Biophysical properties of gap junctions between freshly dispersed pairs of mouse pancreatic beta cells

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ABSTRACT Coupling between beta cells through gap junctions has been postulated as a principal mechanism of electrical synchronization of glucose-induced activity throughout the islet of Langerhans. We characterized junctional conductance between isolated pairs of mouse pancreatic beta cells by whole-cell recording with two independent patch-clamp circuits. Most pairs were coupled (67%, $n = 155$), although the mean junctional conductance (g_j) (215 ± 110 pS) was lower than reported in other tissues. Coupling could be recorded for long periods, up to 40 min. Voltage imposed across the junctional or nonjunctional membranes had no effect on g_j. Up to several hours of treatment to increase intracellular cAMP levels did not affect g_j. Electrically coupled pairs did not show transfer of the dye Lucifer yellow. Octanol (2 mM) reversibly decreased g_j. Lower concentrations of octanol (0.5 mM) and heptanol (0.5 mM) than required to uncouple beta cells decreased voltage-dependent K^+ and Ca^{2+} currents in nonjunctional membranes. Although g_j recorded in these experiments would be expected to be provided by current flowing through only a few channels of the unitary conductance previously reported for other gap junctions, no unitary junctional currents were observed even during reversible suppression of g_j by octanol. This result suggests either that the single channel conductance of gap junction channels between beta cells is smaller than in other tissues (<20 pS) or that the small mean conductance is due to transitions between open and closed states that are too rapid or too slow to be resolved.

INTRODUCTION

In the presence of glucose islet cells exhibit periodic bursts of action potentials (Dean and Matthews, 1970; Atwater et al., 1978), which occur almost synchronously throughout the islet (Meissner, 1976; Meda et al., 1984*a*). Electrical coupling was previously recorded between islets cells when electrode tips were separated by a distance of 35 μm or less; at greater distances coupling could not be recorded, possibly as a consequence of three-dimensional spread of current (Eddlestone and Rojas, 1980; Eddlestone et al., 1984). Despite the wide spread electrical synchrony dye transfer was reported to occur only between small groups of islet cells (Michaels and Sheridan, 1981; Meda et al., 1986*b*), raising the possibility that islet cells are organized in small coupled compartments (Orci, 1982). Synchronization between compartments might then be mediated through cyclic accumulations of K^+ in the intercellular space that occur simultaneously with the bursts of spikes (Pérez-Armendariz et al., 1985), which are also synchronous throughout the islet (Pérez-Armendariz and Atwater, 1986). However, given the small size of gap junctions connecting islet cells (Orci et al., 1973) it was also possible that dye transfer was slow or limited and observed dye coupled domains underestimated electrical ones. To answer this question simultaneous measurements of both kinds of coupling were needed.

The number of gap junctions particles between islet cells was reported to be increased by treatment for several hours with substances that enhance intracellular cyclic AMP levels (I'nt Veld et al., 1985). Also, raising the glucose concentration shifted the distribution of linearly packed to polygonally packed gap junction arrays within minutes (In't Veld et al., 1986). Correspondingly, the strength of electrical coupling was increased by forskolin and glucose within minutes (Eddlestone et al., 1984; Santos and Rojas, 1987). However, the three-dimensional interconnectedness of the islet cells has prevented quantification of effects of these compounds on junctional conductance under experimental conditions that very possibly lead to major changes in conductance of the nonjunctional membrane.

Intercellular communication may be involved in the control of secretion (Meda et al., 1987, 1988). Isolated beta cells release less insulin than reaggregated or intact islets, suggesting that intercellular communication or contact potentiates insulin release (Halban et al., 1982; Pipeleers et al., 1982; Bosco et al., 1989). In the islet of Langerhans an increase in incidence of gap junctions was observed during stimulation of insulin secretion (Meda et al., 1979, 1980). Alkanols, such as octanol and heptanol, reduce the extent of intercellular diffusion of Lucifer yellow, reduce the number of particles per gap junction in intact islets, and decrease glucose-stimulated insulin release (Meda et al., 1986*a*). However, the

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concentration required to uncouple beta cells was not quantified, nor was it determined whether these compounds block voltage sensitive channels, as in other tissues (Pozos and Oakes, 1987), and affect secretion by this mechanism.

