PERSPECTIVES IN DIABETES

"Take Me To Your Leader": An Electrophysiological Appraisal of the Role of Hub Cells in Pancreatic Islets

Leslie S. Satin,[1] Quan Zhang,[2] and Patrik Rorsman[2,3]

The coordinated electrical activity of β-cells within the pancreatic islet drives oscillatory insulin secretion. A recent hypothesis postulates that specially equipped "hub" or "leader" cells within the β-cell network drive islet oscillations and that electrically silencing or optically ablating these cells suppresses coordinated electrical activity (and thus insulin secretion) in the rest of the islet. In this Perspective, we discuss this hypothesis in relation to established principles of electrophysiological theory. We conclude that whereas electrical coupling between β-cells is sufficient for the propagation of excitation across the islet, there is no obvious electrophysiological mechanism that explains how hyperpolarizing a hub cell results in widespread inhibition of islet electrical activity and disruption of their coordination. Thus, intra-islet diffusible factors should perhaps be considered as an alternate mechanism.

Plasma insulin oscillates with a period of 5–10 min (1). A similar pulsatility is observed in isolated islets obtained from healthy human donors (2). There is evidence that the loss of the insulin pulses, via permanent elevation of the insulin receptors, can contribute to insulin receptor desensitization and insulin resistance (1).

At the level of the β-cell, pulsatile insulin secretion is driven by a stereotypical pattern of electrical activity that consists of groups of action potentials superimposed on slower depolarized plateaus that are separated by hyperpolarized/electrically silent intervals (3). According to the "consensus model" of glucose-induced insulin secretion, glucose uptake and metabolism culminate in an increase in the cytoplasmic ATP/ADP ratio that in turn closes ATP-sensitive potassium (K_{ATP}) channels. The activity of the

K_{ATP} channels maintains a negative membrane potential (approximately -80 to -70 mV) in the β-cell under low-glucose conditions, so closure of K_{ATP} channels by elevated glucose metabolism is required for membrane depolarization (i.e., a less negative membrane potential). When the membrane potential is more positive than -50 mV, voltage-sensitive Ca^{2+} channels open, and the associated increase in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) triggers the exocytosis of insulin-containing secretory granules (4) (Fig. 1A). After an initial phase of spiking activity, the islet settles into a rhythmical bursting pattern (Fig. 1B) that drives pulses of insulin exocytosis. Most of the characterization of β-cell electrical activity was carried out on mouse cells, but it appears that similar processes operate in human β-cells and increasing glucose elicits bursting electrical activity also in β-cells in intact human pancreatic islets (5) (Fig. 1B). Although the exact correlation between the bursts of electrical activity and insulin secretion seen in individual islets and the appearance of plasma insulin oscillations in vivo remains enigmatic, studies of islet rhythmicity can provide insights that can help to explain why plasma insulin oscillations are either smaller in amplitude or not observed at all in patients with type 2 diabetes (6).

In 2016, Johnston et al. (7) proposed that glucose-dependent oscillations in β-cell cytosolic free calcium ($[Ca^{2+}]_i$), which are driven by glucose-dependent electrical bursting activity and linked to pulsatile insulin secretion, are initiated by the activation of specially equipped "hub cells," pacemaker-like cells that initiate and coordinate the oscillatory network of the islet β-cell syncytium. In a subsequent study, these cells were renamed "leader cells" (8). Activation of these hub/leader cells (for example, using the light-activated channel channelrhodopsin) may trigger

