fully blocking the Ca^{2+} channels with external cadmium (Satin and Cook, 1988). A representative long-duration Ca^{2+} difference current is shown in Fig. 1 (*upper*). Inspection of current recordings such as these revealed no Ca^{2+} current activation during the hyperpolarized interburst phase, while a small inward Ca^{2+} current of variable amplitude averaging 3.04 ± 1.64 pA (mean ± SE; $N = 5$; range = 0–9.2 pA) was observed to activate in response to the plateau depolarization in some of the cells.

In contrast, a prominent inward Ca^{2+} current was elicited by the spike depolarizations. The first (and largest) voltage spike of the burst triggered 20.85 ± 3.36 pA of Ca^{2+} current (mean ± SE; $N = 5$). These findings are as expected from the activation characteristics of HIT cell Ca^{2+} channels, as revealed by the typical I_{Ca}-V in the inset of Fig. 1 (Satin and Cook, 1988). This I_{Ca}-V relation shows no measureable inward current at voltages characteristic of the silent phase while its lower current threshold is near voltages characteristic of the plateau.

In addition, peak Ca^{2+} spike current slowly decayed in amplitude for the duration of the plateau, as shown by Fig. 2 *A*, which shows the plateau voltage command and an example of the inward Ca^{2+} spike currents on a faster time scale (acquired at 2 kHz). The individual Ca^{2+} spike currents rapidly activated and deactivated in response to the voltage spikes (Fig. 2 *B*). Fig. 2 *B* also shows that when the K-S model was subjected to the burst waveform, the model qualitatively reproduced the rapid kinetics of the Ca^{2+} spike currents. Despite the fact that the K-S model assumes that $I_{Ca,slow}$ activates instantaneously with voltage, good agreement was obtained between the model and the measured spike difference currents.

Fig. 2 *C* presents results pooled from 5 cells, and shows that peak Ca spike currents decayed during the plateau. The model (open triangles) predicted a biexponential decay with a fast time constant of 270 ms and a slow time constant of 5.85 s. The decay of the observed currents (filled squares) suggested there were two components present, one fast and one slow. The fast component occurs ca. 100 ms while the slow could be fitted with the same time constant, 5.85 s. Comparing the theoretical predictions with the actual data shows that the model underestimated the extent of inactivation, due to predicting a considerably smaller fast component than was observed.

An advantage of clamping cells to a physiological waveform is that Ca influx during the waveform can be estimated. In the case of the HIT cell, digital integration of net Ca^{2+} current during the imposed burst of spikes yielded a net Ca^{2+} entry of 35.77 ± 16.6 pcoulombs of Ca^{2+} charge. By integrating the capacity transients elicited by hyperpolarizing commands, and assuming 1 μF/cm^2 intrinsic membrane capacitance, HIT cell diameter was estimated as 19.92 ± 2.74 μm ($N = 5$). This allowed us to estimate cell volume and hence estimate that total cell [Ca] would increase by 64.74 ± 32.36 μM ($N = 5$) during a single burst of 40 spikes, if Ca^{2+} efflux or sequestration are neglected.

Some decay of the spike-elicited Ca^{2+} currents is expected because the peaks of the voltage spikes decreased by 3.10

