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Magnitude and Modulation of Pancreatic β-Cell Gap Junction Electrical Conductance *In Situ*

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Abstract. The parallel gap junction electrical conductance between a β-cell and its nearest neighbors was measured by using an intracellular microelectrode to clamp the voltage of a β-cell within a bursting islet of Langerhans. The holding current records consisted of bursts of inward current due to the synchronized oscillations in membrane potential of the surrounding cells. The membrane potential record of the impaled cell, obtained in current clamp mode, was used to estimate the behavior of the surrounding cells during voltage clamp, and the coupling conductance was calculated by dividing the magnitude of the current bursts by that of the voltage bursts. The histogram of coupling conductance magnitude from 26 cells was bimodal with peaks at 2.5 and 3.5 nS, indicating heterogeneity in extent of electrical communication within the islet of Langerhans. Gap junction conductance reversibly decreased when the temperature was lowered from 37 to 30°C and when the extracellular calcium concentration was raised from 2.56 to 7.56 mM. The coupling conductance decreased slightly during the active phase of the burst. Activation of adenylate cyclase with forskolin (10 μM) resulted in an increase in cell-to-cell electrical coupling. We conclude that β-cell gap junction conductance can be measured *in situ* under near physiological conditions. Furthermore, the magnitude and physiological regulation of β-cell gap junction conductance suggest that intercellular electrical communication plays an important role in the function of the endocrine pancreas.

Key words: Pancreatic islet — Cell-to-cell coupling — Calcium — Burst — Synchrony — Insulin

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Introduction

Changes in the rate of insulin secretion from the islet of Langerhans are closely correlated with changes in intercellular communication among β-cells as indicated by gap junction morphology (Meda, Perrelet & Orci, 1979; in't Veld, Schuit & Pipeleers, 1985; in't Veld, Pipeleers & Gepts, 1986; Meda, 1989), spread of fluorescent tracers (Kohen et al., 1979; Michaels & Sheridan, 1981; Kohen, Kohen & Rabinovitch, 1983; Meda et al., 1983; Michaels et al., 1987; Sorenson et al., 1987; Meda, 1989; Meda et al., 1990), and gap junction electrical conductance measurements (Eddlestone et al., 1984; Meda et al., 1984; Meda et al., 1991; Pérez-Armendariz et al., 1991). Furthermore, isolated β-cells and those within intact islets display vastly different electrical (Rosman & Trube, 1986; Falke et al., 1989; Rojas et al., 1990; Kukuljan, Gonçalves & Atwater, 1991) and secretory responses to glucose (Halban et al., 1982; Pipeleers et al., 1982; Meda, 1989), indicating that cell-to-cell communication plays an important role in shaping islet glucose responsiveness. Nevertheless, the biophysical properties of β-cell gap junctions and the functional roles of intercellular communication within the endocrine pancreas remain poorly understood. Dye and electrical coupling studies indicate that both the cultured rat islet and the microdissected mouse islet are organized into small domains of 1–6 coupled cells (Kohen et al., 1979; Michaels & Sheridan, 1981; Meda et al., 1982; Kohen et al., 1983; Meda, Santos & Atwater, 1986; Michaels et al., 1987; Meda, 1989), while the almost totally synchronous β-cell electrical (Meissner, 1976; Eddlestone et al., 1984; Meda et al., 1984; Santos et al., 1991; Gilon & Henquin, 1992; Roe et al., 1993; Valdeolmillos et al., 1993) and secretory (Scott, Atwater & Rojas, 1981; Rosario, Atwater & Scott, 1995) activity within an islet support the notion of widespread cell-to-cell communication. The apparently

restricted extent of intercellular communication within the islet of Langerhans has led to the suggestion that an extracellular factor, such as periodic accumulations of ions in the interstitial space (Pérez-Armendariz & Atwater, 1986), may be responsible for synchronizing islet electrical activity (Stokes & Rinzel, 1993).

To characterize the nature of electrical coupling among β-cells under physiological conditions, we have modified an intracellular voltage-clamp technique used to study membrane ionic currents from β-cells *in situ* (Rojas et al., 1995), to measure the parallel electrical conductance between one β-cell and its nearest neighbors. The results indicate that electrical communication within the islet of Langerhans is more extensive than dye transfer studies predict. Furthermore, we have studied the effects of changes in calcium concentration and temperature, as well as addition of the adenylate cyclase activator forskolin, on β-cell gap junction conductance. We conclude that electrical coupling among pancreatic β-cells is subject to physiological regulation and plays a significant role in the observed synchronous activity within the islet of Langerhans.

Materials and Methods

Theory (Sherman, Xu & Stokes, 1995)

In the presence of stimulatory glucose concentrations, neighboring β-cells within the islet of Langerhans undergo periodic oscillations in membrane potential, or bursts, which are nearly identical in magnitude and phase (Eddlestone et al., 1984; Meda et al., 1984; Santos & Rojas, 1987). A single β-cell within the islet can be impaled with an intracellular microelectrode and the potential clamped at a hyperpolarized level so that it is no longer able to burst. The holding current required to maintain this potential is given by

$$I_h = I_m + I_c \tag{1}$$

where I_m is the sum of the membrane currents and I_c is the sum of the currents into neighboring cells through gap junctions. The junctional current is driven by the difference between the holding potential of the impaled cell and the membrane potential of the coupled neighbors:

$$I_c = \sum_{k\in\Omega} g_{j,k}(V_h - V_{n,k}) \tag{2}$$

where V_h is the holding potential, $V_{n,k}$ is the membrane potential of the k^{th} neighboring cell, $g_{j,k}$ is the gap junction conductance between cell k and the impaled cell, and the sum is taken over all neighboring cells. Since the surrounding cells have approximately identical membrane potential, and β-cell gap junction conductance is voltage independent (Pérez-Armendariz et al., 1991), the holding current can be expressed as

$$I_h = I_m + (V_h - V_n)\sum_{k\in\Omega} g_{j,k} = I_m + (V_h - V_n)G_j \tag{3}$$

where V_n is the potential of the neighboring cells and G_j is the sum of the individual cell-to-cell conductances between the clamped cell and neighboring β-cells. The surrounding cells are free to burst, so the potential V_n cycles periodically between the active and silent phase levels, and the holding current record consists of "bursts" of inward current reflecting the electrical activity of neighboring cells. The silent and active phase levels of the holding current can be expressed in terms of the corresponding membrane potential levels in surrounding cells:

$$\begin{aligned} I_{h,s} &= I_m + G_j(V_h - V_{n,s}) \\ I_{h,a} &= I_m + G_j(V_h - V_{n,a}) \end{aligned} \tag{4}$$

where the subscripts s and a denote silent and active phase, respectively. Subtracting Eq. 4 and rearranging yields an expression for the parallel gap junction conductance

$$G_j = \frac{I_{h,s} - I_{h,a}}{V_{n,a} - V_{n,s}} \tag{5}$$

The electrical behavior of the neighboring β-cells is not measured directly, but because nearby cells display identical electrical activity (Eddlestone et al., 1984; Meda et al., 1984; Santos & Rojas, 1987), the burst pattern of the neighboring cells can be estimated by recording a few voltage bursts from the impaled cell in current clamp mode. The active to silent phase voltage change so recorded can then be used in conjunction with the magnitude of the current bursts obtained after switching to voltage clamp in order to calculate G_j. Beta cells also form gap junctions with α-cells (Orci et al., 1975), and electronic spread of current from neighboring, active β-cells causes small oscillations in α-cell membrane potential (Meda et al., 1986). However, these oscillations are not large enough to make significant contributions to the current bursts, and hence G_j as calculated with Eq. 5 does not include coupling conductance between the impaled cell and neighboring α-cells.