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[1]Department of Pharmacology, Brehm Center for Diabetes, University of Michigan Medical School, Ann Arbor, MI
[2]Oxford Centre for Diabetes, Endocrinology & Metabolism, Radcliffe Department of Medicine, University of Oxford, Churchill Hospital, Oxford, U.K.
[3]Metabolic Physiology, Department of Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
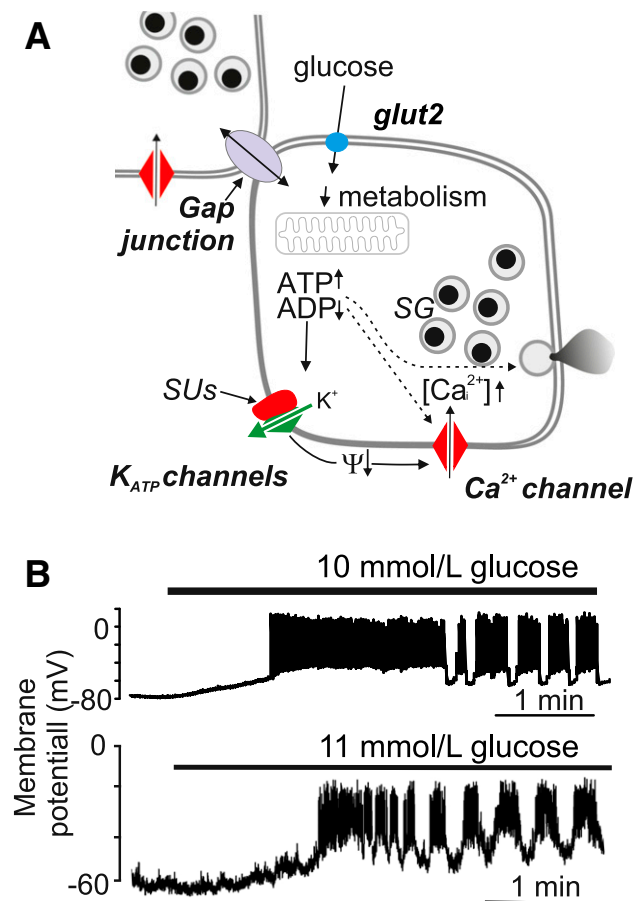
Corresponding author: Leslie S. Satin, lsatin@umich.edu

Figure 1—*A*: Stimulus-secretion coupling of the pancreatic β-cell ("consensus model"). The arrows (↑,↓) indicate an increase or decrease of the indicated parameter. Glucose initiates insulin secretion following its metabolism with a resultant increase in cytoplasmic ATP-to-ADP ratio, closure of K_{ATP} channels, membrane depolarization, opening of voltage-gated Ca^{2+} channels, and initiation of Ca^{2+}-dependent exocytosis of the insulin-containing secretory granules (SG). In addition, the β-cells are electrically coupled to neighboring β-cells via gap junctions consisting of connexin-36. Ψ, membrane potential. Sulfonylureas (SUs) stimulate insulin secretion by closing the K_{ATP} channels. *B*: Glucose-induced electrical activity recorded from a β-cell in intact mouse (top) or human (bottom) islets when the glucose concentration was increased from 1 to 10 or 11 mmol/L (as indicated by horizontal bars).

$[Ca^{2+}]_i$ waves that extend to the other cells within the islets ("follower cells") (9). The propagation of the $[Ca^{2+}]_i$ waves depends on the metabolic state of the β-cell, K_{ATP} channel activity, and cell coupling (10). These coordinated increases and decreases in $[Ca^{2+}]_i$ drive pulses of insulin secretion from individual islets (11).

In this article, we will review the existence and role of hub/leader cells in islets from an electrophysiological viewpoint and highlight some of the earlier studies (some of which were conducted ~40 years ago), which are difficult to reconcile with the leader/follower cell concept and that we feel have not received the recent attention they deserve. As we shall point out, some aspects of the hub/leader cell concept are incompatible with electrophysiological theory and it may

therefore be necessary to consider other possibilities (including intraislet diffusible factors).