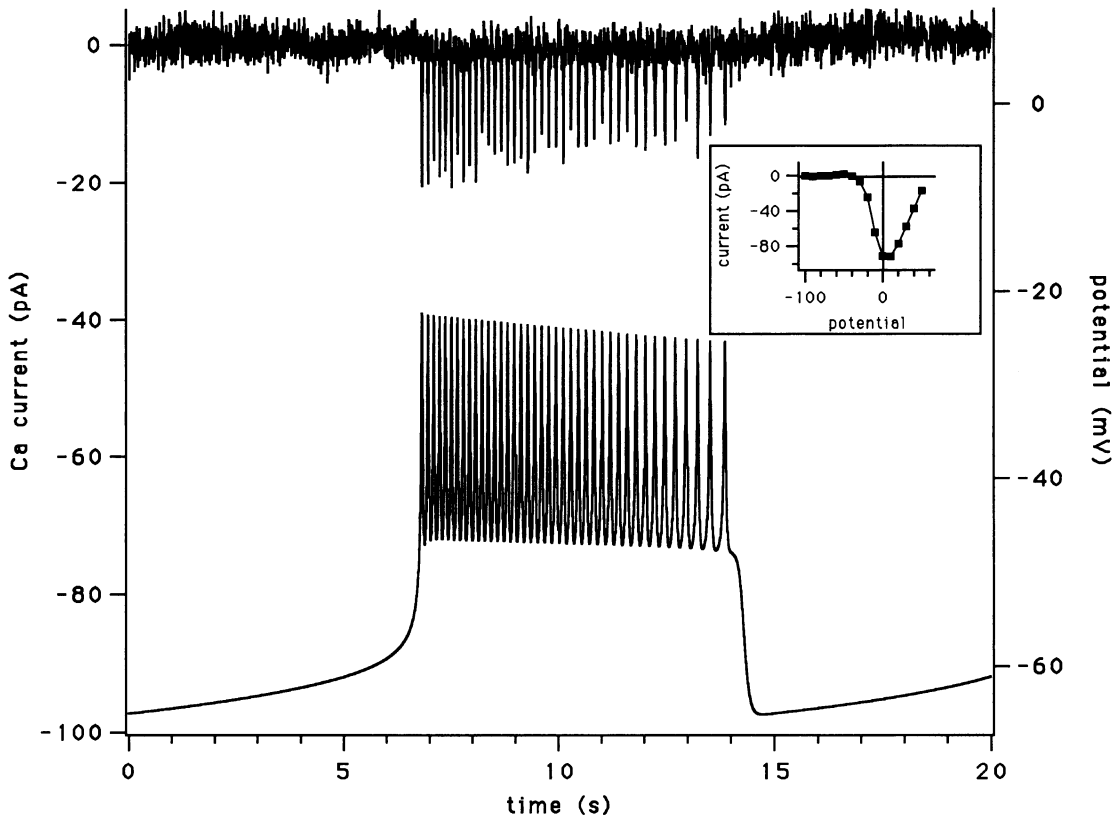

FIGURE 1 Burstwave voltage command (*lower*) used to voltage clamp a single HIT cell, and Ca^{2+} difference current obtained for one simulated burst cycle (*upper*). Bursts were simulated using the computer model of Sherman et al. (1988). No inward Ca^{2+} current activated during the interburst period while robust inward currents were obtained in response to each brief spike depolarization. Current data acquired at 500 Hz after filtering at a cut-off frequency of 250 Hz. In some cells, the plateau itself elicited a small, slowly inactivating Ca^{2+} current component. *Inset:* A representative HIT cell I-V obtained from a holding potential of −65 mV, using the solutions and techniques described in Methods.

mV during the burstwave command. Successive spikes would thus activate less Ca^{2+} current as the burst progressed, and thus might account for some of the Ca current decrease we are ascribing to inactivation. In order to more fully separate slow inactivation from decreasing channel activation, we used another protocol, modifying the burstwave command by adding twenty 20-ms test pulses to +10 mV. The test pulses were applied once per s (Fig. 3, *lower*) and elicited a robust Ca^{2+} current (45.66 ± 14.05 pA; $N = 5$) that could be readily monitored to determine the time course of inactivation during the burst commands. Because peak Ca currents can be cleanly distinguished from capacity currents during a 20-ms test pulse, this modification also simplified data analysis. As shown in Fig. 3 (*upper*), application of this waveform from a holding potential of −65 mV inactivated test-pulse Ca^{2+} currents (filled squares) to 0.804 ± 0.026 of control ($N = 5$). Applying repetitive test pulses to +10 mV from −65 mV at 1 Hz in the absence of a burst depolarization had no effect on HIT cell Ca currrent (data not shown). In addition, the burstwave command did not cause significant cell leakage or changes in the Ca current-voltage relation. The data were normalized to 1.00 to facilitate comparison. Inactivation of the Ca current elicited by test pulses in Ca-containing external solution resembled the prediction of the K-S model (open diamonds), when the model was numerically subjected to this same voltage command protocol, although the observed inactivation appeared to be somewhat faster in Ca-containing solution than was predicted by the model. Thus, while Ca current was observed to decrease for 2–4 s of the active phase of the burst, the K-S model requires decay to occur during the entire plateau, because repolarization at the end of the plateau occurs once there is sufficient inactivation.