We have now studied isolated pairs of islet cells by a dual patch-clamp approach. A high incidence of electrical coupling was found in agreement with a high degree of synchronization of bursting electrical activity throughout the islet. Electrically coupled pairs were found not to be dye coupled, suggesting that electrical coupling could be more widespread than inferred from previous studies using dye transfer techniques. Gap junctions between beta cells did not exhibit voltage dependence and appeared not to be modulated by cAMP-dependent protein kinase in the short term. Lower concentrations of octanol and heptanol than required to uncouple the cells decreased voltage sensitive Ca^{2+} and K^+ currents substantially, affecting the interpretation of mode of action of this agent on insulin secretion. Although junctional conductance was lower than in tissues studied previously, no evidence of single channel fluctuations was found, even when uncoupling was induced by octanol; the conductance of channels between these cells is small, fast flickering between open and closed states is unresolved or these transitions are too slow to be recognized.

Isolated cell pairs allow evaluation of possible modulatory effects of metabolic intermediates on beta cell coupling and of the role of coupling in insulin release. Part of this work has been presented in abstract form (Pérez-Armendariz et al., 1988, 1989).

METHODS

Islet cell preparation

Islets of Langerhans were obtained from 2–5 mo old CD1 mice with a collagenase dissociation technique previously described (Lacy and Kostianovsky, 1967). Pancreata were minced and incubated for 15 min in 2.5 mg collagenase (Serva Fine Biochem. Inc., Westbury, NY) in 1 ml buffered Krebs solution (solution A) with 3% albumin per pancreas, and washed in Krebs 0.5% albumin. For beta cell preparation ~150 islets were dissociated in a two step protocol: 10 min. incubation in a 0-Ca^{2+} solution (Spinner; Sigma Chemical Corp., St. Louis, MO), with 16 mM glucose, 3 mM EGTA, 3% albumin solution at 37°C, followed by 3 min incubation in 0 Ca^{2+} solution (Spinner) with 16 mM glucose, 3% albumin and 0.2 U/ml papain (United States Biochemical Corp., Cleveland, OH) in the presence of 16 mM glucose. Cells were mechanically dispersed by trituration with a Pasteur pipette, washed and plated on glass coverslips, and maintained in culture medium (RPMI 1460; Gibco Laboratories, Life Tech. Inc., Grand Island, NY), 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco Laboratories), 16 mM glucose with or without 1 mM dibutyryl-cAMP (Sigma Chemical Co.) in a 5% CO_2/air incubator at 37°C for 6–9 h before recording.

Pancreatic exocrine acinar cells

As a control for dye transfer and electrophysiological properties, exocrine acinar cells were prepared. Minced pancreata were exposed to 1.0 mg of collagenase (Type I; Sigma Chemical Co.)/ml buffered Krebs solution (solution A) for 15 min. The tissue was washed and incubated for 10 min in a 0-Ca^{2+} solution (Spinner) with 5 mM glucose and 3% albumin in the presence of 6 U/ml papain (United States, Biochemical Corp.). Cells were obtained by trituration with a Pasteur pipette, washed and plated on glass coverslips in buffered Krebs solution, and recorded from in the next 2 h.

Electrophysiological recordings

Cells pairs and clusters were common in the freshly dissociated pancreatic islet tissue. Recordings were obtained from pairs only during the 6–9 h after dissociation because thereafter cells tended to reaggregate, packing so closely that pairs could not be unambiguously identified. Each cell of a pair was voltage clamped, usually to a holding potential of −70 mV, with an Axo-patch amplifier (Axon Instruments, Inc., Burlingam, CA) and patch pipette glass. High resistance seals (typically >5 GΩ) were formed on each cell by application of gentle suction through polished pipettes (resistances 6–8 MΩ). Access to the cell interior was gained by further brief suction. Series resistance (Rs) was usually 10 MΩ which was <10% of the parallel sum of input and seal resistances, so Rs compensation had little impact on the recordings and was not employed routinely.

Solutions

The external recording solution was Krebs saline (solution B) 2.5 mM $CaCl_2$, 16 mM glucose, 2 mM $NaHCO_3$, buffered with 10 mM Hepes, pH 7.4 unless otherwise specified. A perfusion chamber of 2 ml was used and medium could be changed within 1.0 min. When used, heptanol and octanol were sonicated for 5 min in the external solution just before application to the bath. Internal pipette solutions used are summarized in Table 1.