Evidence for the Existence of Hub Cells in Pancreatic Islets

Johnston et al. (7) reported that a subset (10%) of "super-connected" β-cells controls electrical activity (and thus $[Ca^{2+}]_i$ and secretion) in the β-cells of the remainder of the islet. This "connectedness" was inferred from pairwise correlation analysis of glucose-induced $[Ca^{2+}]_i$ oscillations. Depolarization of thus identified hub cells (using the light-activated ion channel channelrhodopsin) evoked electrical activity across the entire network of connected β-cells. A key observation of this article was the demonstration that hyperpolarizing these "hub cells" (i.e., making the β-cell membrane potential more negative by photoactivating the light-sensitive Cl^- pump halorhodopsin) disrupted the entire network of oscillating follower β-cells. Notably, photoinhibition of the leader cell did not abolish the $[Ca^{2+}]_i$ oscillations in the followers but they became uncoordinated. In a follow-up study, photoablation of the leader cells (but not follower cells) using laser light reduced $[Ca^{2+}]_i$ in the small islets of zebra-fish larvae (8). The hub cells so identified were also reported to exhibit a characteristic metabolic signature suggesting that they are highly active metabolically with increased ATP production and increased glucokinase expression. In addition, they had reduced insulin content and reduced expression of β-cell–specific "markers" (like Pdx1 and Nkx6.1), suggesting they may be less mature than the other β-cells of the islet. The possible significance of these hub or leader cells for diabetes is that if their activity was important for glucose-induced insulin secretion, their selective dysfunction could in turn contribute to the loss of glucose homeostasis that is a hallmark of type 2 diabetes (12,13). However, it is not immediately evident how hub/leader cells would participate in the synchronization of the pulsatile secretion of 1,000 (mouse) (14) or 1,000,000 (human) (15) islets within the whole pancreas (16), although how well synchronized the electrical bursts of different islets are in vivo is not clear (17).

Cell Coupling

In elevated glucose, widespread oscillations in $[Ca^{2+}]_i$ that correlate with electrical activity and insulin secretion are observed (18–20). The $[Ca^{2+}]_i$ oscillations seen in islets are synchronized by electrical coupling between adjacent β-cells (21). Thus, electrical activity that occurs in one β-cell results in the injection of current into its neighboring β-cells, and a membrane depolarization that may be sufficient to trigger their electrical activity results from this cell-cell communication. Electrical coupling is mediated by proteins known as gap junctions (Fig. 1*A*). Connexin-36 is the only connexin expressed at significant levels in either mouse or human islets (21). The central role of the electrical coupling and the connexins in the coordination of the $[Ca^{2+}]_i$ waves is illustrated by the complete loss of their synchronization and loss of pulsatile insulin secretion after genetic ablation of connexin-36 (21). Importantly, individual cells continue to oscillate.

Electrophysiology allows quantitative measurements of the electrical coupling between islet β-cells. As discussed above, glucose stimulation leads to the initiation of bursting electrical activity in the β-cells. This electrical activity measured with patch-clamp or sharp intracellular electrodes reports the behavior of the single β-cell within the islet that impaled with or connected to the recording electrode and can be suppressed by injecting negative current to clamp the membrane potential at −70 mV regardless of any currents flowing through the membrane (a technique called "voltage-clamp"). This membrane potential is too negative to activate any of the voltage-gated ion channels of the β-cell membrane. Nevertheless, oscillations in the holding current that look like inverted bursts of electrical activity remain observable when the prevailing glucose concentration is >7 mmol/L or so. The interpretation of this phenomenon is that the current oscillations occur because voltage-clamping can only suppress electrical activity in the cell connected to the recording electrode but is insufficient to control the membrane potential of the neighboring β-cells. Thus, the other cells "escape" voltage control and are free to fire action potentials that can spread into the voltage-clamped β-cell via the gap junctions (22,23) (Fig. 2A and B).

