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Modulation of the frequency of glucose-dependent bursts of electrical activity by HCO_3/CO_2 in rodent pancreatic B-cells: experimental and theoretical results

P. B. Carroll, A. Sherman, R. Ferrer, A. C. Boschero, J. Rinzel, and I. Atwater

Laboratory of Cell Biology and Genetics and Mathematical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Building 8, Room 326, Bethesda, MD 20892, USA

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Abstract. The burst pattern of electrical activity recorded from pancreatic B-cells in response to 11 m*M* glucose shows a large islet to islet variability. The relationship between burst frequency and glucose sensing (the threshold for electrical activity and the graded increase in electrical response to glucose, i.e. active phase %) has not been investigated within the same islet. In this work, we show that low HCO_3 (5 m*M*) Hepes buffered solutions reversibly reduce the frequency of bursts compared to control (25 m*M*) HCO_3 buffered solutions in the same islet. There was no change in the threshold or active phase (%). Using the mathematical model of Sherman et al. 1988, we explored mechanisms for a change in frequency independent of a change in active phase (%). Increased exchangeable calcium pool size and increased cell to cell coupling were the two theoretical treatments which could reproduce the experimental data. We conclude that burst frequency can be modulated independent of the active phase and that alteration of a calcium pool size best fits the experimental data.

Key words: Pancreatic B-cell – Burst frequency modulation – HCO_3/CO_2 – Mathematical model

Introduction

Pancreatic B-cells show a characteristic "burst" pattern of electrical activity in response to glucose (Dean and Matthews 1970; Meissner and Schmeltz 1974; Atwater and Beigelman 1976) (Fig. 2A). This activity correlates closely with insulin secretion (Scott et al. 1981). The burst pattern in 11 m*M* glucose is variable from islet to islet, but is consistent between cells within a single islet (Meda et al. 1984). Another experimental observation is that from islet to islet, the burst frequency in 11 m*M* glucose is variable. This is independent of the relative time that the cell is depolarized and spiking (active phase, or active phase (%)). Experiments indicated that these two parameters (burst frequency and active phase) were independently regulated since their distribution in a population study of 250 islets was different (Atwater et al. 1980).

Feedback between B-cell membrane permeability and intracellular free calcium concentration is proposed to regulate the characteristic burst pattern and has been modelled mathematically (Chay and Keizer 1983; Himmel and Chay 1987; Rinzel et al. 1986; Sherman et al. 1988; Chay and Kang 1988). Previous theoretical treatment of the observed islet to islet variability in the burst frequency suggested that a change in the exchangeable calcium pool size could produce a change in frequency without affecting the active phase (%) (Atwater and Rinzel 1986). Because of this interaction between experiment and theory we wanted to find conditions which would selectively alter burst frequency and allow us to examine effects on the threshold and graded response of the cell. While studying the effect of raised extracellular calcium on the burst pattern we noted such a condition. We had to change from a HCO_3/CO_2 based buffer to a Hepes based buffer in order to avoid calcium precipitation.

As a control we studied the glucose evoked electrical activity in standard (25 m*M* HCO_3) buffer compared with low (5 m*M* HCO_3) Hepes buffer. We noted that lowering the HCO_3/CO_2 in the perifusate, while keeping external pH constant (7.4), reduced the burst frequency without changing the active phase (%) within the same islet. We thus tested the effect of this buffer on the threshold and graded increase in active phase induced by glucose. Using mathematical modelling of B-cell electrical activity, we examined mechanisms which could fit the experimental results and provide further insights.

Methods

Experimental: intracellular voltage recordings

We used tail islets microdissected from normal fed Swiss Webster mice (NIH colony 6–14 weeks). We measured

Offprint requests to: P. B. Carroll

membrane potential using standard electrophysiological techniques (Atwater et al. 1978). Using a point 1/2 the way up to the plateau potential, we calculated the relative duration of the active phase (active phase (%)). We used the last 3 min of record obtained during a 10 min exposure to each glucose concentration for these calculations. We also used the last 3 min of steady state record to calculate active phase (%) in the two experimental conditions. Using the equation $I = -Cm\,dV/dt$, we estimated the peak inward (calcium) and outward (potassium) currents from the last 3 min of expanded steady state record in 11 m*M* glucose in each condition. Cm (capacitance) was taken as 5.4 pF (Rorsman and Trube 1986) and dV/dt represented the time derivative of the action potentials (Nassar et al. 1988). Approximately 300 action potentials were analyzed.