These results demonstrate that the burstwave reversibly suppressed Ca^{2+} channel activity for the duration of the plateau wave, and that recovery of the current followed during the interburst phase.

We have suggested that the Ca^{2+} currents of HIT and mouse β cells have both fast and slow components of inactivation, with fast inactivation being Ca^{2+}-influx dependent and slow inactivation being voltage-dependent (Satin and Cook, 1989; Hopkins et al., 1991). In order to test how these processes might be manifested during our physiological burst commands, we replaced Ca^{2+} with Ba^{2+} in our external solutions. Both divalents can carry inward current through Ca^{2+} channels, but inactivation is much less in Ba^{2+} (Satin and Cook, 1989; Hopkins et al., 1991; Plant, 1988; Eckert and Chad, 1984). As shown in Fig. 4, Ba^{2+} substitution re-

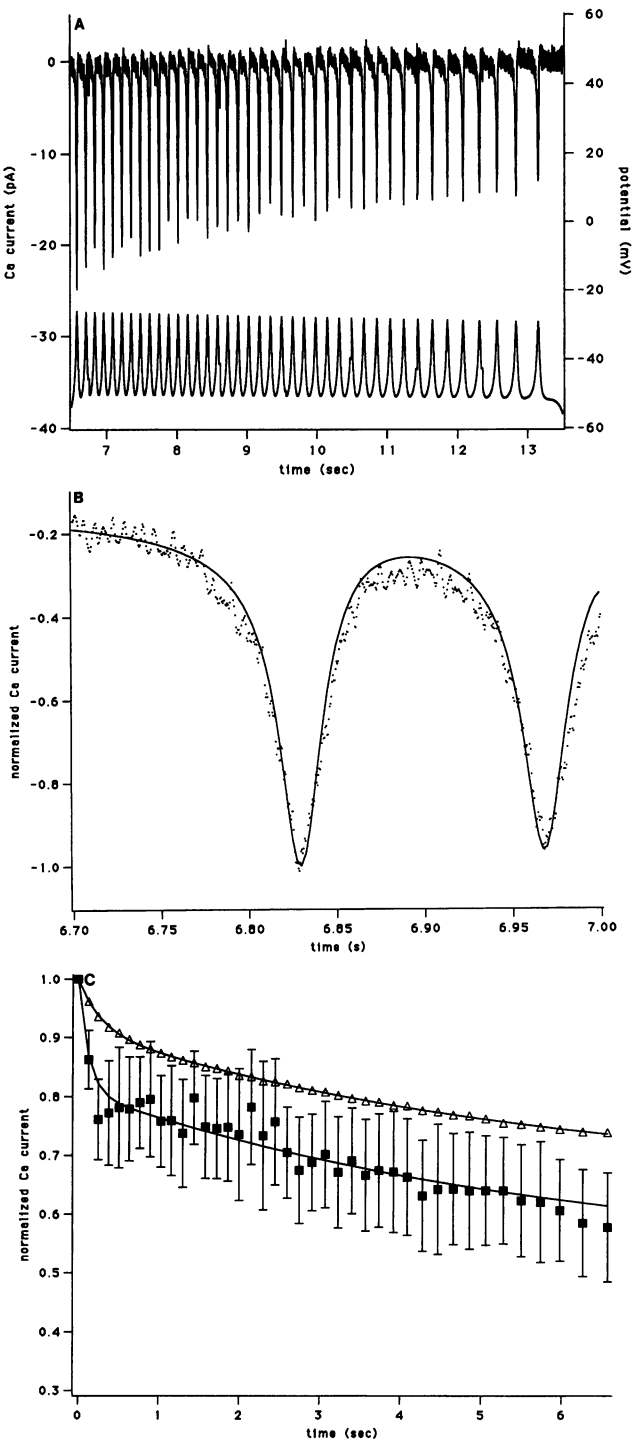

FIGURE 2 Slow decay of spike-elicited Ca^{2+} currents during the plateau depolarization. (*A*) Ca^{2+} difference current data (*upper*) shown after filtering at 1 kHz and acquisition at 2 kHz. Current spikes activated in response to each voltage spike command (*lower*). The spikes declined in amplitude as the burst progressed. Small "steps" in the command potential array elicited rapid capacity transients used to check synchronization of the current pairs to be subtracted. (*B*) Two individual current spikes (*points*) shown on an expanded time scale, with the prediction of the K-S model shown for comparison (*solid line*). Currents activated and deactivated rapidly, and the general form of the spike currents was well approximated by the model. Model and experimental amplitudes were normalized to −1.0. (*C*) Spike current decay had both fast and slow components in five cells (mean ± SE shown). The time shown starts at the beginning of the plateau depolarization. The decay of the modeled (△) and experimental currents (■) are shown.