Comparing membrane potential and membrane current recordings from the same β-cell allows us to quantitate the extent of the cell-to-cell coupling. The plateaus that are observed are ~20 mV depolarized relative to the most repolarized membrane potentials observed and inject ~20 pA of current into these neighboring cells. From these values, we can estimate that the total gap-junctional

conductance (G_j) experienced by a β-cell in an intact islet is ~1 nS (22) (Fig. 2B). Relating this value to the coupling conductance measured between an isolated pair of β-cells (~200 pS [24,25]) suggests that a β-cell within an intact mouse islet is coupled to an average of approximately five other β-cells.

What do these biophysical data mean, and what are their implications for the hub cell concept? They suggest that although β-cells are unarguably electrically coupled, this coupling is not very strong. Thus, only 30% of the voltage change imposed on the voltage-clamped cell will extend to its neighbors, and there will be a similar degree of attenuation for every layer of β-cells that is situated away from the recording electrode; 9% and 2.7% of the command will propagate to the 2nd and 3rd tier of β-cells, respectively (Fig. 3A). This argument is consistent with direct measurements made by Eddlestone et al. in the early 1980s (26). In those experiments, the same mouse islet was impaled with two fine-tipped glass microelectrodes placed 10–100 μm apart. It was observed that current injected into one cell only elicited a change in membrane potential in the second cell if the distance between them was less than ~35 μm (i.e., equivalent to ~2–3 β-cell diameters).

Importantly, the efficiency of electrical coupling (measured as G_j [see above]) also depends on the resting conductance (G_r) of the β-cell membrane (that at low glucose concentrations principally reflects K_{ATP} channel activity): the greater the G_r, the weaker the impact of G_j will be. For example, in a cell with a G_r of 1 nS (corresponding to a resistance [R] of 1GΩ), a 20-pA current (I) injected into a β-cell from its electrically active neighbors via the gap junctions will produce a 20-mV change in membrane potential (V) by Ohm's law ($V = I \times R$), which would be just sufficient to trigger electrical activity in the neighbor. However, if G_r increases to 3.5 nS (equal to 0.3GΩ: equivalent to what would be seen in the absence of glucose, when K_{ATP} channel activity is high), the current will only produce a 6-mV depolarization in its neighbor, which would be insufficient to induce regenerative electrical activity in an electrically inactive (i.e., hyperpolarized) β-cell. These considerations explain an observation made in mice expressing a gain-of-function mutation in the K_{ATP} channel that was expressed in 60% of the β-cells (27). Although the fraction of β-cells (~40%) that expressed normal K_{ATP} channels displayed glucose-induced $[Ca^{2+}]_i$ responses, these were not synchronized across the islet. This is so because the cells with high K_{ATP} channel activity (i.e., those cells expressing the mutant gain-of-function channels) function as electrical shunts that prevent propagation of the depolarizing wave (Fig. 3B). Similarly, loss of gap-junctional coupling in connexin-36 knockout mice blocks the spread of active currents (28) (Fig. 3C).