In addition to the assumption that neighboring β-cells exhibit identical electrical activity, it has also been assumed that the membrane holding current (I_m) is constant and that clamping the potential of the impaled cell has no effect on the magnitude of the voltage bursts of the surrounding cells. Control experiments in which the K^+ concentration was increased to suppress the bursts in surrounding cells and experiments in which the holding potential was altered were used to verify that errors caused by making these assumptions are small. Furthermore, based on a mathematical model of coupled β-cells, in which this theory was originally proposed, Sherman and colleagues (1995) predict that the error in conductance measurement due to the effect of voltage clamping on the electrical activity of surrounding cells will be less than 5% for single cell-to-cell conductances of 100 nS and less than 25% for conductances of 400 nS. These values cover much of the range of β-cell gap junction conductance measured in dual whole-cell voltage clamp studies (Meda et a., 1991; Pérez-Armendariz et al., 1991).

Experimental Methods

The details of the electrophysiological technique for recording membrane potential from microdissected mouse pancreatic islets have been presented elsewhere (Atwater, Ribalet & Rojas, 1978). Briefly, islets of Langerhans were removed by hand from the pancreata of 2–5 month old albino mice and pinned to the sample chamber. The perifusion medium was a modified Krebs solution containing, in mM, 120 NaCl, 24 $NaHCO_3$, 5 KCl, 2.56 $CaCl_2$, 1.13 $MgCl_2$, and was equilibrated with 95% O_2: 5% Co_2 to maintain the pH between 7.4 and 7.5 at 37°C. Glucose was added without adjusting for changes in osmolarity. Calcium and potassium were increased where indicated by making additions from 1 M stock solutions of $CaCl_2$ or KCl, again without adjusting

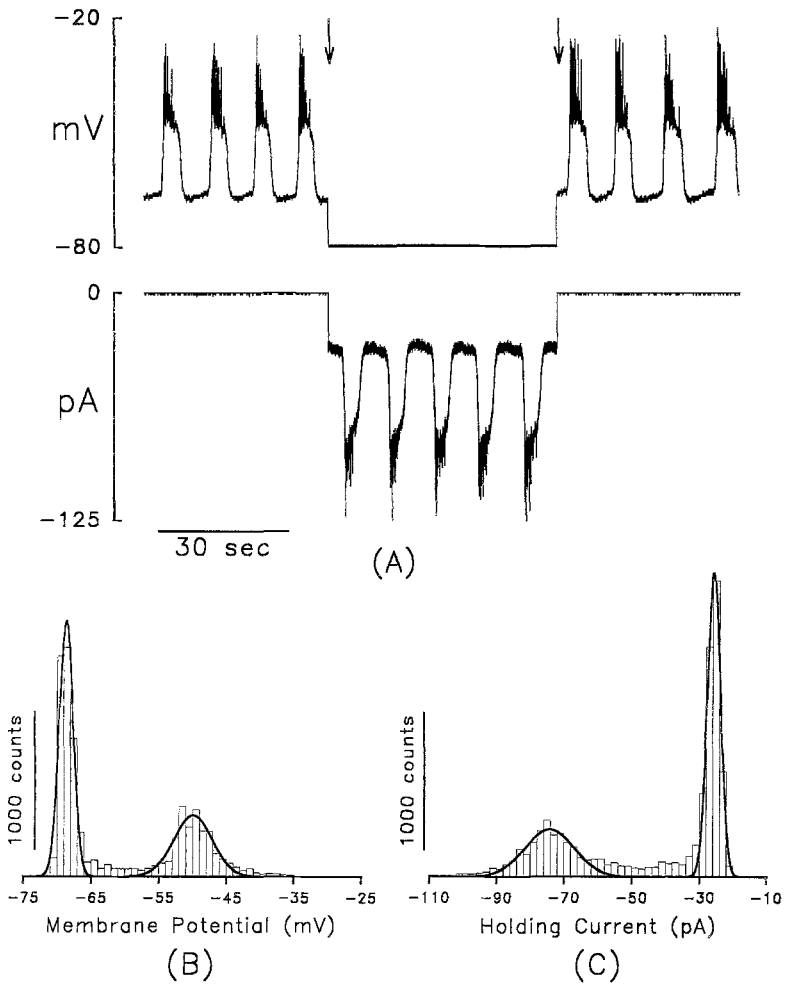

Fig. 1. *In situ* coupling measurement. (*A*) Simultaneous recordings of membrane potential (top) and holding current (bottom) from a single β-cell during an *in situ* coupling conductance experiment. The arrow at the left indicates switch from current clamp ($I_h = 0$) to voltage clamp ($V_h = -80$ mV). Arrow at the right indicates return to current clamp mode. (*B*) Membrane potential histogram created from th digitized current clamp record from the top trace of 1*A*. Gaussian fit to the data (solid curve) has peaks at −68.7 mV (V_s) and −49.9 mV (V_a). (*C*) Holding current histogram from the bottom trace of 1*A* during voltage clamp. Peaks are at −25.7 pA (I_s) and −74.2 pA (I_a). Parallel coupling conductance from Eq. 5 was 2.58 nS.

for osmolarity. In experiments involving forskolin, the drug was added from a 10 mM stock solution in DMSO, and the control Krebs solution was augmented with DMSO to the same concentration. The temperature of the perifusate was controlled with a Peltier heating element located proximal to the chamber inflow, and was monitored continuously by means of a miniature thermistor (Model HH-80 E, Omega Engineering, Stamford, CT) placed in the sample chamber.

Membrane potential measurements were made between two Ag-AgCl wires, one in the microelectrode and the other in the sample chamber. Microelectrodes were constructed from borosilicate capillary glass having 2 mm external diameter and 1 mm internal diameter (FHC, Brunswick, ME) on a vertical puller (Narashige Instruments, Tokyo, Japan). The electrodes were filled with a 1:1 mixture of 1 M KCl and 1 M K-citrate and had a tip resistance of 150–200 MΩ. The large potential difference between the electrodes was offset by connecting the chamber electrode to a variable voltage source which had one terminal grounded. The output of the current clamp amplifier was adjusted to zero using this voltage source prior to penetration of a cell (Rojas et al., 1995).