duced but did not abolish total inactivation (Ca^{2+}: filled squares; Ba^{2+}: open squares). The amount of Ca^{2+} current that did not inactivate changed from 0.804 ± 0.026 of control (in Ca^{2+}; $N = 5$) to 0.885 ± 0.018 of control (in Ba^{2+}; $N = 3$) on changing from Ca^{2+}-containing to Ba^{2+}-containing solutions. This suggests that Ca^{2+} influx modulates the total inactivation process but is not absolutely required for significant inactivation to occur.

The dotted line shown represents the inactivation predicted by the K-S model of bursting using Ca^{2+} under control conditions, and the solid line shows the prediction obtained using Ba^{2+} to block fast inactivation. We simulated Ca^{2+} replacement with Ba^{2+} in the K-S model by setting the forward rate constant for fast inactivation to zero (Keizer and Smolen, 1991). In contrast to the results obtained with Ca^{2+}, the time course of inactivation of the mean data obtained using Ba^{2+} were not well fit by the model. Thus, while the K-S model could account for the degree of inactivation (11.5%) observed in Ba^{2+} by the end of the plateau depolarization, the onset of inactivation in Ba^{2+} was considerably faster than predicted by the model. However, there was significant variability among the cells, with some cells showing slower inactivation in Ba^{2+} than the mean data. The inset to Fig. 4 shows one example of this, where the changes observed in the current were better fit by the K-S model. This variability might be expected if HIT cells have a variable ratio of fast versus slowly inactivating Ca^{2+} current, in accord with our suggestion that these components reflect the activity of two distinct Ca^{2+} channels (Satin and Cook, 1988; 1989).

Another factor that may make Ba^{2+} inactivation appear faster than predicted from the model is that the forward inactivation rate constant for the rapidly inactivating Ca^{2+} channels in Ba^{2+} may be finite rather than zero, and thus it may not be appropriate to simulate Ba^{2+} substitution. In support of this possibility, the fast inactivation of HIT cell Ca^{2+} current obtained in a three-pulse protocol decreased from 60% per 40-ms conditioning pulse to ≈15% (rather than zero) in 40 ms upon switching from 3 mM Ca to 3 mM Ba (Satin and Cook, 1989).