Two important conclusions can be drawn from these observations. First, the electrical activity of electrically "insulated" β-cells occurs independently and does not require hub cells. This is in agreement with studies of dispersed islet cells,
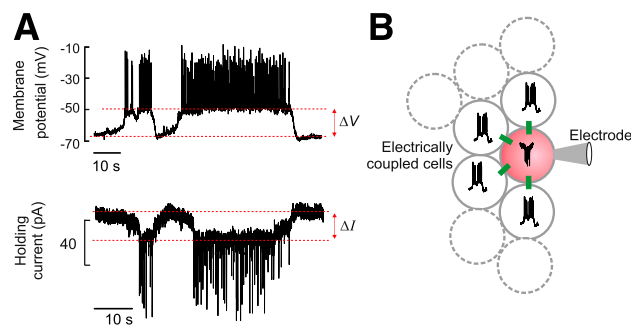
Figure 2—*A*: Membrane potential (top) and holding current (bottom) recorded from the same β-cell under current- and voltage-clamp conditions, respectively. The voltage-clamp recording was obtained at −70 mV. *B*: Schematic of electrical coupling between β-cells in an intact islet. The green rectangles indicate gap junctions. One β-cell within the islet is voltage-clamped at −70 mV through the recording electrode (pink), and regenerative electrical activity in this cell is therefore prevented. Spontaneous electrical activity in neighboring electrically coupled cells results in an inward current that resembles an inverted burst of action potentials in the voltage-clamped cell. Assuming that electrical activity recorded in the β-cell connected to the patch electrode prior to voltage-clamping approximates that of its neighbors, the total gap-junctional conductance (G_j) can be estimated from the equation $G_j = \Delta I/\Delta V$, where ΔV and ΔI represent the current and voltage differences between the plateau current/potential and the most repolarized voltage/least negative current, respectively (as indicated in *A*).
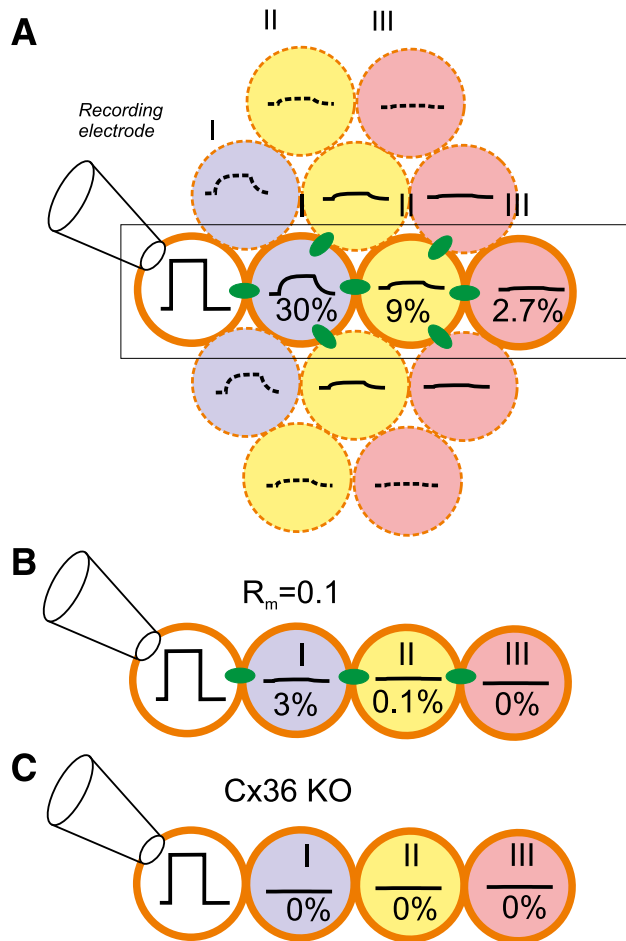
Figure 3—Attenuation of electrical signaling via gap junctions. *A*: A voltage step is applied to a voltage-clamped β-cell in an islet (white) (connected to recording electrode). The change in membrane potential enters neighboring cells (blue) (layer II) via the gap junctions but is attenuated by 70%, and its time course is "filtered" by the resistive-capacitive properties of the β-cell. The voltage change in layer I extends into layer II (yellow) but undergoes additional attenuation, and only 9% remains. In layer III (pink), the signal has declined to <3% of the original value. *B*: Impact of increased K_{ATP} channel activity in a cell within layer I (due to, for example, expression of a gain-of-function mutant K_{ATP} channel or impaired ATP production). If the input resistance in layer I is reduced by 90%, then the voltage change in this and cells in layers II and III will be correspondingly smaller. *C*: Genetic ablation of connexin-36 (Cx36 KO) (the β-cell gap junctions) will abolish electrical coupling. *B* and *C* highlight the part of *A* indicated by rectangle.

which show that 80% of the cells (similar to the percentage of islet cells that are identified as β-cells) respond to glucose with electrical activity and/or an increase in $[Ca^{2+}]_i$ (29). Given that nearly every β-cell in the islet has the capacity of producing spontaneous glucose-induced electrical activity, it would be peculiar if silencing a single β-cell would suffice to silence the entire islet. Indeed, as remarked above, optical silencing of leader/hub cells did not abolish glucose-induced $[Ca^{2+}]_i$ oscillations in the individual cells—it merely led to the disruption of synchronicity. Second, the coordination of electrical activity across the islet syncytium requires electrical connections between β-cells.