Solutions. We performed control experiments using a modified Krebs bicarbonate buffered solution containing (m*M*): 120 NaCl, 5.0 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2$, 1.1 $MgCl_2$, equilibrated with a gas mixture of 95% O_2 5% CO_2 to give a pH of 7.4 at 37 °C.

The low bicarbonate experimental solution contained (m*M*): 120 NaCl, 5.0 KCl, 20 Hepes, 5.0 $NaHCO_3$, 2.5 $CaCl_2$, 1.1 $MgCl_2$ equilibrated with room air, pH 7.4 (adjusted with NaOH) at 37 °C.

Net uptake of $^{45}Ca^{2+}$. We measured $^{45}Ca^{2+}$ uptake into islets isolated by collagenase digestion of the pancreas from adult fed Wistar rats. $^{45}Ca^{2+}$ uptake was measured over 90 min (Malaisse-Lagae and Malaisse 1971). The solutions used for the calcium uptake experiments contained only 1.0 m*M* $CaCl_2$, but were otherwise identical to those used in voltage recording experiments.

Statistics. The experimental results are expressed as mean ± SEM and significance of difference was assessed by Student's t-test.

Theoretical

In this work, we apply the model of Sherman et al. (1988), which incorporates individual stochastic calcium activated K-channel (K-Ca channel) events and has been extended to allow for finite coupling conductance, to examine two hypotheses for the effect of low HCO_3. We performed calculations with a $4 \times 4 \times 4$ cube of cells, each of which is coupled to its 6 neighbors, except for the edge cells which have fewer neighbors. The theoretical parameters which we have varied to account for the data are "*f*" the ratio of free to total intracellular Ca^{2+} and "*g*", the gap junctional conductance between each pair of neighboring cells. (See Appendix for details).

Results

Experimental: intracellular voltage recordings

Effects of low HCO_3 solutions on the frequency of glucose-evoked bursts. Figure 1 compares the steady state effects

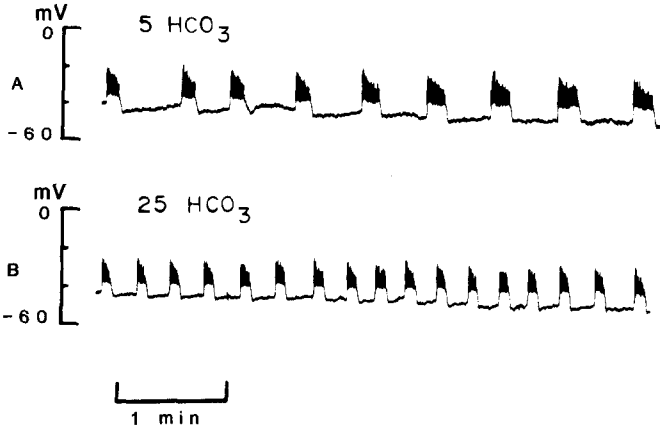

Fig. 1 A, B. Effect of low HCO_3 on glucose-evoked burst frequency in the mouse B-cell. The figure represents steady state portions of the voltage recording from a single B-cell. 11 m*M* glucose was present throughout; HCO_3 concentration was as indicated, pH = 7.4 in both conditions. (This experiment is representative of 7 similar experiments)

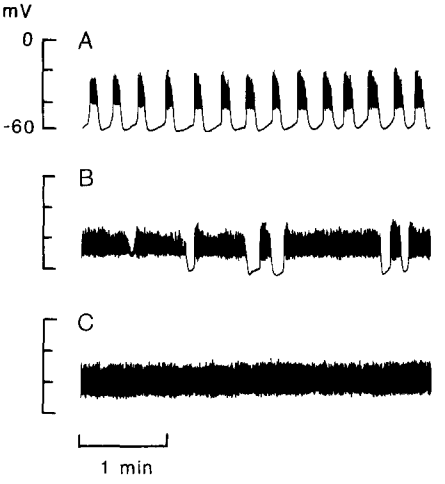

Fig. 2 A–C. Graded increase in active phase (%) in low HCO_3. The figure represents steady state portions of voltage recording from a single B-cell exposed to progressively increasing glucose concentrations from 11 m*M* (**A**) to 16.7 m*M* (**B**) and 22 m*M* (**C**). (This experiment is representative of 5 similar experiments)

of low HCO_3 and control solutions on the glucose evoked burst pattern recorded from the same cell. Lowering the HCO_3/CO_2 content of the perifusion solution decreased the burst frequency in all experiments. This was always reversible upon return to the control solution. Additionally, we compared results from control solutions with low HCO_3 solutions in different islets. The burst frequency was 4.65 ± 0.25 bursts/min in control ($n = 21$) and 2.48 ± 0.18 bursts/min ($p < 0.001$) in low HCO_3 solutions ($n = 21$). The active phase (%) was 49% in the low HCO_3 experiments and was not different from control.