As in Keizer and Smolen (1991), we could more adequately fit the burstwave-derived data by changing several of the parameters of the Ca^{2+} current equations of the model. Specifically, the activation curves $m_{f,\infty}$ and $m_{s,\infty}$ had to be steepened and the rate constant for fast inactivation increased. These changes are also necessary to reproduce the steep *I-V* relations (Fig. 1, *inset*) and more closely account for the data of Satin and Cook (1989). However, as in Keizer and Smolen (1991), integrating the model equations with these parameters failed to produce measureable bursts (data not shown).

DISCUSSION

We developed a novel physiological burst waveform command, and applied it to voltage clamped HIT cells. The waveform command was computed from a theoretical model of a

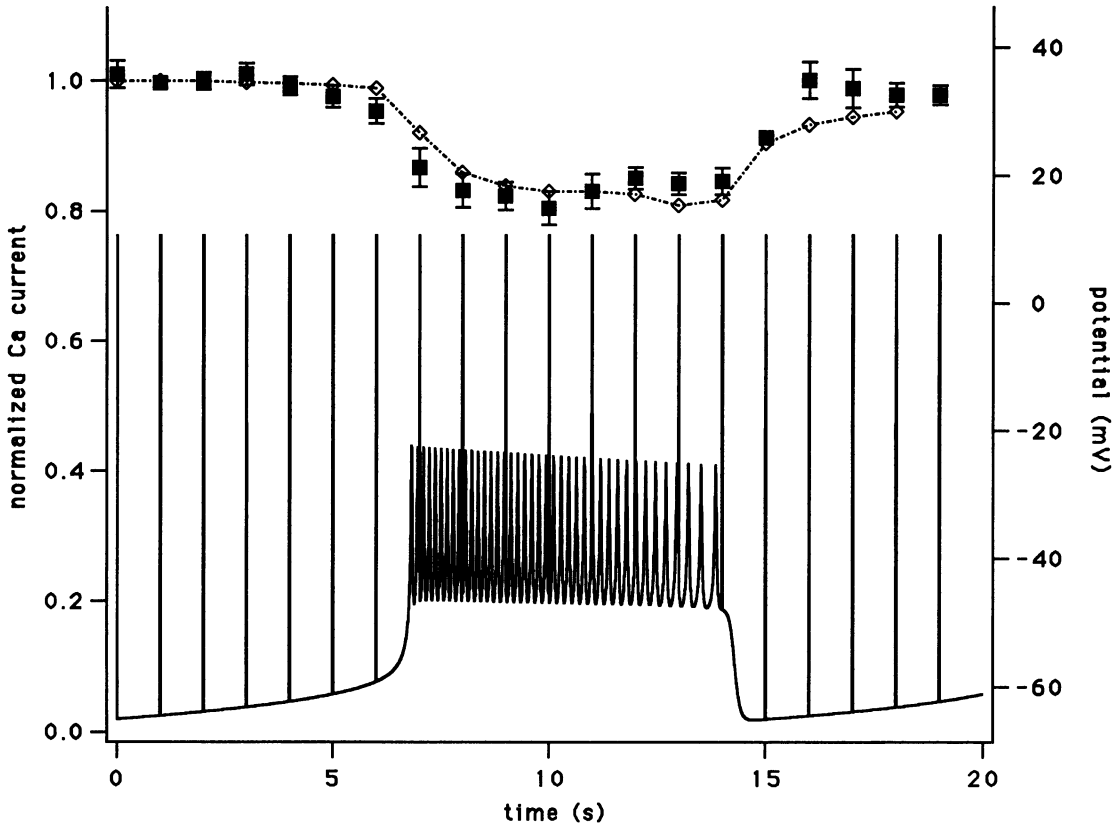

FIGURE 3 Modified burst command includes standard 20-ms test pulses to the peak of the Ca^{2+} I-V (+10 mV) applied at 1 Hz (*lower*). Test pulse current was stable during the interburst but then slowly decreased as the plateau commenced, and slowly recovered as the plateau repolarized to −65 mV (*upper*). Data (■) were well simulated by the prediction of the K-S model (– – ◇ – –). Data represent mean ± SE of five cells obtained from a initial holding potential of −65 mV.