To establish whether hub cells exist will require the simultaneous measurement of electrical activity in all of many β-cells in an islet. This feat may eventually be achieved using multiarray electrode measurements of islet β-cell electrical activity (30,31) or optical measurements of membrane potential. Figure 4*A* shows an example where we have monitored spontaneous electrical oscillations in an islet expressing the membrane potential sensor Optopatch2 (32) in its β-cells. The example shown is representative of several measurements made from multiple islets obtained from several mice (J. Tolö, H. Dou, and P.R., unpublished observations). The islet in this example was exposed to 11 mmol/L glucose, and the three measurements shown are taken from three different regions of the islet (R01–R03). It is clear that the β-cells in this case generate bursts of electrical activity that closely resemble those recorded using patch pipettes (compared with Fig. 1*B*). We analyzed the spread of electrical activity during three bursts (shown as B01–B03). The pseudocolor images shown in Fig. 4*B* depict the delay that occurs between burst initiation and electrical activity in different parts of the islet. During burst B01, for example, electrical activity was first detected in RO1 and then spread across the islet within 700 ms. However, the next burst (B02) was initiated in R02 (>60 μm away from R01) and the third burst (B03) was first detected in R03 (>100 μm away from R02). Clearly, in this case at least, there is no hub cell having a fixed location, demonstrating that even if there
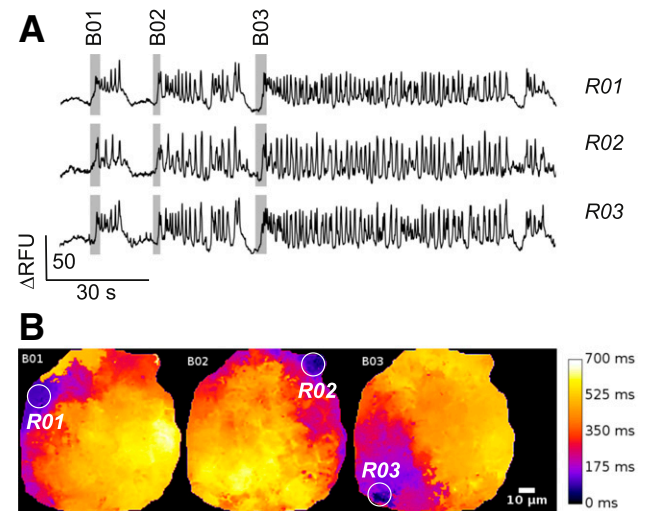

Figure 4—No evidence of hub cells from optical imaging of β-cell electrical activity. *A*: Electrical activity in three regions (R01–R03) within an islet expressing the membrane potential sensor Optopatch2. Responses are shown as the changes in relative fluorescence units (RFU). The gray rectangles superimposed on the traces highlight three successive bursts of electrical activity (B01–B03). *B*: Spread of electrical activity across the islet. The delay with respect to the first point at which electrical activity was detected is displayed in pseudocolors with black corresponding to no delay and white to delay of 700 ms. Note that bursts B01, B02, and B03 are initiated in three different spatially separated areas of the islet (R01–R03: white circles). The measurements in *A* were taken from these regions.

are tendencies for certain regions or certain individual β-cells to fire first in response to glucose stimulation, such roles are not absolute. Ultimately, the resolution to the question of whether hub cells exist will require rapid three-dimensional imaging of electrical activity in every β-cell within the entire islet.