Low HCO_3 solutions do not affect the dose response to glucose. Pancreatic B-cells show a threshold for glucose-induced electrical activity and a graded increase in the active phase (%) (Beigelman et al. 1977). We assessed if the alteration of frequency of glucose evoked bursts was associated with any change in the threshold or active phase (%). Figure 2 shows the graded electrical response

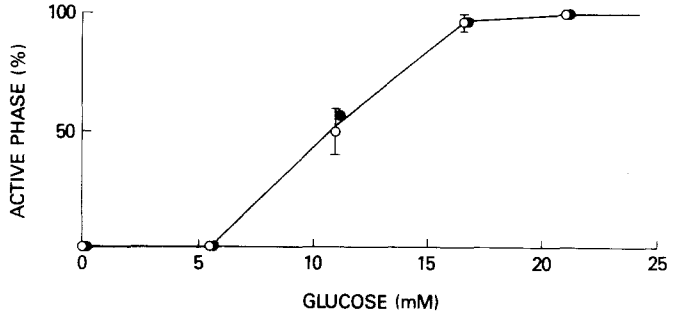

Fig. 3. Threshold and range of response to glucose in low HCO_3 solutions is similar to control. The figure graphically presents the graded increase in % active phase in response to glucose in 5 m*M* HCO_3, Hepes-buffered, solutions (open circles) compared with previously published results in control solutions (Rosario et al. 1985) (filled circles). ($n=5$)

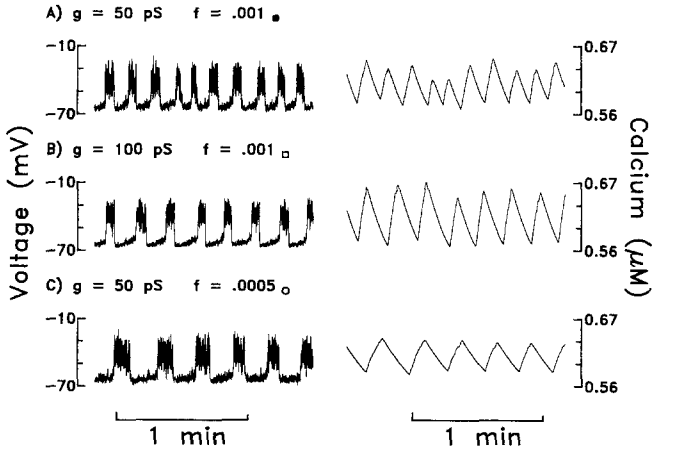

Fig. 4 A–C. Effect of increasing coupling conductance (g) or lowering the ratio of free to total calcium (f) on modelled burst frequency and free cytosolic calcium. Using the mathematical model described in the Appendix, time courses of voltage (left panels) and predicted free intracellular Ca^{2+} (right panels) were computed for one cell in a cube of 64 B-cells coupled by gap junctions of conductance g. Traces for one representative interior cell are displayed because the cells are synchronized. For each case, all other parameters are fixed and the removal rate for intracellular Ca^{2+} (k_{Ca}), which regulates the active phase for each case, $=0.03\ ms^{-1}$. **A** control: pairwise coupling conductance 50 pS and the ratio of free to total intracellular Ca^{2+}, $f=0.001$. **B** Lowering HCO_3 simulated by doubling g. **C** Lowering HCO_3 simulated by halving f to 0.0005. Note that the absolute levels of intracellular Ca^{2+} predicted by the model (right panels) are somewhat arbitrary; we wish to focus attention on the relative changes predicted by changing f and g

to glucose recorded from a single B-cell within an islet perifused with low HCO_3 solutions. Increasing glucose from 11 m*M* (Fig. 2A), to 16.7 m*M* (Fig. 2B) and 22 m*M* (Fig. 2C), produced an increase in the active phase (%). Figure 3 illustrates the full range of the response from 0–22 m*M* glucose. The graph compares the active phase (%) recorded in low HCO_3 solutions to control. The reduced burst frequency in low HCO_3 solutions was not associated with any significant alteration of the threshold or graded response to glucose. There was no apparent change in the distribution of size of spikes along the burst. In addition, maximal peak inward and outward currents were 6.4 ± 0.42 pA and 7.5 ± 0.46 pA respectively. Average peak inward current was 3.6 ± 0.16 pA. Average peak outward current was 4.5 ± 0.44 pA. These values were not significantly different in low HCO_3 experiments (not shown).