Dye injection

Individual cells were injected with Lucifer yellow through patch electrodes (30 mM dissolved in K^+ internal solution [solution F]). During injection (1–26 min) beta cells were only considered when outward currents, and usually inward as well, were recorded in response to depolarization and a resting membrane potential of at least −55 mV was recorded. Under these conditions Lucifer yellow (LY) diffused into the cells within seconds. 15–26 min after rupturing the patch, injected cell pairs were viewed and photographed under fluorescent illumination on an inverted Olympus microscope with FITC excitation and emission filters. LY at 4% in 150 mM LiCl solution buffer with Hepes at pH 7.2 was also injected through 70 mΩ microelectrodes by passing hyperpolarizing pulses of 20 nA, 900 ms for 1–2 min.

Cell identification

Antibodies against insulin were used to identify beta cells. Briefly, cells were fixed with Zamboni's Fixative (Stefanini et al., 1967) for 30 min at room temperature, washed several times with PBS, permeabilized by dehydration and rehydration in an ethanol series, and treated with 0.03% H_2O_2 in PBS for 20 min to suppress endogenous peroxidase activity. Cells were incubated with guinea pig anti-pig insulin antibodies, 1:200 (Cambridge Isotope Laboratorie, Woburn, MA), over-

TABLE 1

	External solutions					Internal solutions		
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
NaCl	135	135	135	120	145	—	—	—
$NaHCO_3$	2	2	2	2	—	—	—	—
M_gCl_2	1.2	1.2	1.2	1.2	—	—	—	—
$CaCl_2$	1.0	2.5	2.5	10	0.5	—	—	0.5
Hepes	10	10	10	10	10	10	10	5
EGTA	—	—	—	—	—	10	10	5
Glucose	5	16	16	16	16	—	—	—
KCl	5	5	5	5	—	35	—	35
K-Asparate	—	—	—	—	—	100	—	100
CsOH	—	—	—	—	—	—	110	—
Asparate	—	—	—	—	—	—	100	—
TEA	—	—	—	—	—	—	20	—
Mg-ATP	—	—	—	—	—	3	3	3
TTX	—	—	—	200 *nM*	200 *nM*	—	—	—
Cd^+	—	—	100 μ*M*	—	100 μ*M*	—	—	—
CPK	—	—	—	—	—	—	50 *U/ml*	—
pH	7.4	7.4	7.4	7.4	7.4	7.16	7.16	7.16
PC	—	—	—	—	—	—	10	—

CPK Creatine phosphokinase
PC Phosphocreatine

night at 4°C, washed and incubated with Protein A conjugated to HRP, 1:100, for 1 h at room temperature. After several washes, cells were again passed up and down in an ethanol series at room temperature to suppress endogenous peroxidase activity further, and then exposed to DAB reaction solution (Metz et al., 1988). The bound HRP was visualized by reaction with 0.02% DAB, 0.02% H_2O_2, 0.024% nickel ammonium sulfate (0.24 g/100 ml) in 0.05 M Tris-HCl, pH 7.4 (Fig. 1, *C* and *D*). Generally control experiments were done following the same protocol but incubating cells without primary antibody, and with nonimmune guinea pig serum. Negative internal controls were observed in cell cultures at 36 h, when some fibroblast cells had grown (Fig. 1 *D*).

Electron microscopy of freeze fractured specimens

Pellets of 100–150 isolated islets as well as isolated clumps, pairs, and individual cells, cultured for 24 h, were fixed for 60 min in 2% glutaraldehyde with 0.1 M cacodylate buffer containing 1 mM $CaCl_2$ (pH 7.4) on ice, then washed with the same buffer. The samples were treated with 30% glycerol in distilled water, frozen in liquid nitrogen-cooled Freon 22 and fractured using a double replica device in a Balzer freeze-etch unit at −135°C. The replicas were cleaned overnight in bleach, washed in several changes of distilled water and collected on formvar-coated slot grids. Electron microscopic examination of the replicas was carried out on a Phillips 300 transmission electron microscope at 60 kV.