Cells were impaled using a current clamp amplifier equipped with a "cell puncture circuit," and β-cells were identified based on their bursting electrical activity in 11.1 mM glucose. Once a β-cell had been impaled, a manual switch was used to disconnect the preparation from the current clamp amplifier and connect it to an amplifier (EPC-7, List Electronics, Darmstadt-Eberstadt, Germany), which was used for both voltage and current clamp measurements. Voltage and current records were printed on a chart recorder (Model 2400S, Gould, Cleveland, OH), and stored on magnetic tape (Store 4, Racal Recorders, Irvine, CA) for future analysis.

Coupling measurements were made by first recording several membrane potential bursts under current clamp conditions to estimate the electrical behavior of neighboring cells and then switching the amplifier to voltage clamp mode to record the coupling current. Cells were held at −80 mV unless otherwise indicated, and all experiments were performed with 11.1 mM glucose in the perifusion medium. The voltage clamp amplifier was used with series resistance compensation, which generally corrected 50–70% of the voltage drop across the high access resistance of the intracellular microelectrode. For analysis, membrane potential records containing at least 3 bursts were digitized at 200–500 Hz using a commercial software package (Axotape 2.0, Axon Instruments, Foster City, CA). A second software package (PCLAMP 5.5, Axon Instrs.) was used to construct histograms of membrane potential from the digitized records. These histograms had two peaks corresponding to the average silent phase and active phase plateau potentials. Bimodal distributions were fit to the data and the voltage levels of the two peaks were taken as $V_{n,s}$ and $V_{n,a}$. The holding current records were analyzed in an identical manner to determine $I_{h,s}$ and $I_{h,a}$, and coupling conductance was calculated using Eq. 5.

To detect changes in coupling, the protocol described above was first performed with normal Krebs solution as the perifusate, then the modulator of interest was added to the solution and the measurement was repeated 5 to 10 min later. To test for reversibility, the islet was

perifused with normal Krebs solution for at least five min before the protocol was repeated. Significance levels of the observed changes in coupling conductance were determined using the paired student's *t*-test.

Results

In situ Coupling Measurements

Figure 1*A* shows data from an *in situ* gap junction conductance experiment, with membrane potential on the upper trace and holding current on the lower trace. The left-most portion of the upper trace illustrates typical bursting electrical activity in the presence of 11.1 mM glucose, recorded in current clamp mode. When the potential of the cells was clamped at −80 mV, as indicated by the arrow on the left, bursts of inward current resulting from synchronized bursting in surrounding, coupled β-cells were recorded. The shape of the current bursts is nearly identical to that of the previously recorded voltage bursts, providing evidence that the behavior of neighboring cells can indeed be estimated from the membrane potential record of the impaled cell. Returning to current clamp mode (arrow at right of Fig. 1*A*) confirmed that the burst magnitude and frequency were not altered by the voltage clamp protocol. Figures 1*B* and *C* show, respectively, the membrane potential and holding current histograms generated from the digitized records in Fig. 1*A*, along with Gaussian fits to the data. The average voltage change during the bursts (measured as the potential difference between the locations of the two peaks in Fig. 1*B*) was 18.8 mV; the average current change (Fig. 1*C*) was 48.5 pA. From Eq. 5, the parallel coupling conductance between this cell and neighboring β-cells was 2.58 nS. Note that this measurement gives no information about the magnitude of individual cell-to-cell conductances or the number of cells with which this β-cell communicates.

Similar "inverted" bursts of current were seen in each of over 35 β-cells which were clamped in the presence of 11.1 mM glucose, suggesting that no electrically active β-cell exists in isolation within the intact islet. In some experiments the electrode tip clogged during the impalement, as indicated by a gradual decrease in the baseline holding current and tip resistance measurements of greater than 300 MΩ upon removal from the cell. Figure 2 shows a coupling conductance magnitude histogram generated from twenty-six recordings in which the baseline holding current remained constant throughout the impalement and the electrode resistance was less than 275 MΩ at the end of the experiment. The histogram has two peaks, and the data were fit with a bimodal Gaussian distribution having peaks at 2.49 (SD 0.25 nS) and 3.47 nS (SD 0.25 nS), as shown in Fig. 2. The maximum and minimum parallel coupling conductances were 4.11 and 2.10 nS, respectively.

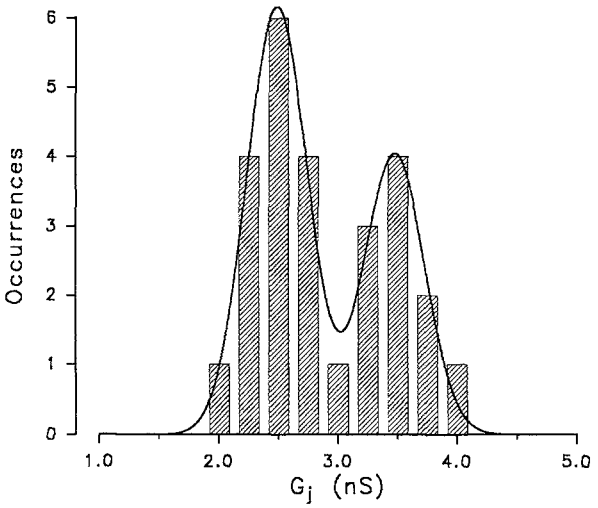

Fig. 2. Histogram of parallel coupling conductance magnitude from 26 *in situ* coupling experiments. Bimodal Gaussian fit to the data (solid curve) has one peak at 2.49 nS (SD 0.25 nS) and one peak at 3.47 nS (SD 0.25 nS). Fit was made using a commercial software package (Sigmaplot 5.0, Jandel Scientific, San Rafael, CA).

Testing the Assumptions Underlying the *In Situ* Method

In situ gap junction conductance measurements are based on the assumptions that (i) neighboring β-cells exhibit identical electrical activity, (ii) the β-cell membrane current (I_m) is constant at a constant holding potential, and (iii) clamping the potential of the impaled cell does not affect the electrical activity of surrounding cells (Sherman et al., 1995). While identical electrical activity in nearby β-cells has been well established using simultaneous intracellular recordings (Eddlestone et al., 1984; Meda et al., 1984; Santos & Rojas, 1987), the latter two assumptions require validation.