Low HCO_3 solutions do not alter $^{45}Ca^{2+}$ uptake. The uptake of $^{45}Ca^{2+}$ in 0-glucose was 4.05 ± 0.32 pmol/islet/90 min in control and 3.28 ± 0.37 pmol/islet/90 min (N.S.) in low HCO_3 solutions. Net uptake in 16.7 m*M* glucose was 7.25 ± 0.32 pmol/islet/90 min in control and 6.63 ± 0.45 pmol/islet/90 min (N.S.) in low HCO_3 solutions.

Theoretical

Burst frequency decreases with a decrease in the ratio of free to total intracellular Ca^{2+} ("f"). In the Chay-Keizer model and its variations (Chay and Keizer 1983; Keizer 1988), some ionic currents are dependent on the instantaneous level of cytosolic calcium, which provides a mechanism for slow feedback from cytoplasm to membrane activity. The model is summarized in the Appendix. In order to understand how the burst frequency in the model depends on calcium buffering we examine the calcium handling mechanism. Total calcium, free plus bound, increases owing to inward current through Ca^{2+} channels and decreases owing to first-order removal, say by pumping or sequestration:

$$\frac{dCa_T}{dt} = -\alpha I_{Ca}(V) - k_{Ca}\,Ca_i$$

where k_{Ca} is calcium removal rate, Ca_i is free cytosolic Ca^{2+} and Ca_T is total calcium. (Note that I_{Ca} is negative so that the first term on the right hand side is positive). Also note that the second term involves Ca_i since only free calcium is accessible to the pump. The term k_{Ca} refers to calcium removal rate. We assume that Ca_i is in instantaneous equilibrium with a pool of high affinity exchangeable calcium binding sites. Under this assumption, the ratio of Ca_i to Ca_T is a constant, denoted "f". Therefore the rate of change of Ca_i is given by

$$\frac{dCa_i}{dt} = f(-\alpha I_{Ca}(V) - k_{Ca}\,Ca_i)$$

We conclude that the rate of increase of Ca_i due to influx, the decrease due to efflux and the net rate of change of Ca_i are proportional to f.

During the silent phase of a burst, the membrane is hyperpolarized and the influx of Ca^{2+} through the channels is smaller than the efflux due to removal. Hence, the net Ca^{2+} flux is outward, and both bound and free Ca^{2+} decrease. When Ca_i reaches a critical minimum value, the cell depolarizes, opening Ca^{2+} channels and initiating the active phase. The Ca^{2+} influx through the channels exceeds the efflux due to removal, so the net flux is inward, and bound and free Ca^{2+} increase. When Ca_i reaches a critical maximum value, the cell repolarizes. These critical values do not depend on f. Therefore, the silent and active phase durations are inversely proportional to f (provided f is small), and reducing f increases burst period without affecting the percent active phase. For example, reducing f from 0.001 to 0.0005 reduced the burst frequency by half (Fig. 4A, C).

The effect of coupling on burst frequency. It is unlikely that lowering HCO_3 affects calcium-insensitive parameters such as the ionic conductances (channel densities, reversal potential and time constants) since varying these would typically change the percent active phase and/or the peak ionic currents. We therefore considered another possibility: cell to cell coupling. Pancreatic B-cells are electrically coupled by gap junctions (Eddlestone et al. 1984; Meda et al. 1984; Meda et al. 1986; Perez-Armendariz et al. 1988).