RESULTS

Gap junctions remain between freshly isolated islet cells

In collagenase treated mouse islets, freeze fracture showed gap junction aggregates coexisting with tight junctions (Fig. 1 *A*). Similar gap junctional arrays were observed in small clusters of cultured islet cells (Fig. 1 *B*). These results show that gap junction aggregates are present in islet cell preparations similar to those used for measurements of junctional current.

Cell identification

After 12 h in culture almost all cells (estimated >95%) were identified as beta by immunostaining with anti insulin antibodies (Fig. 1, *C* and *D*). Negative internal controls were observed in cell cultures at 36 h where some clear morphologically distinctive cells, possibly fibroblasts, had grown (Fig. 1 *D*). This result indicates that most cultured cells used for electrical recordings were beta cells. Furthermore junctional currents were recorded from pairs of cells that exhibited inward and outward currents characteristic of beta cells (see below).

Typical nonjunctional membrane channels are retained in freshly isolated beta cells

Glucose induces membrane depolarization in pancreatic beta cells by inducing synthesis of $[ATP]_i$ which closes sensitive K^+ channels, ("G-K Channel" [Ashcroft et al., 1984]; ATP-K channel [Cook and Hales, 1984]). This depolarization activates voltage sensitive Ca^{2+} and K^+ channels (Dean and Mathews, 1970; Rorsman and Trube, 1986; Satin and Cook, 1985; Hiriart and Mathes-