The issue of constant membrane current arises from suggestions that the cycling of the β-cell membrane potential between the active and silent phases in the presence of glucose may be controlled by a voltage-independent, metabolically regulated ion channel in the plasma membrane (Henquin, 1988; Cook & Ikeuchi, 1989; Rojas et al., 1990). Metabolically induced oscillations in whole-cell conductance could cause errors in the junctional conductance measurement by adding an inward component to the current bursts that is not due to coupling current. To search for cyclic membrane currents, the voltage clamp was performed in elevated extracellular potassium, a condition which suppresses glucose induced bursting in mouse islets (Dean & Matthews, 1970; Dawson, Atwater & Rojas, 1984). With the changes in coupling current abolished, any oscillations in holding current could only be attributed to nonjunctional events. Figure 3 shows a current record from such an experiment. The holding potential was −80 mV, and in normal potassium (5 mM), bursts of coupling current

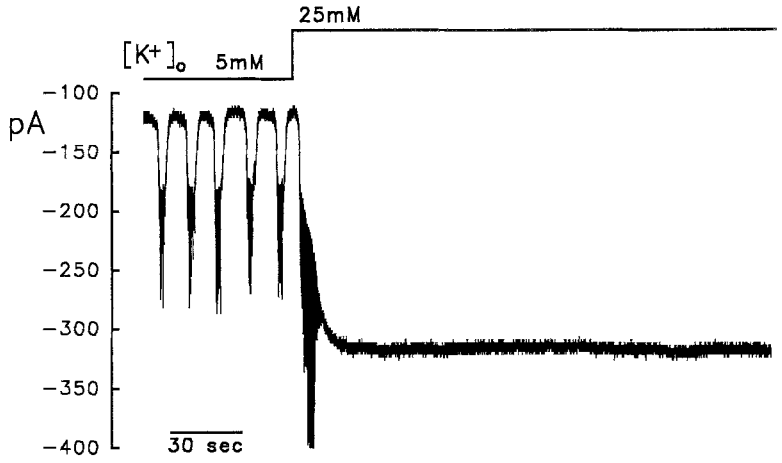

Fig. 3. Effect of increasing extracellular potassium on *in situ* holding current record. Switching to current clamp in elevated potassium confirmed that bursting was abolished by this condition (*not shown*).

were observed. When the extracellular potassium was increased to 25 mM, the holding current increased and the bursts disappeared. The same effect was seen in 11 other β-cells, indicating that any cyclic membrane current must have magnitude less than about 5 pA. Therefore an insignificant error is introduced to the coupling measurement by assuming that the entire current burst results from changes in junctional current.

The effect of the voltage clamp protocol on the electrical activity of neighboring β-cells was determined by varying the holding potential (V_h). Figure 4*A* shows the results of an experiment in which V_h was varied in steps of 40 mV from −80 to 0 mV. At a holding potential of −80 mV, the current bursts resembled the voltage activity recorded during current clamp (*not shown*). Changing V_h to −40 mV dramatically increased the size of the spikes in the active phase of the current bursts. At 0 mV, the spike size returned to that seen at −80 mV, but the current change from the silent to active phase was greatly reduced. The modifications in the shape of the current bursts were reversible upon returning to a holding potential of −80 mV. The same results were seen in 6 of 6 experiments, demonstrating that the electrical activity of neighboring β-cells changes when the impaled cell is voltage-clamped. However, as illustrated in Fig. 4*B*, small changes in holding potential in the range of −90 to −70 mV had very little effect on the shape of the current bursts. In five experiments, the magnitude of the current bursts ($I_{h,a} - I_{h,s}$) changed by less than 3% when the holding potential was varied in the range −70 to −100 mV. These results indicate that hyperpolarized holding potentials should be used to minimize the effect of voltage-clamping on the electrical activity of surrounding cells, thus yielding the most accurate measurement of coupling conductance.

Effect of Temperature on Electrical Coupling

Temperature-dependent modulation of voltage and current bursts is illustrated in Fig. 5. The upper traces show membrane potential recordings from a β-cell made at 37 and 30°C. The increased burst duration and reduced burst frequency at lower temperature agree with previous results (Atwater et al., 1984). The bottom traces show the holding current records obtained when the voltage of the same cell was clamped at the two different temperatures. While the active-silent phase voltage change was the same in the membrane potential records at the two temperatures, the corresponding current change during voltage clamp was much smaller at 30°C. The coupling conductances were 2.44 and 1.84 nS at 37 and 30°C, respectively. The conductance returned to its initial value after the temperature was restored to 37°C (*data not shown*). In 5 of 6 experiments the coupling conductance decreased by an average of 21% ($P < 0.01$) when the temperature was lowered from 37°C to 28–30°C. The one cell that did not illustrate this trend underwent large changes in coupling conductance that were not correlated with temperature, and may have been due to an unstable electrode resistance. In the two experiments in which the electrode stayed in the cell long enough to return to 37°C, the change in coupling conductance was reversible.

Effect of Calcium on Electrical Coupling

The effect of extracellular calcium concentration on intercellular communication in the islet was determined by measuring the coupling conductance in both normal (2.56 mM) and high (7.56 mM) calcium. The results of one such experiment are illustrated in Fig. 6. While the active to silent phase voltage difference increased when calcium was elevated (top traces), the corresponding current changes were very similar at the two calcium levels (bottom traces). The net result for this experiment was a decrease in coupling conductance from 3.01 nS in normal calcium to 2.52 nS in 7.56 mM calcium. In 6 of 8 experiments, the coupling conductance was reduced in elevated calcium ($P < 0.01$) with a mean change of