Cell Coupling and the Propagation of Electrical Activity/$[Ca^{2+}]_i$ Waves in Islets

As discussed above, electrical activity in a "leader" cell will inject a depolarizing current into neighboring cells via the gap junctions. In those neighbors with sufficiently low G_r, this may produce sufficient depolarization to exceed the voltage threshold for action potential initiation, which is approximately −50 mV (22). Around this voltage, there is an ∼0.2 s delay, which largely reflects the time between depolarization and the initiation of an action potential (only 5% is due to the time needed for the gap junctions to charge the neighboring cell). Given that β-cells have a diameter of ∼15 μm, we can estimate an upper limit of the spread of electrical activity/$[Ca^{2+}]_i$ as 75 μm/s for mouse islets, in good agreement with what we observed experimentally (22) and similar to the 50–75 μm/s reported by others (7,9). Electrical activity in the "new" cell amplifies the "signal" to its original full amplitude, as action potentials are self-regenerative so that they can be propagated to the next coupled cell, etc. (22), in a way similar to the use of relays in early telegraphs or to the nodes of Ranvier that display saltatory action potential propagation. Thus, unless the signal is continuously regenerated (i.e., by the initiation of new electrical activity) it will decay to very low values within 1–2 cell diameters and thus die out. It is implicit from this argument that the follower cells have to be close to threshold and likely would have fired soon anyway. This is consistent with the weak spread of $[Ca^{2+}]_i$ waves at substimulatory glucose concentrations (9).

Oscillatory Electrical Activity Can Be Reset by Current Injection

The concept that electrical activity in leader cells propagates across the islet via gap junctions and successive electrical activation of follower cells explains why it is possible to reset the rhythmicity of glucose-induced electrical activity by experimentally injecting large currents into islets (33). It was shown by Cook et al. (33) in the early 1980s that the injection of exogenous currents into the islet while simultaneously recording electrical activity from a β-cell within it could either prematurely trigger an oscillation during the silent phase between bursts or prematurely hasten its deactivation and repolarization by applying depolarizing or hyperpolarizing currents, respectively. Notably, the currents required to influence β-cell electrical activity in these experiments were very large in amplitude (∼10 μA, i.e., >100,000-fold larger than those used to identify hub cells) and had to be applied over a large area of the islet to be effective. This means that many islet cells had to be electrically stimulated or inhibited to significantly alter the observed bursting pattern.

The finding that membrane depolarization can induce widespread Ca^{2+} waves (now confirmed in islets by optogenetic means [7]) is uncontroversial, and these waves are initiated and propagated as we discussed above. However, the finding that a 50-mV hyperpolarization applied to a single hub cell can abolish the activity of the rest of the islet is difficult to reconcile with what is known about β-cell coupling and electrical activity, as this hyperpolarization would be largely restricted to the cell to which it is applied. This is because there is no obvious mechanism that can regenerate a hyperpolarization as it spreads across the islet, and the effect on membrane potential would quickly decline to very low values: the 50-mV hyperpolarization in the leader cell produced by activation of halorhodopsin would decrease to 15 mV in the first layer neighbors, <5 mV in the second layer, and ∼1 mV in the third layer, etc.