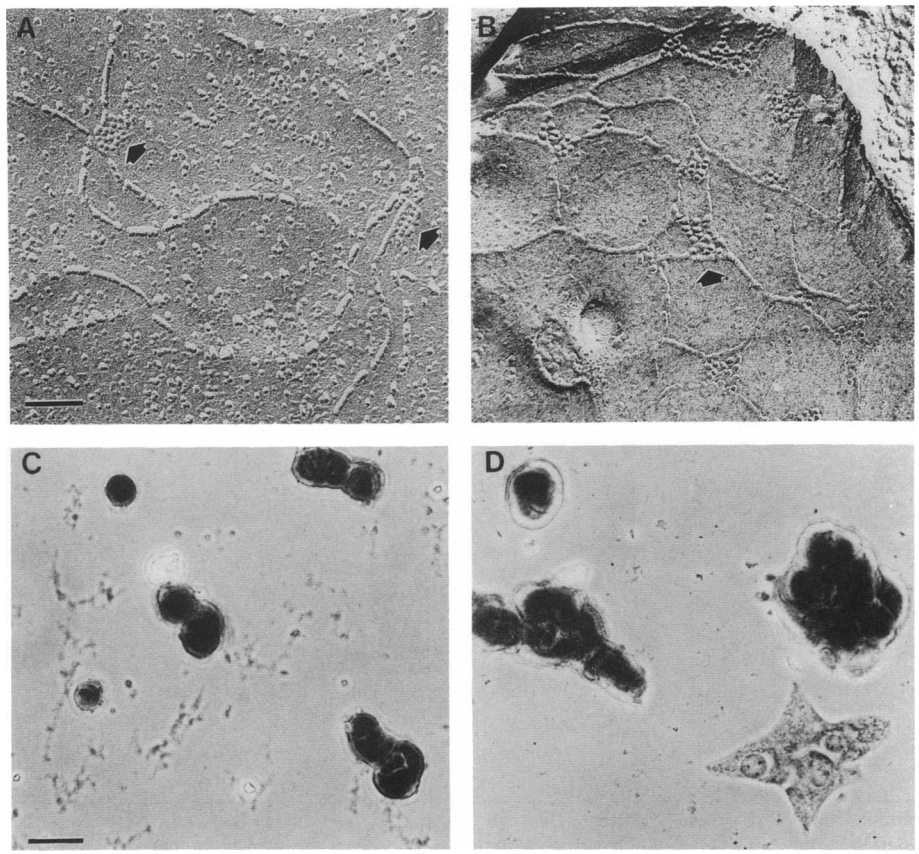


FIGURE 1 Ultrastructure of appositional areas of cultured cells and immunostaining with anti-insulin antibodies. (*A*) Freeze fracture replicas obtained from an isolated mouse islet (*A*) and mouse islet cells cultured for 24 h after dissociation (*B*). Both tight junction strands and gap junction particle aggregates (*arrowhead*) are present, indicating that these junctions were preserved after enzymatic treatment. P-face replicas are shown. Immunostaining with anti-insulin antibodies of mouse islet cells cultured for 12 h (*C*) and 36 h (*D*) after dissociation. Cells were immunostained with guinea pig anti-insulin antibodies incubated with protein A conjugated to HRP and visualized by reaction with DAB (see Methods). After 12 h of culture almost all cells were identified as beta, indicating that electrical recordings belong mainly to this cell type. At 36 h (*D*) a morphologically distinctive cell, a possibly fibroblast, is shown as an internal negative control. Scale bars (*A*, *B*) 0.1 μm (*C*, *D*) 20 μm.

son, 1988). Voltage-sensitive outward currents were always recorded in freshly dispersed cells (Fig. 2 *A*) and could be almost completely suppressed by external application of 20 mM TEA, or by substitution of Cs^+ (solution G) for K^+ (solution F) in the internal pipette solution but not by external application of 100 μM tolbutamide or Cd^{2+} (not shown) suggesting that this current was mainly due to the delayed rectifier and was not Ca^{2+}-activated or ATP-sensitive K^+ currents. The threshold and the activation curve of the outward current were similar to those for the potassium delayed rectifier current previously reported for mouse islet cells in culture (Rorsman and Trube, 1986). Voltage-sensitive inward currents (Fig. 2 *C*) were also commonly observed when high calcium (10 mM) and 200 nM TTX were perfused in the external solution (solution D) and when K^+ was replaced by Cs^+ and 20 mM TEA in the internal pipette solution (solution G). These currents were blocked by 100 μM Cd^{2+}, and the threshold and the activation curve were similar to those previously reported for Ca^{2+} currents in cultured beta cells (Rorsman and Trube, 1986). I–V curves formed in part with records shown in Fig. 2, *A* and *C*, are shown in Fig. 2, *B* and *D*. Some pairs of cells exhibited spike bursts under current clamp (Fig. 2 *E*), suggesting that bursting activity is a property of single cells as recently demonstrated (Smith et al., 1990).

Acinar cells of the exocrine pancreas did not attach to coverslips if isolated with protocols used for islet cells. Moreover, when acinar cells were obtained with a brief isolation procedure (see Methods), no voltage-dependent currents were recorded. These results indicate that characteristic voltage-dependent membrane channels in beta cells are still present after acute enzymatic dissocia-