Coupling is apparently not only important for synchronization of the bursts but possibly also for generating the burst pattern (Sherman et al. 1988; Atwater et al. 1983). Isolated cells exhibit random spiking (Rorsman and Trube 1986), and only B-cells coupled together in an islet, or cell cluster, burst. The model of Sherman et al. (1988) accounted for the behavior of both single cells and islets by treating some channels stochastically and assuming cells are coupled by zero-resistance gap junctions. Further computations with this model show that increased coupling, (in the sense of increased cluster size), reduced burst frequency without affecting the active phase. Here we explore the effects of varying coupling by incorporating gap junctions with finite conductance, g, between each pair of neighboring cells. For the control case of 25 m*M* HCO_3, we set $g=50$ pS and let f be 0.001 (Fig. 4A). We simulated lowering of HCO_3 by doubling the coupling conductance, g, to 100 pS (Fig. 4B). Burst frequency decreased by 26% while the active phase decreased 18% in Fig. 4C, we simulated lowering of HCO_3 using the alternative mechanism of halving f to 0.0005. In this case burst frequency decreased 43% with little effect on active phase. The traces correspond to a representative cell in the cube, and should be compared to the experimental results of Fig. 1.

Comparison of the graded response to glucose. Theoretical treatments. In the model, glucose level is represented by calcium removal rate, k_{Ca}, and is the same in all three cases in Fig. 4. Figure 5 summarizes the effects of changing f and g on the glucose dose-response properties of the model (active phase as a function of k_{Ca}). It corresponds to the experiments on glucose dose response depicted in Figs. 2 and 3. Increased coupling (high g) caused a small but consistent decrease in percent active phase, while increasing the pool size (low f) did not significantly alter the active phase (%).

Effect of altering the ratio of free to bound calcium (f) or coupling (g) on burst frequency at different glucose concentrations. Figure 6 shows the effects of f and g on burst frequency at different glucose levels but the plotting method used here (burst frequency versus active phase) compensates for the shift in the dose response curves of Fig. 5. Decreasing f or increasing g, both reduce the burst frequency; however, decreasing f has a stronger effect. Moreover, the f effect can be tuned by further decreases in f, while the coupling effect appears to saturate and falls off slightly for very large g (not shown). We have not found any examples which produced a greater decrease in frequency by increased coupling than the one presented here. Another interesting effect of increasing g is that it increased the mean peak to peak amplitude of the predicted calcium oscillations (compare Fig. 4 right panels).

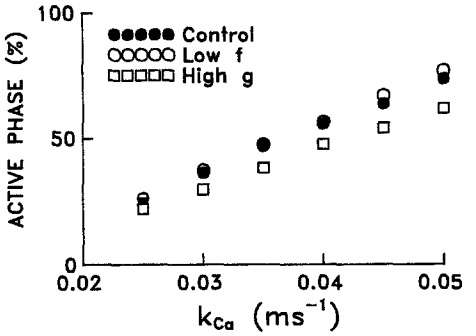

Fig. 5. Theoretical effects of raising g or lowering f on glucose dose response, k_{Ca} is taken to reflect glucose level. The data points for the case $k_{Ca}=0.03$ are from the simulations of Fig. 4. For the control case (filled circles) $g=50$ pS and $f=0.001$. In the high coupling case (squares) corresponding to Fig. 4B $g=100$ pS and $f=0.001$. In the low f case (open circles), corresponding to Fig. 4C, $g=50$ pS and $f=0.0005$

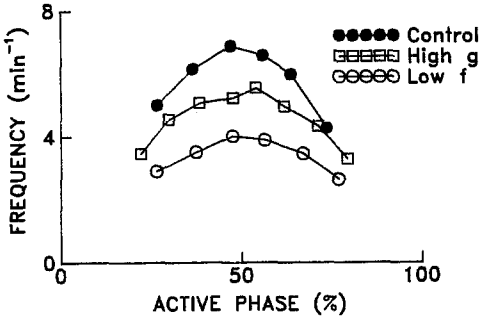

Fig. 6. Theoretical effects of raising g or lowering f on glucose induced burst frequency. We compensated for the shifts in dose response noted in Fig. 6 by plotting frequency against active phase instead of against k_{Ca}. The coupling effect is simulated (squares) as well as the effect of changing exchangeable bound calcium (open circles) as noted previously

Discussion

The ionic mechanisms underlying the characteristic glucose-sensitive burst pattern of the pancreatic B-cell continue to be explored in detail. Most factors such as glucose, calcium, cholinergic and adrenergic agonists which alter burst frequency also affect active phase (%) (Chay 1985; Atwater et al. 1980; Cook et al. 1981; Cook and Perara 1982). We have shown here that these two parameters can be independently controlled. Analysis of burst frequency may provide insights into modulatory influences underlying the rhythmic oscillations the B-cells exhibit in the presence of glucose. It may be important to identify which parameters are most sensitive in controlling the burst frequency. It is likely that factors other than ion permeability control burst frequency since changes in ion channel permeability will produce changes

in active phase (%). In this work we have explored this theoretically and found that there are few parameters that will selectively alter burst frequency. These are alterations in exchangeable calcium buffering or coupling.