We used the oscillations in holding current seen in voltage-clamped cells described above to indirectly monitor electrical activity in the neighbors when the β-cell connected to the recording electrode is experimentally hyperpolarized (Fig. 5). We found that in islets exposed to 10–20 mmol/L glucose, when the β-cells are firing action potentials from a plateau potential of approximately −40 mV, hyperpolarizing the β-cells' membrane potential to −70 ($n =$ 33 [20 mmol/L glucose]) or less than or equal to −110 mV ($n = 10$ [10 mmol/L glucose]) had no effect on islet electrical activity. From these observations, we can conclude with 99% certainty that hub cells do not operate via electrical coupling; there is a 1% chance ($100 \times [1 − 0.1]^{43}$%) that we would have missed the "hub" cells in 43 trials if they represented 10% of the islet cells.
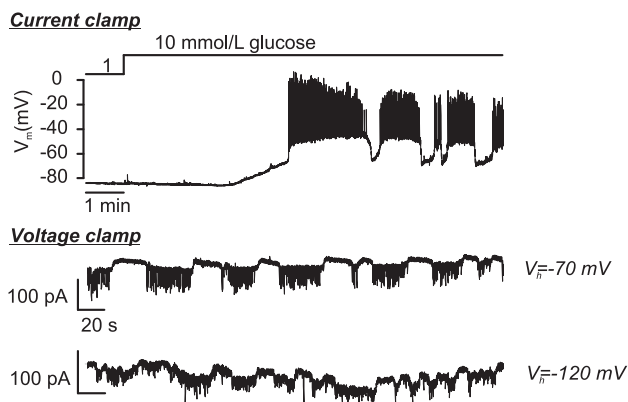

Figure 5—Hyperpolarization of β-cells does not affect electrical activity in neighbors. Changes in β-cell membrane potential when increasing glucose from 1 to 10 mmol/L recorded under current-clamp conditions (top) and under voltage-clamp conditions when holding the membrane potential at −70 mV (middle) or −120 mV (bottom). The current oscillations reflect glucose-induced electrical activity in neighboring (unclamped) β-cells. The bottom trace appears noisier at −120 mV because of membrane breakdown (partial seal rupture) around the recording electrode at this unphysiologically negative membrane potential.

Possible Role of Diffusible Factors

Collectively, the findings reviewed above argue that the disruption of the coordination observed upon optical (halorhodopsin) hyperpolarization is unlikely to rely solely on an electrical signal and may perhaps (at least in part) reflect diffusible paracrine signals released within the islet. Photoactivation of β-cells in islets exposed to 5 mmol/L glucose has previously been shown to evoke action potential firing in δ-cells and stimulate somatostatin secretion (34). Possibly, hyperpolarization of β-cells may hyperpolarize δ-cells electrically connected to the hub cell, which may result in a paradoxical stimulation of δ-cell electrical activity and somatostatin secretion by reactivation of channels that have undergone voltage-dependent inactivation. Thus, studies using somatostatin receptor antagonists may be rewarding.

In more recent work, it was reported that photoablation of leader cells abolishes glucose-induced β-cell $[Ca^{2+}]_i$ oscillations in zebrafish islets (8). However, in other studies the β-cell glucose-induced $[Ca^{2+}]_i$ oscillations in zebrafish islets were poorly correlated, and thus islets in this species might lack strong gap-junctional coupling (35). It is therefore of interest that connexin-35b, the zebrafish ortholog of connexin-36, is not detected in zebrafish islets (35). It would be interesting to know whether the suppression of the $[Ca^{2+}]_i$ oscillations was permanent of if they resumed if the islets were allowed sufficient time to recover. It is also noteworthy that in mouse islets, ablation of the "1st responder" (the leader cell) did not affect the capacity of the islet to respond to glucose with an increase in $[Ca^{2+}]_i$ and a new 1st responder emerged (36). If these preliminary data can be confirmed, it would reinforce our reservations on electrophysiological grounds about the hub/leader cell concept.

Coda

We have reviewed the role of leader/hub cells from an electrophysiological perspective. Whereas depolarization of these cells may suffice to trigger electrical activity and $[Ca^{2+}]_i$ waves in the rest of the islet, it is difficult for one cell to supply enough current to repolarize the entire islet.

While there is no direct evidence yet, an alternative hypothesis might be that diffusible factors and not electrical coupling may be preferentially released by specialized cells or groups of cells within islets. Future research should concentrate on the molecular identification of such putative diffusible factor(s). Evidence has appeared for possible roles of released nitric oxide (37), carbon monoxide (38), and GABA (39,40), but these will need to be evaluated much more thoroughly within the context of hub cells and β-cell–β-cell synchronicity.

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