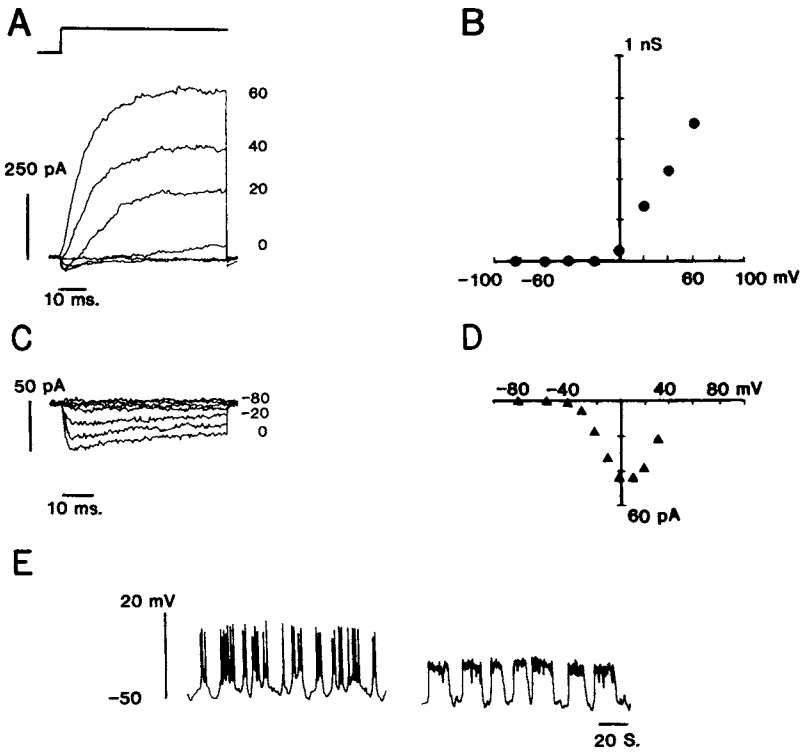


FIGURE 2 Potassium and calcium currents and electrical activity in freshly dispersed mouse islet cells. Families of leakage corrected currents recorded with patch pipettes in the whole cell mode, from two different single cells when membrane potential was stepped in small increments starting at a holding potential of −80 mV. (*A*) Outward currents were recorded with solutions B and F as the extenal medium and patch pipette solution. (*C*) Inward currents were recorded when Cs^+ replaced K^+ in the internal pipette solution (solution G) and in the presence of 10 mM $CaCl_2$ and 200 nM TTX (solution D). (*B*) I–V curves formed in part with the maximum outward currents from records shown in *A*. (*D*) I–V curves formed in part with peak clacium currents shown in *C*. (*E*) Electrical activity from one of a pair of cells under current clamp. Solution H was used in the internal pipette.

tion, and that their activation permits electrophysiological identification of endocrine cell pairs.

Values of junctional conductance between beta cells

Junctional conductance (g_j) was measured between voltage-clamped cell pairs with high input resistances (3–10 GΩ) and from which voltage-sensitive Ca^{2+} and/or K^+ currents could be recorded. g_j values were measured within 1 min after whole-cell recording was obtained in the second cell. A typical recording of junctional currents between such a cell pair is shown in Fig. 3 *A*, where depolarizing steps in one cell induced proportional junctional currents in the other cell, in contrast with nonjunctional currents where a nonproportional increment is induced with higher depolarizing steps, due to activation of K^+ outward currents. Of 155 pairs recorded from, 67% were electrically coupled and 33% were not. For the coupled pairs g_j was 200 pS or less in 56% and between 200 and 600 pS in the remaining. The mean g_j was 215 ± 110 pS (SD). Fig. 3 *B* shows the histogram of g_j values from 100 doubly voltage clamped pairs of cells.

Gap junctions between beta cells are insensitive to voltage

Voltage dependence of g_j between beta cells were evaluated with the double voltage clamp technique. When 0.5–2 s command voltage steps of either polarity were applied to one cell, a linear relation was obtained between the magnitudes of I_j and V_j (Fig. 4 *B*). Linearity in g_j was also observed in 14 pairs in which slow voltage ramps (20 mV/s) between −90 and +20 mV were applied to cell 1, while voltage was held constant in cell 2. Junctional current (I_2) was always linear (Fig. 4 *A*) whereas nonjunctional current (I_1) exhibited a clear outward rectification beginning at a voltage of ~ −35 mV, which is near the threshold of voltage-dependent K^+ channels in these cells. When Cs^+ replaced K^+ in the