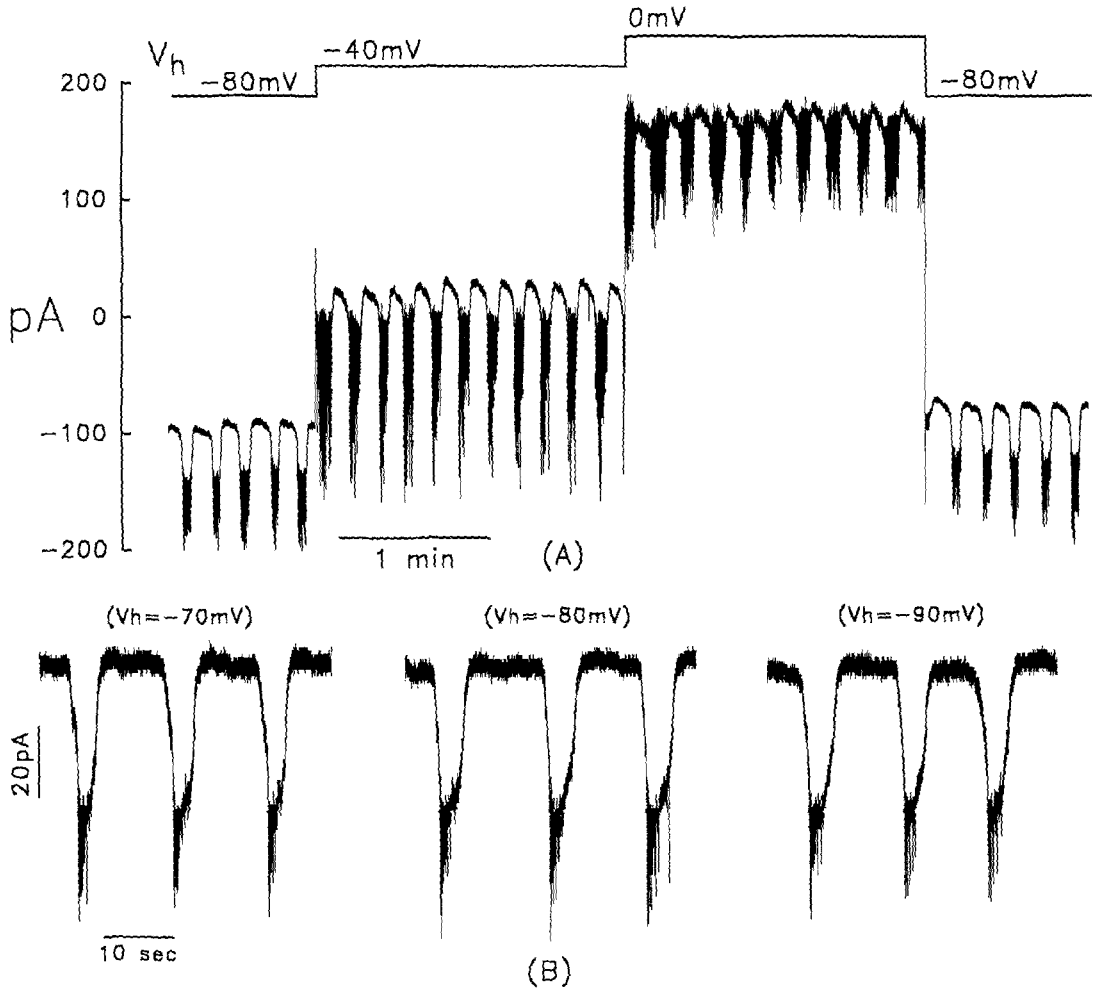

Fig. 4. Effect of holding potential on coupling current record. (*A*) Large changes in holding potential alter the shape of coupling current bursts. Holding potential was under computer control. Membrane potential levels for this cell under current clamp were $V_s = -52$ mV and $V_a = -42$ mV. (*B*) Small changes in holding potential at hyperpolarized levels have little effect on current burst magnitude. To facilitate comparison of burst magnitude, traces have been shifted to align silent phase levels. Time scale applies to all three traces. Membrane potential levels for this cell under current clamp were $V_s = -56$ mV and $V_a = -44$ mV.

17.5% in the six cells showing an effect. When the impalement lasted long enough to return to normal calcium concentration, the change in coupling conductance was reversible (n = 4).

Calcium blocks gap junction channels in many coupled tissues (Oliveira-Castro & Loewenstein, 1971; De Mello, 1975; Rose & Loewenstein, 1975; Loewenstein & Rose, 1978; Dahl & Isenberg, 1980; Noma & Tsuboi, 1987; Lazrak & Peracchia, 1993). Since the active phase of β-cell bursting involves calcium influx via voltage-gated calcium channels (Dean & Matthews, 1970; Ribalet & Beigleman, 1980; Atwater et al., 1981; Santos et al., 1991; Atwater, Kukuljan & Pérez-Armendariz, 1994), we were interested to see if coupling conductance decreased during the burst due to neighboring cells loading with calcium. To accomplish this, the junctional conductance was calculated from the voltage and current changes occurring at the transition from silent to active phase and compared to that measured using the transition from active to silent phase. In each of 4 cells tested, the magnitude of the coupling conductance was smaller at the end of the burst than at the beginning. The decrease in coupling conductance during the burst ranged from 6.6% to 14.2%, with an average decrease of 10.9% ($P < 0.01$) for the four cells.

Effect of Forskolin on Electrical Coupling

Figure 7 shows the effects of the adenylate cyclase activator forskolin on voltage and current bursts. Addition of 10 μM forskolin to the perifusion medium resulted in an increase in burst frequency and a reduction in the active to silent phase voltage difference in the membrane potential records (top traces of 8*A* and *B*), as observed

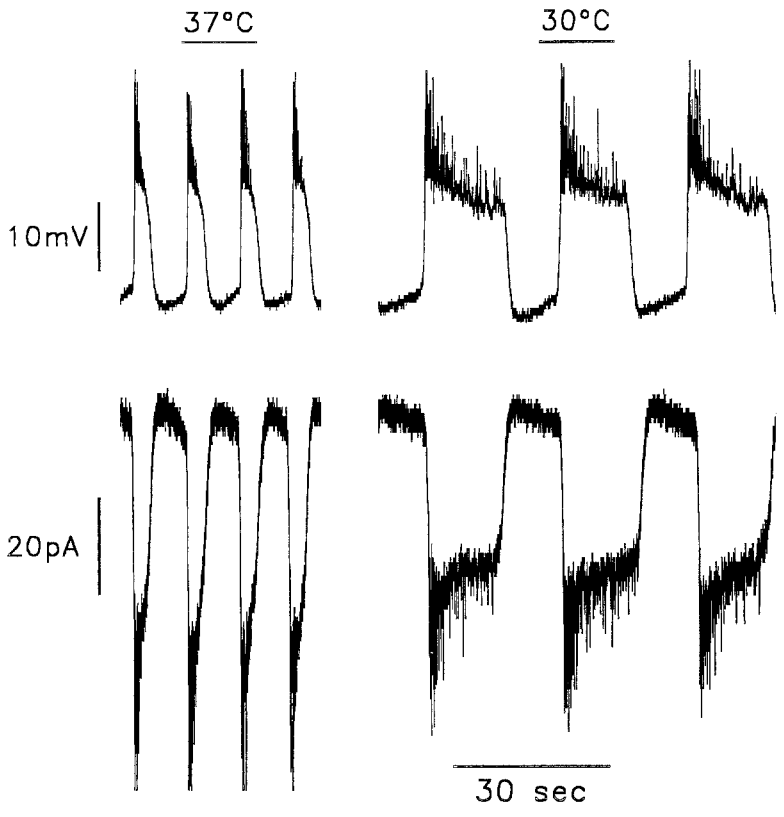

Fig. 5. Lowering temperature reduces coupling conductance. Top traces show membrane potential recordings at 37 and 30°C, and bottom traces show corresponding holding current records when the voltage was held at −80 mV. Silent phase levels of the traces have been aligned to facilitate comparison of burst magnitude. Eight min elapsed between recording of traces on left and right. Parallel coupling conductance was 2.44 nS at 37°C and 1.84 nS at 30°C.

previously (Henquin, Schmeer & Meissner, 1983; Ikeuchi & Cook, 1984; Santos & Rojas, 1987). The reduction in the magnitude of the voltage bursts in the presence of forskolin was accompanied by a slight increase in the active to silent phase difference of the current bursts (lower traces of 8*A* and *B*), such that the coupling conductance increased from 2.39 to 3.05 nS for this cell. Similar results were observed in 4 of 4 experiments, with a mean increase in coupling conductance of 24% for the group ($P < 0.005$). In two experiments in which forskolin was subsequently removed from the perifusate, the coupling conductance approached the original levels, although the conductance was still elevated up to 7 min after return to normal Krebs solution. The burst frequency also remained elevated at these times, indicating that the effects of forskolin are slowly reversible.