Whether alterations in frequency reflect physiologically important differences between islets remains to be determined. There is some general evidence that modulation of oscillatory frequency may have physiological consequences (Nordman and Stuenkel 1986; Martiel and Goldbeter 1987). Additionally, compared to normal B-cells which have a bimodal distribution of burst frequency (Atwater et al. 1980), B-cells from the ob/ob diabetic mouse (a model for type II diabetes) show a unimodal distribution of burst frequency, with only slow frequency bursting patterns observed. The distribution of the active phase (%) is similar to cells from normal mice (Rosario et al. 1985).

Experimentally, lowering HCO_3/CO_2 decreased burst frequency reversibly within the same cell. The graded electrical response to glucose, calculated currents and ^{45}Ca uptake were not affected. One explanation of our experimental results would be that low HCO_3 solutions increase the size of a bound calcium pool in the B-cell. Decreasing f, the ratio of free to bound calcium, in the mathematical model reduced burst frequency with no effect on active phase percent, spike amplitudes, spike frequencies, or peak inward and outward currents. This is consistent with experimental results. Whether reducing CO_2 increases a calcium pool in the B-cell has not been investigated experimentally. In muscle, CO_2 reduces the calcium stores in the sarcoplasmic reticulum (Lea 1986).

An alternative explanation for the experimental results is that cell-to-cell coupling in the islet is increased by lowering HCO_3. One possible explanation for an increase in gap junctional conductance caused by lowering of external HCO_3/CO_2 would be a change in pH_i. Changes in pH_i modulate gap junction conductance (Spray and Bennet 1985). We do not favor this explanation since changes in intracellular pH would alter metabolism and hence would be expected to alter glucose sensing which we did not observe here.

Total removal of HCO_3/CO_2 alkalinizes the cytosol of islets (Lindstrom and Sehlin 1986). Under such conditions activation of the K-ATP channel may be seen (Gillis et al. 1988) (Carroll et al. 1988). An additional difference is that total removal of HCO_3/CO_2 inhibits glucose induced insulin secretion whereas lowering HCO_3/CO_2 does not (Henquin and Lambert 1976). Measurements of pH_i under conditions of low HCO_3/CO_2 in islets have not been made.

As a mechanism for decreasing frequency without changing active phase (%), decreasing f is somewhat more robust than increasing g since it allows a greater frequency range without noticeably affecting the active phase. A variety of models have been proposed to describe B-cell electrical activity (Chay and Keizer 1983; Rinzel et al. 1986; Keizer 1988; Sherman et al. 1988; Chay and Kang 1988), but they all rely on slow calcium feedback on membrane properties, and the effects of changes in "f" and "g" would be similar. Neither mechanism violates the observed insensitivity of calcium uptake or peak ionic currents. The model indicates an interesting difference between the two mechanisms which could be explored experimentally. The mean peak to peak amplitude of the predicted calcium oscillations was increased by enhanced coupling. Since changing f had no effect on mean, minimum or maximum calcium, the two hypotheses might be distinguished experimentally by measuring free Ca^{2+} in bursting B-cells under the two conditions. Alternatively, if the rapidly exchangeable pool could be selectively accessed pharmacologically, depleting or increasing the pool size should reproduce the effects on burst frequency. In addition, if this pool is important for secretion and could be accessed, increased secretion might be noted under conditions which predict a larger mobilizeable calcium pool size (decreased f) i.e. slowed burst frequency. These studies may be difficult since pharmacological agents are not so selective. In addition, insulin secretion from rodent islets is critically dependent on HCO_3 (Henquin and Lambert 1976). It should be possible to detect experimentally if low HCO_3 solutions enhance electrical coupling.