burst of rapid spikes riding on a slow plateau depolarization and was digitally converted to a voltage clamp command with a hardware/software combination that allowed arbitrary waveforms to be created and converted to voltage protocols. We could apply the command, in addition to conventional square pulse commands, before and after fully blocking Ca^{2+} channels with Cd^{2+}, to obtain a difference current reflecting the activity of only the Ca^{2+} ion channels. In addition, we applied the same burstwave to drive the K-S model of bursting. Using parameters that produce bursting in this model, the model was found to predict inactivation. Inactivation was also observed in spike- or test pulse-elicited HIT cell Ca^{2+} currents. However, the model differed from the data in that we observed more fast, Ca^{2+}-dependent inactivation than predicted, and this inactivation phase proceeded at a faster rate than expected (Fig. 2 *C*). In addition, slow, voltage-dependent inactivation that we observed in Ba^{2+} was faster than predicted by the model in most but not all of the cells (Fig. 4). The preponderance of faster forms of Ca^{2+} channel inactivation under our experimental conditions may be correlated with the observation that burst-like activity in cultured mouse β cells and HIT cells at room temperature is also fast (2–4 s; Satin, unpublished; Keahy et al., 1989; Rorsman and Trube, 1986).

It may be problematic that we found no measurable Ca^{2+} current during the silent phase and only a small, variable Ca^{2+} current at the plateau potential. Some inward current during the silent phase is necessary to trigger the next plateau, and a sustained inward current is necessary for its maintenance. This issue was partially addressed by Keizer and Smolen (1991) who noted that use of the whole-cell patch technique and/or conducting experiments at 20 degrees may have masked a low-threshold Ca^{2+} current that helps to trigger the plateaus. Even very small currents (on the order of 1–2 pA) during the silent phase would be sufficient for plateau depolarization. The parameters used here for the K-S model are such as to give this requisite small inward Ca^{2+} current. Recently, several metabolically-regulated Ca^{2+} channels which could pass such a current have been observed in insulin-secreting cells (Rojas et al., 1990; Smith et al., 1990; Velasco et al., 1988). The contribution of these currents might have been underestimated in our studies if dialysis of the cell by the whole-cell pipette disrupted the relevant metabolic pathways.

Smolen and Keizer (1992) have also emphasized that it is difficult to obtain bursting if the voltage-dependent Ca^{2+} current has a sharp lower threshold of activation, as observed for HIT cells (Satin and Cook, 1988; 1989; Keahy et al., 1989) and adult mouse β cells (Plant, 1988; Hopkins et al., 1991; Rorsman and Trube, 1986) in patch clamp studies. Thus, for example, the descending limb of the *I-V* shown in the inset to Fig. 1 was fit by a Boltzmann equation having