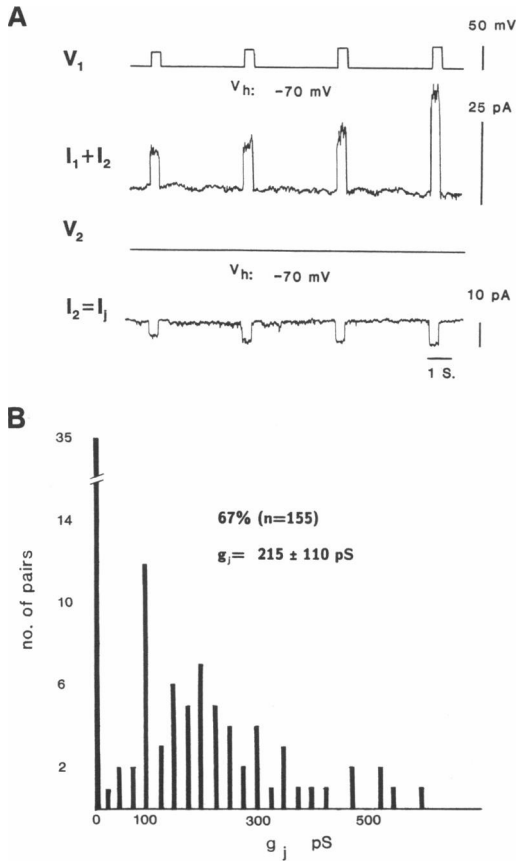


FIGURE 3 Junctional currents between beta cell pairs are small. (*A*) Simultaneous voltage and current recordings from a pair of islet cells under double voltage clamp. V_1 and V_2 show the voltage recordings of cell one and cell two, I_1 and I_2 show their corresponding currents. I_1, representing the sum of junctional and nonjunctional currents, increased nonlinearly with depolarizing steps, due to activation of outward currents. I_2, representing junctional current, was proportional to the positive command voltages applied to V_1 and did not decay during 500 ms voltage steps. Nonjunctional conductances in this record were 430 and 670 pS and g_j was 200 pS. (*B*) Histograms of g_j values from 100 doubly voltage clamped pairs of cells. Bin width 25 pS.

internal pipette solution and slow voltage ramps were applied, junctional currents remained linear with similar g_j values, and nonjunctional currents became linear as well (not shown). In three other experiments the holding potentials of both cells were changed simultaneously to different values while continuing to apply a ramp to one of them; the slope of the $I_j - V_j$ curve (g_j) remained constant (Fig. 4 *C*). These results show that gap junctions between isolated pairs of beta cells from adult mice are not affected by transjunctional voltage (V_j) or voltage between cytoplasm and exterior (V_{i-o}). A similar conclusion was reached by showing constancy of the coupling coefficient, V_2/V_1 between pairs of islet cells (Eddlestone et al., 1984) over a smaller range of voltage than studied here. However, under current clamp recording nonlinearity of junctional membrane over large voltage ranges would be expected to interfere with measurements of this kind.

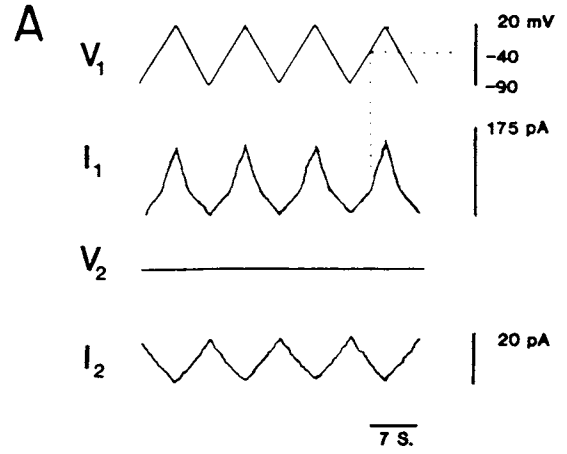
Electrotonically coupled pairs of beta cells are not detectably dye coupled

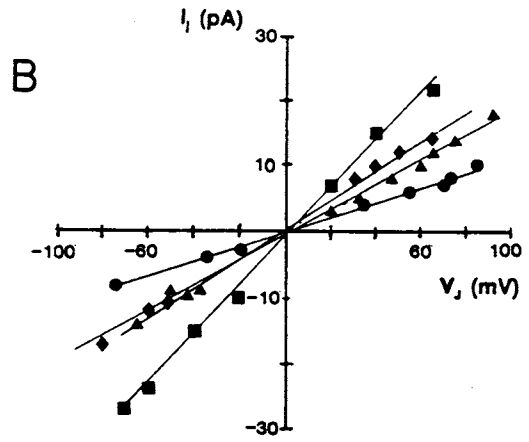
Dye transfer was found by others to be limited to two to five cells within rat and mouse islet (Michaels and Sheridan, 1981; Meda et al., 1986*b*) and in rat monolayers or clumps (Meda et al., 1982; Kohen et al., 1983). Also, in both preparations about one-third of the cells are not dye coupled to any others (Meda et al., 1982, 1986*b*). Furthermore, junctional specializations could be found between noncommunicating and communicating cells in a monolayer (Meda et al., 1982). However, electrical coupling was not studied between beta cells that were not dye coupled. Because we found a 67% incidence of electrical coupling between freshly isolated pairs, we evaluated the incidence of dye coupling by injecting LY into one of the cells with a microelectrode (n = 50) or patch pipette (n = 41) (see Methods). No dye transfer was detected when fluorescent excitation was applied 10–25 min later, independently of the method used for dye injection. To evaluate if dye and electrical coupling correspond, simultaneous measurements were done. Phase contrast and fluorescence micrographs of a representative cell pair are shown in Fig. 5 *A* and a recording of junctional current is shown in Fig. 5 *B*. These results show that absence of observed dye transfer does not preclude electrical coupling and that widespread electrical coupling could exist between islet cells without being evident from dye coupling studies. In contrast to these results dye coupling was rapidly detected (17.7 ± 8.46 S; n = 9) between electrically coupled acinar exocrine cells with g_j of tens of nanosiemens (19.5 ± 11.4 nS; n = 9). Phase contrast and fluorescent micrographs are shown in Fig. 5 *C* and electrical measurements in Fig. 5 *D*.