With the experimental data, we can use the model to estimate calcium concentration in intracellular compartments and the magnitude of the proposed changes in bound calcium. Assuming free Ca^{2+} is 0.5 μM (Fig. 4A) and f is 0.001, then the concentration of calcium in the rapidly exchangeable compartment, taken over the entire cell volume, would be about 0.5 mM. Halving the burst frequency requires halving f to 0.0005. This would double the size of the bound pool to 1 mM. Assuming a cell volume of 1000 μm^3, the size of the bound pool when $f=0.001$ would thus be about 0.5 pMol. Since the actual values of Ca_i and f are not known, these estimates must be regarded as provisional. Using the experimental values of total islet calcium and assuming 1000 cells per islet and a fraction of labelled calcium equal to 0.001, we estimate total calcium per cell to be about 7 pMol in 16.7 mM glucose. This is an order of magnitude larger than that of the bound pool in our model. The dynamics of calcium flux from islets are consistent with at least two compartments: a small fast compartment and a large, slow one (6.7%/min and 1.2%/min respectively) (Frankel et al. 1978). Moreover, if the large compartment were insensitive to HCO_3, then net uptake into the cells over 90 min would not show the dependence of the smaller rapidly exchangeable bound pool on HCO_3/CO_2.

We conclude that burst frequency can be modulated independently of the threshold and graded glucose induced increase in active phase. Of the two mechanisms explored theoretically, a change in an exchangeable calcium pool size reproduced the experimental data best. In addition, the interaction between experiment and theory has proven fruitful in suggesting possible differences between these two mechanisms which can be further pursued experimentally.

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Appendix

Chay and Keizer (1983) presented a model to account for the bursting electrical activity of pancreatic β-cells: active phases of rapid spiking alternating slowly with hyperpolarized silent phases. The period of the spikes is hundreds of milliseconds, while that of the bursts is tens of seconds. (See Fig. 1 for an example.) Most versions of the model have the following generic form:

$$C_m \frac{dV}{dt} = -I_{Ca}(V) - I_K(V, n) - g_{K-Ca}(V - V_K) \quad (1)$$

$$\frac{dn}{dt} = \lambda \left[\frac{n_\infty(V) - n}{\tau_n(V)} \right] \quad (2)$$

$$\frac{dCa_i}{dt} = f(-\alpha I_{Ca}(V) - k_{Ca} Ca_i) \quad (3)$$

The independent variables are V, the membrane potential; n, the fraction of potassium channels open; and Ca_i, the concentration of free intracellular calcium. The parameters are C_m, the whole-cell capacitance; λ, a pseudo-temperature parameter to fine-tune the time constant of n; f, the ratio of free to total intracellular Ca^{2+}; α, a factor to convert units of current to units of concentration per time; and k_{Ca}, the rate of removal of free Ca^{2+} from the cytosol due to pumping or sequestration. The particular parameter values and functional forms used here are the same as in Sherman et al. (1988). More detailed analysis of the equations may also be found there.

Equations (1) and (2) generate the spikes during the active phase. The alternation between active and silent phases is the result of variations in the calcium-activated potassium conductance, g_{K-Ca}, which in turn depends on Ca_i:

$$g_{K-Ca} = \bar{g}_{K-Ca} \frac{Ca_i}{K_d + Ca_i}$$

These variations are slow because f, the ratio of free to total Ca^{2+}, in (3) is small (0.001).

Equations (1)–(3) describe the behavior of a cell in an intact pancreatic islet. Sherman et al. (1988) extended the model to account for the erratic spiking of isolated cells by making the calcium-activated potassium (K-Ca) channels stochastic:

$$g_{K\text{-}Ca} = \bar{g}_{K-Ca}\, p$$

where p is determined by a stochastic process such that

$$\langle p \rangle = \frac{Ca_i}{K_d + Ca_i}.$$

This model can also account for the behavior of clusters of cells, which becomes regular like the behavior of whole islets as the number of cells increases, if one assumes that the cells are so tightly coupled that they effectively share a common pool of K-Ca channels. Then p is the fraction of channels open in the cluster, and its fluctuations become smaller as the population size increases.

In this study we use a further extension of the stochastic model of Sherman et al. (1988) in order to study the effects of varying coupling strength. We assume that each pair of neighboring cells in a cube is connected by a gap-junctional conductance, g. The voltage in the jth cell satisfies

$$C_m \frac{dV_j}{dt} = -I_{ion}(V_j, n_j) - \bar{g}_{K-Ca}\, p_j (V_j - V_k) - g \sum_{i \in \Omega_j} (V_j - V_i)$$

where

$$I_{ion}(V, n) = I_{Ca}(V) + I_K(V, n)$$

and Ω_j is the set of neighbors (six for interior points, fewer for boundary points).

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