Discussion

In situ Technique Allows Measurement of Gap Junction Conductance under Physiological Conditions

The *in situ* gap junction conductance measurement technique is similar in principle to the dual whole-cell voltage clamp method that is used to study gap junction conductance in cell pairs (Veenstra & Brink, 1992). In the latter technique, the tissue of interest is dispersed to form cell pairs, and the voltage across the junction between the two cells is controlled via separate whole-cell voltage clamp amplifiers. A potential step is applied to one cell and the change in coupling current is measured directly from the holding current record of the other cell. Junctional conductance is calculated by dividing the current change by the size of the voltage pulse (Spray, Harris & Bennett, 1981). In the *in situ* technique, the potential of a single β-cell is held constant, and the synchronized glucose-induced oscillations in membrane potential of the neighboring β-cells create the periodic voltage changes necessary to measure coupling conductance. The conductance so measured is not that of a single cell-to-cell junction, but the total, parallel conductance between the clamped β-cell and immediately adjacent, active β-cells. The membrane potential of the surrounding cells must oscillate, and hence coupling can only be measured in glucose concentrations which support bursting. Similarly, only modulations in coupling brought about by stimuli which do not suppress glucose-induced bursting can be studied.

The two methods are somewhat complementary in that isolated cell pair experiments yield the conductance of a single cell-to-cell junction without any information about the number of neighbors to which a given β-cell is coupled within the islet, while the *in situ* method provides a measurement of the total conductance between

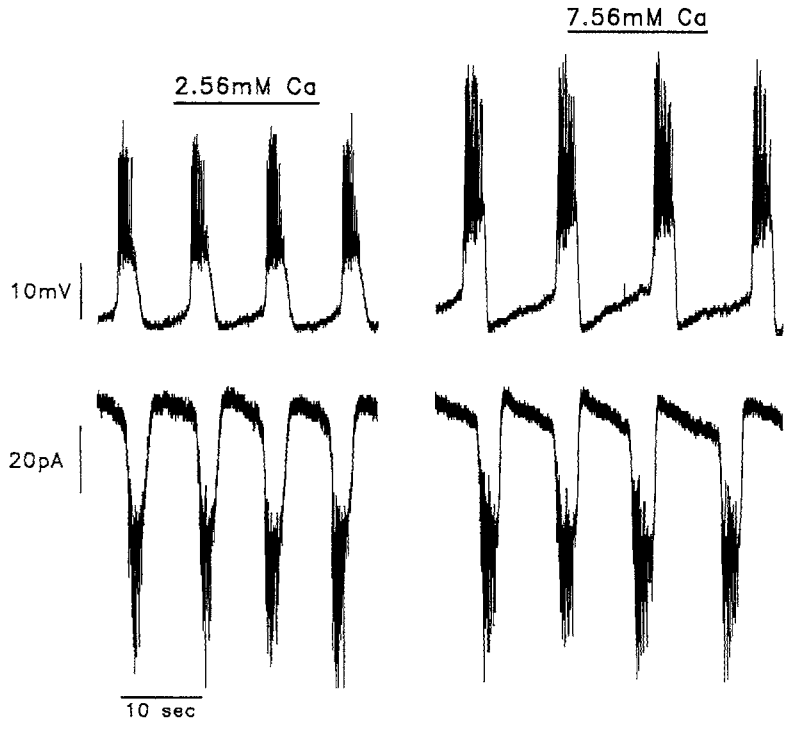

Fig. 6. Effect of extracellular calcium concentration on membrane potential and holding current records. Silent phases have been aligned for purposes of comparison. Top traces show membrane potential recorded in 2.56 and 7.56 mM calcium, and bottom traces show the corresponding holding currents. Five min elapsed between recording of left and right traces. Parallel coupling conductance was 3.01 nS in 2.56 mM Ca^{2+} and 2.52 nS in 7.56 mM Ca^{2+}.

β-cell and its neighbors, with no information regarding the conductance of individual junctions. The major advantage of the *in situ* measurements is that they are made with the β-cells in their native environment under near physiological conditions (bicarbonate buffer and 37°C), whereas dual whole-cell measurements require dispersal of the islet, and are generally carried out at room temperature with HEPES buffer (Meda et al., 1991) to facilitate patch formation.

Elevated Potassium Abolishes Glucose-induced Current Oscillations

The current bursts observed while voltage-clamping a β-cell in 11.1 mM glucose (Fig. 1*A*) are assumed to result solely from coupling current due to the electrical activity of surrounding cells, that is, nonjunctional membrane current is assumed to be constant. While the hyperpolarized holding potential assures that the voltage-dependent ion channels resident in the membrane of the impaled cell will remain inactive (Ashcroft & Rorsman, 1989; Atwater et al., 1994), the β-cell membrane may possess a metabolically regulated, voltage-independent ion channel with cyclically varying conductance in the presence of 11.1 mM glucose (Henquin, 1988; Cook & Ikeuchi, 1989; Rojas et al., 1990). Such a channel could be the "clock" responsible for controlling burst duration. If a portion of the current bursts resulted from these membrane current changes, errors in the gap junction conductance measurement would result. Two candidate channels, which are active at hyperpolarized potentials and subject to metabolic regulation, have been observed experimentally in pancreatic β-cells (K_{ATP} and Ca_G), although to date no basis for oscillatory activity of these channels has been described (Cook & Hales, 1984; Rojas et al., 1990).

The high potassium experiments illustrated in Fig. 3 were aimed at elucidating any such cyclic membrane currents. In 25 mM potassium and 11.1 mM glucose, β-cells depolarize to a potential above that of the active phase and become silent, presumably owing to inactivation of the voltage gated ion-channels involved in bursting (Dawson et al., 1984). The voltage, and hence the current, across the intercellular junctions no longer changes periodically, but the hypothetical voltage-independent "clock" of the β-cell should remain active, because glucose is still present. In all 11 experiments, the current bursts were completely abolished by 25 mM potassium, and no periodic membrane currents were observed. These results indicate that voltage-independent, metabolically driven oscillations in membrane current, if present, have magnitude below the resolution of the *in situ* voltage clamp technique (about 5 pA), and do not cause significant errors in the coupling measurements made with this method. Theoretical studies predict that bursts can be driven by whole-cell currents as small as a few pA (Sherman, Rinzel & Keizer, 1988), so the postulated currents may still play an important role in controlling β-cell electrical activity.