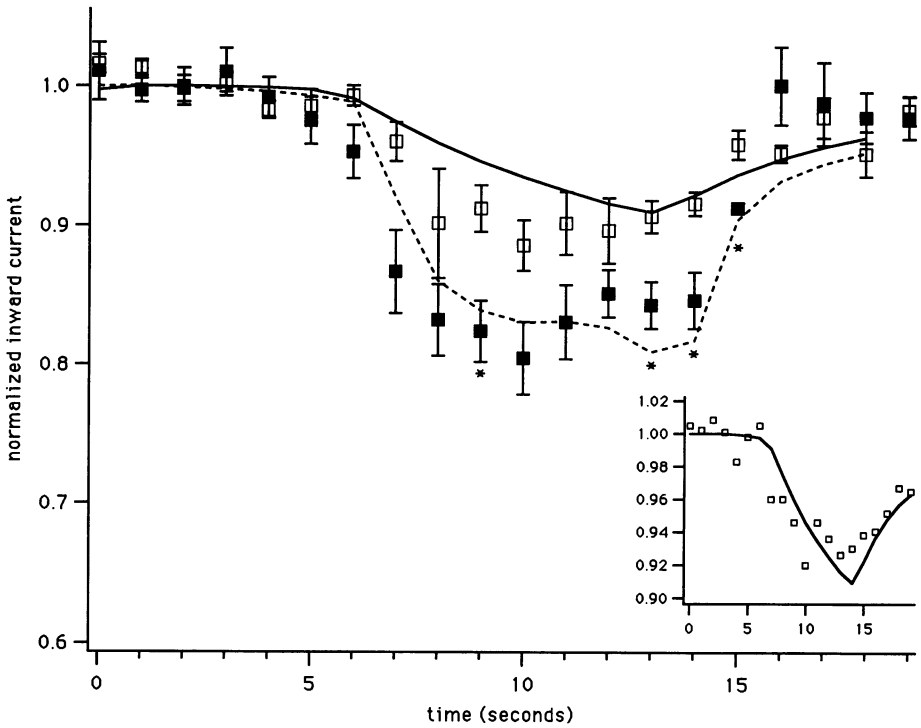

FIGURE 4 Dependence of inactivation on permeant divalent species. Replacing all external Ca^{2+} (■; $N = 5$) with Ba^{2+} (□; $N = 3$) reduced burst-associated inactivation. The K-S model predicted the amount of inactivation observed in Ca^{2+} (·····) or Ba^{2+} (——) but the time course observed using Ba^{2+} was faster than predicted theoretically. Data shown are mean ± SE of determinations made by subjecting HIT cells to a burstwave voltage from −65 mV. Asterisks indicate differences that were significant at $P < 0.05$ level (determined using Student's *t*-test). The *inset* (*lower right*) shows a cell that slowly inactivated in Ba^{2+}. Open squares indicate normalized Ba^{2+} current, and the solid line is the prediction of the K-S model. Same axes as for main figure.

a half-activation voltage of −14.8 mV and a steepness factor of +5.08 mV. In contrast, the comparable half-activation and steepness parameters used in the version of the K-S model that produced bursting were −25 mV and +10 mV, respectively. Smolen and Keizer (1992) suggest that the steep voltage activation of HIT and β cell Ca^{2+} channels may mean that, in addition to the voltage-dependent inactivation of Ca^{2+} channels, another slow process might be necessary. They hypothesize that Ca^{2+} influx during the burst plateau may slowly activate K-ATP channels by decreasing mitochondrial respiration and thus decreasing ATP/ADP. Although there is some indirect support for this hypothesis from whole-islet studies (Henquin, 1990), voltage-clamp analysis needs to be done to test it more fully.

This preliminary study was intended to demonstrate the utility of a new protocol and it demonstrates the existence of HIT cell Ca^{2+} current inactivation with properties qualitatively similar to those needed to drive bursting in a mathematical model, although slow inactivation was not seen in all cells. Considerable cell-cell variability was observed experimentally. We could only closely fit the mean data (Fig. 4) using parameters for the K-S model that showed no clear bursting when the model was integrated. Similar results were reported by Keizer and Smolen (1991), who modelled HIT cell Ca currents elicited using conventional pulse protocols (Satin and Cook, 1989). They found, as we do, that steeper activation curves and a larger rate constant for fast inactivation were required to fit the whole-cell data than to produce bursting. This similarity is significant, because the present findings were obtained using a more physiological approach. Thus, we conclude that although a final successful model of bursting may include multiple components and possibly subtle changes in several ionic currents, slow voltage-dependent Ca channel inactivation is likely to play an important role under physiological conditions. In addition, this new approach can be easily extended for testing other models of bursting based on different slowly varying ion channel currents, as long as selective blockers for the channels are available. It will be especially critical to extend these studies by using the burstwave technique to analyze the ion currents of adult mouse β-cells at physiological temperatures.

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