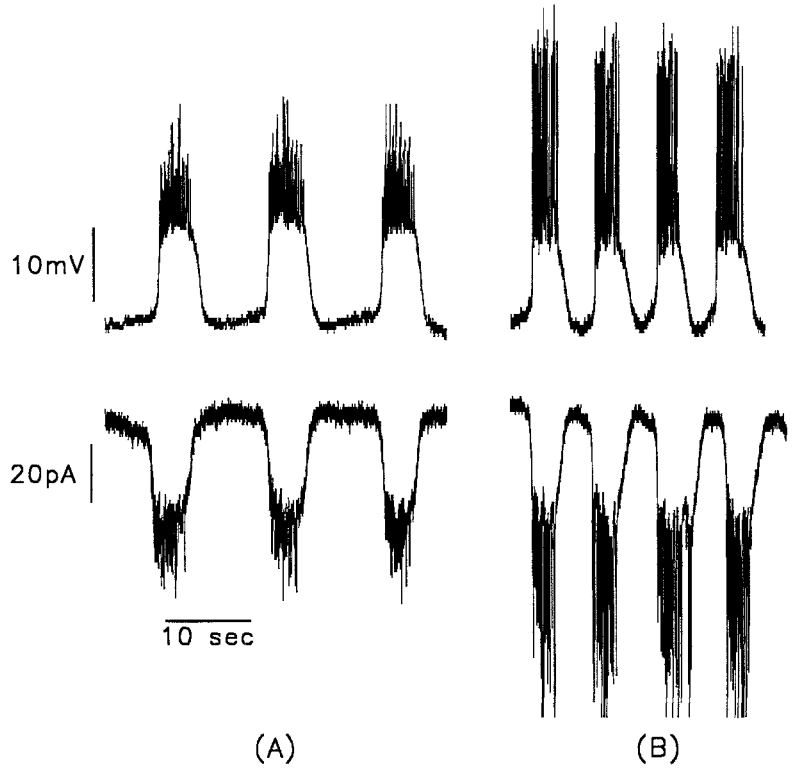

Fig. 7. Effect of forskolin on coupling conductance. (*A*) Voltage (top) and coupling current (bottom) bursts in the absence of forskolin. (*B*) Corresponding records 6.5 min after addition of 10 μM forskolin. Silent phases aligned to facilitate comparison. Parallel coupling conductance was 2.39 nS under control conditions, and 3.05 nS in the presence of forskolin.

Hyperpolarized Holding Potentials have Little Effect on Burst Activity of Adjacent β-Cells

The *in situ* method also relies on the assumption that the membrane potential recorded from the impaled cell during current clamp is an accurate estimate of the electrical activity of neighboring cells after switching to voltage clamp mode. While there is much evidence that neighboring β-cells display similar electrical activity (Eddlestone et al., 1984; Meda et al., 1984; Santos & Rojas, 1987), the voltage record of the impaled cell is an accurate representation of the electrical behavior of surrounding cells during voltage clamp only if voltage clamping has no effect on the burst pattern of neighboring cells. In particular, the voltage clamp protocol must not alter the active-silent phase voltage difference in the surrounding cells, because this quantity is used to compute coupling conductance (Sherman et al., 1995). Clamping the impaled cell causes current to flow into coupled cells, with the magnitude and direction depending on the difference between the holding potential and the membrane potential of the neighboring cells. The effect of injecting current into bursting β-cells has been studied previously (Atwater et al., 1978; Atwater, Gonçalves & Rojas, 1982). In the cited studies it was noted that positive current injections dramatically decrease the active-silent phase voltage change, while negative current injections suppress the spikes somewhat but, importantly, do not alter the voltage change from silent to active phase. In agreement with these reports, Fig. 4*A* shows that when positive current flows from the impaled cell into the neighbors ($V_h = -40$ mV and $V_h = 0$ mV), the change in current from the active to the silent phase is reduced, but when negative current flows from the clamped cell ($V_h \leq -70$ mV, Fig. 4*B*), there is little change (less than 3%) in the active-silent phase current difference. These results indicate that the active-silent phase voltage changes in the neighboring cells are unmodified by the negative holding potentials used in these experiments, and the voltage record of the impaled cell provides a reasonable estimate of the behavior of the coupled cells during subsequent voltage clamping.

Coupling Conductance Histogram is Bimodal

The bimodal nature of the coupling conductance histogram in Fig. 2 indicates that at least two subpopulations of β-cells, differing in terms of extent of electrical communication with neighboring β-cells, exist within the islet. Such a difference could be brought about by differences in the physical number of neighboring β-cells. While the exact location of each impaled β-cell was not quantified, many of the conductance measurements were made from cells near the surface of the islet, where the population of α-cells is high (Meda, 1989). As discussed previously, membrane potential oscillations in α-cells contribute little to current bursts, and hence coupling

conductance between the impaled cell and neighboring α-cells is not included in the parallel conductance measurement. Thus the lower mode of the coupling conductance histogram may represent cells that were in contact with several α-cells. Also, β-cells on the very surface of the islet are in physical contact with fewer cells than those below the surface, and may account for the group of cells having lower coupling conductances. A second possibility for the two junctional conductance peaks is variations in the number or functional properties of gap junctions between neighboring cells at different locations within the islet. Meda and coworkers (1980) found twice as many gap junctions per unit area on the membranes of β-cells near the surface of the mouse islet as compared to more centrally located cells. Conversely, dye transfer in cultured rat islets has been shown to be more extensive at the core than in the periphery (Michaels & Sheridan, 1981). While the basis of the difference in morphological and functional coupling is not known, both results are consistent with the bimodal distribution of coupling conductances observed in this experiment. Thus the two modes of the junctional conductance histogram may represent peripherally and more centrally located cells.

There were two experiments in which multiple coupling conductance measurements were made in the same islet. In both cases two cells in the same islet had very different junctional conductances (3.5 and 2.5 nS in one islet, 3.8 and 2.6 nS in another). These data indicate that both the high and low coupling conductance populations exist within the same islet of Langerhans. The physiological significance of the bimodal coupling conductance distribution remains to be determined.

Electrical Coupling is Extensive within the Islet of Langerhans

Every β-cell clamped in the presence of 11.1 mM glucose (n = 35) was electrically coupled to at least one other active β-cell, as indicated by the occurrence of current bursts. This contrasts some dye transfer studies in which fluorescent tracers injected into one β-cell sometimes did not spread to any surrounding cells (Kohen et al., 1979; Michaels & Sheridan, 1981; Meda et al., 1982; Meda et al., 1986; Meda, 1989). The dye may have damaged the injected cell, causing it to be uncoupled from surrounding cells, or, more likely, the permeability of the gap junctions to the dye was too low for intercellular spread to occur. Indeed, β-cell pairs that are electrically coupled are often not dye transferring (Pérez-Armendariz et al., 1991).

In one previous study using the dual whole-cell voltage-clamp method, 67% of β-cell pairs were coupled with a mean junctional conductance of 215 pS (Pérez-Armendariz et al., 1991). Adjusting for the approximately 30% change in coupling conductance owing to temperature differences between the two experimental conditions (Fig. 5), and assuming the islet to consist of packed spheres of β-cells (12 neighbors for each cell), one would expect an average parallel coupling conductance of about 2.2 nS. This calculated value agrees reasonably well with the lower conductance peak (2.5 nS) in Fig. 2. The slightly higher value observed here may result from additional experimental differences such as pH buffers used, or it may be that the dispersal procedure used in the cell pair study preferentially selected uncoupled pairs such that the incidence of coupling is greater than 67% *in situ*. Interestingly, in the cell-pair study several high conductance (400–600 pS) cell-to-cell junctions were observed. Nonrandom distribution of these high conductance junctions throughout the islet could account for the high conductance mode of the histogram in Fig. 2. In another study, only 16% of rat β-cell pairs were electrically coupled (Meda et al., 1991). The vast difference between the extent of coupling in that study and one presented here may be caused by species variation, although the most likely cause is differences in experimental technique. Interestingly, in one mathematical model, the electrical activity of a group of coupled β-cells could be synchronized by electrical communication alone when gap junction conductances were distributed according to the data of Pérez-Armendariz et al. (1991), but not when the results of Meda et al. (1991) were used (Smolen, Rinzel & Sherman, 1993). Our results support the notion that electrical communication plays a major role in synchronizing the electrical, and hence secretory, activity of β-cells within an islet.

Cell-to-Cell Electrical Conductance is Reduced by Lowering Temperature

The effect of temperature on gap junction conductance is of interest since dual whole-cell voltage-clamp experiments are carried out at room temperature. Reduction of temperature as far as 25°C in these experiments often caused the burst pattern to shift to continuous spiking. Since *in situ* coupling conductance measurements can only be made in bursting islets, the temperature was generally lowered to 28–31°C. As in previous experiments, lowering the temperature caused an increase in the active phase duration and often reduced the active-silent phase voltage difference (Atwater et al., 1984). In 5 of 6 experiments the coupling conductance decreased when the temperature was lowered, with an average conductance change in the five cells of 21%. Similar temperature dependence has been observed for gap junction conductance in pairs of cardiac myocytes from adult guinea pigs (Sugiura et al., 1990) and neonatal rats (Bukauskas & Weingart, 1993), which, like pancreatic β-cells (Meda et al., 1991), express the gap junction channel protein con-

nexin 43 (Bennett & Verselis, 1992). The temperature dependence of the junctional conductance is accounted for by reduction in ion mobility at low temperature, since lowering the temperature from 37 to 29°C results in a 16–23% reduction in conductance of aqueous KCl solution (Robinson & Stokes, 1970). These results indicate that diffusion is the main process governing charge transfer through β-cell gap junction channels, and steady state junctional conductance measurements made at room temperature can be reasonably extrapolated to physiological temperature by adjusting for changes in ion mobility.

Elevated Extracellular Calcium Blocks Gap Junction Electrical Conductance

Gap junction conductance is an inverse function of intracellular calcium concentration at physiological levels in a variety of coupled tissues (Noma & Tsuboi, 1987; Veenstra & DeHaan, 1988; Peracchia, 1990; Lazrak & Peracchia, 1993; Crow, Atkinson & Johnson, 1994), and intracellular calcium measured from the whole islet has been shown to increase when the extracellular calcium concentration is elevated (Silva, Rosario & Santos, 1994). In 6 of 8 cells studied, the coupling conductance decreased by an average of 17.5% when the calcium concentration was raised from 2.56 to 7.56 mM. The effect is most likely caused by an increase in the average cytosolic free calcium level, which may block gap junction channels directly (Loewenstein & Rose, 1978) or activate second messengers which mediate the effect on junctional conductance (Johnston & Ramón, 1981; Peracchia, Bernardini, & Peracchia, 1983). The effect of raising external calcium on islet intracellular calcium is quite variable (Silva et al., 1994), so the absence of junctional conductance change in 25% of the experiments suggests that the intracellular calcium concentration did not reach sufficient levels to cause uncoupling in these cells.

Coupling Conductance Varies during Glucose-induced Bursting

Islet β-cells load with calcium during the active phase of the burst (Santos et al., 1991), and one previous study using simultaneous intracellular impalements and current injections suggested that gap junction conductance may decrease during the active phase (Eddlestone et al., 1984). These results were confirmed in this study, as the parallel coupling conductance of four cells decreased by an average of 10.9% during the active phase. The most likely explanation for this result is that calcium accumulations during the active phase are sufficient to cause partial uncoupling of the cells, although we cannot rule out the possibility that oscillations of other second messengers are responsible for the cyclic nature of β-cell gap junction conductance. Note that with the exception of this section, the coupling conductance measurements reported in this study were made using voltage and current records containing several bursts, and thus represent the average of the junctional conductance at the beginning and end of the burst. Whether oscillations of β-cell electrical coupling plays a role in controlling burst duration or frequency remains to be investigated.

Forskolin Increases β-Cell Electrical Communication

Activation of adenylate cyclase by forskolin consistently resulted in a rapid increase in junctional conductance (Fig. 7). These results agree with a previous study using dual intracellular impalements in intact islets, in which the electrical coupling ratio between two β-cells *in situ* was found to increase within minutes after stimulation with forskolin (Santos & Rojas, 1987). Conversely, in one study of β-cell pairs, no change in gap junction conductance was observed when the cells were exposed to dibutyryl-cAMP (Pérez-Armendariz et al., 1991). The discrepancy between results obtained *in situ* and those observed with the dual whole-cell method could be caused by "washout" of cytosolic constituents in the latter technique, which may have resulted in intracellular depletion of key components involved in mediating the effect of increased cAMP on junctional conductance.

The mechanism by which cAMP increases junctional conductance is not clear. While long-term exposure to elevated cAMP levels enhances coupling (Flagg-Newton, Dahl & Loewenstein, 1981; Weiner & Loewenstein, 1983) by stimulating *de novo* synthesis of gap junction channels (Mehta, Yamamoto & Rose, 1992), the rapid modulation of conductance observed in this and other experiments (Stagg & Fletcher, 1990) probably results from gating of existing connexons (Bennett et al., 1991). A role for protein kinase A (pkA) is indicated by experiments in which injection of the catalytic subunit of this protein into cells expressing connexin 43 leads to a rapid increase in intercellular communication, while inhibition of pkA has the opposite effect (Godwin, Green & Walsh, 1993; Nnamani et al., 1994). However, to date there is no evidence that connexin 43 is directly phosphorylated by pKA (Bennett et al., 1991). Interestingly, agents that elevate cAMP inhibit intercellular communication in some tissues expressing connexin 43, suggesting that cell-specific expression of second messengers that link elevated cAMP to changes in junctional conductance are important in determining the effect of the cyclic nucleotide (Stagg & Fletcher, 1990; Bennett et al., 1991). Since metabolism of glucose is accompanied by production of cAMP (Charles et al., 1973; Sharp, 1979; Prentki & Matschinksy, 1987), our results suggest that

the rapid increase in β-cell electrical coupling observed in dual intracellular experiments following glucose stimulation (Eddlestone et al., 1984) may be mediated by a cAMP-dependent mechanism.

Conclusions

Synchronous glucose-induced bursting electrical activity in β-cells within the islet of Langerhans makes it possible to measure the coupling conductance between a β-cell and its nearest neighbors *in situ* under physiological conditions. The bimodal nature of the coupling conductance histogram indicates heterogeneity in intercellular communication within the islet of Langerhans. Decreases in temperature and elevations in extracellular calcium both result in a reversible decrease in gap junction conductance, while activation of adenylate cyclase by forskolin results in a slowly reversible increase in cell-to-cell electrical communication. The effect of temperature can be reconciled by changes in ion mobility, while the rapid effects of calcium and forskolin probably result from direct or indirect gating of existing gap junctions. The results indicate that electrical coupling among β-cells is quite extensive and subject to physiological modulation, and support the hypothesis that intercellular communication plays an important role in the overall function of the islet.

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