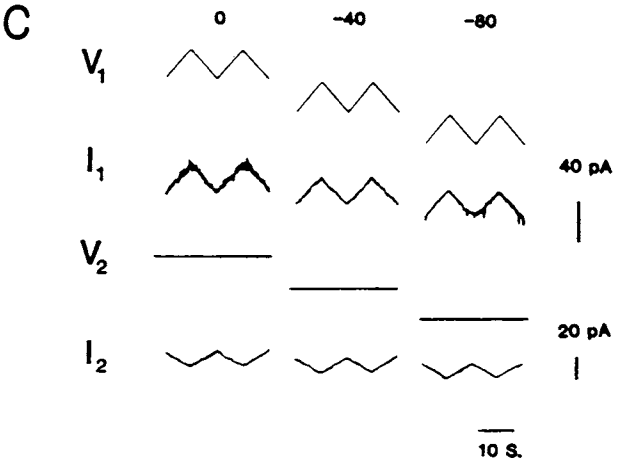
Because g_j between beta cells is particularly small, we estimated the time required for the fluorescent probe to be detected in the second cell, assuming that gap junctions between beta and acinar cell pairs have the same specific permeabilities. Consider dye movement between two cells:

$$dq/dt = (C_1 - C_2)P_j = V_2 dC_2/dt = -V_1 dC_1/dt, \quad (1)$$

where q is quantity of dye crossing, V is volume, C is concentration, subscripts denote the particular cell, and P_j is total permeability of the junctions (provided diffusion across cells is fast compared with that through the







junctions). For channels of a given kind, total permeability will be proportional to g_j.

$$P_j = kg_j.$$

Consider the case of a cell connected to a patch pipette containing dye at a concentration C_o, and that, on rupture of the patch the dye reaches equilibrium in the cell before there is a detectable transjunctional flux. Then, for concentration, C, in the other cell.

$$dC/dt = (C_o - C)P_j/V$$

$$C = C_o(1 - e^{t/\tau}),$$

where

$$\tau = V/kg_j.$$

If the detection threshold in the post cell is $C_d \ll C_o$, the rise to C_d will be nearly linear with slope $1/\tau$, and if t_d is the time for detection:

$$C_d = C_o k g_j t_d / V$$

and

$$k = (C_d/C_o)V/(g_j t_d).$$

We wish to compare two cell types a (acinar) and b (beta), in terms of relative permeability to the dye, i.e., the value of the constant k. Specifically, we ask whether the observation time in which we failed to see dye transfer is long enough to indicate that beta cell junctions are less permeable to the dye than acinar cell junctions. Because C_d will be inversely proportional to cell radius, r, and volume is proportional to radius

FIGURE 4 Junctional currents between beta cell pairs do not exhibit transjunctional or inside-outside voltage dependence. (*A*) Simultaneous recording of junctional and nonjunctional currents in a pair of beta cells in response to slow voltage ramps. I_2 changed in proportion to V_1 over a range from −90 to +20 mV. The nonjunctional membrane current (I_1) displayed outward rectification at voltages positive to −40 mV, which was near the threshold of voltage dependent K^+ channels. (*B*) I_j plotted as a function of transjunctional membrane potential. Curves were constructed by plotting the magnitude of the junctional current induced by voltage steps in the other cell. Each symbol corresponds to a different cell pair. Data points were fitted by linear regression with correlation coefficients higher than 0.99. The different slopes representing g_j are representative of the degree of variation recorded. (*C*) Recordings as in *A*. The mean holding potential of both cells was changed simultaneously to different values (0, −40, −80). Over holding potential, slowly ramps of 40 mV were applied. I_2 remained a constant function of the ramp in V_1, indicating lack of V_{i-o} voltage dependence.

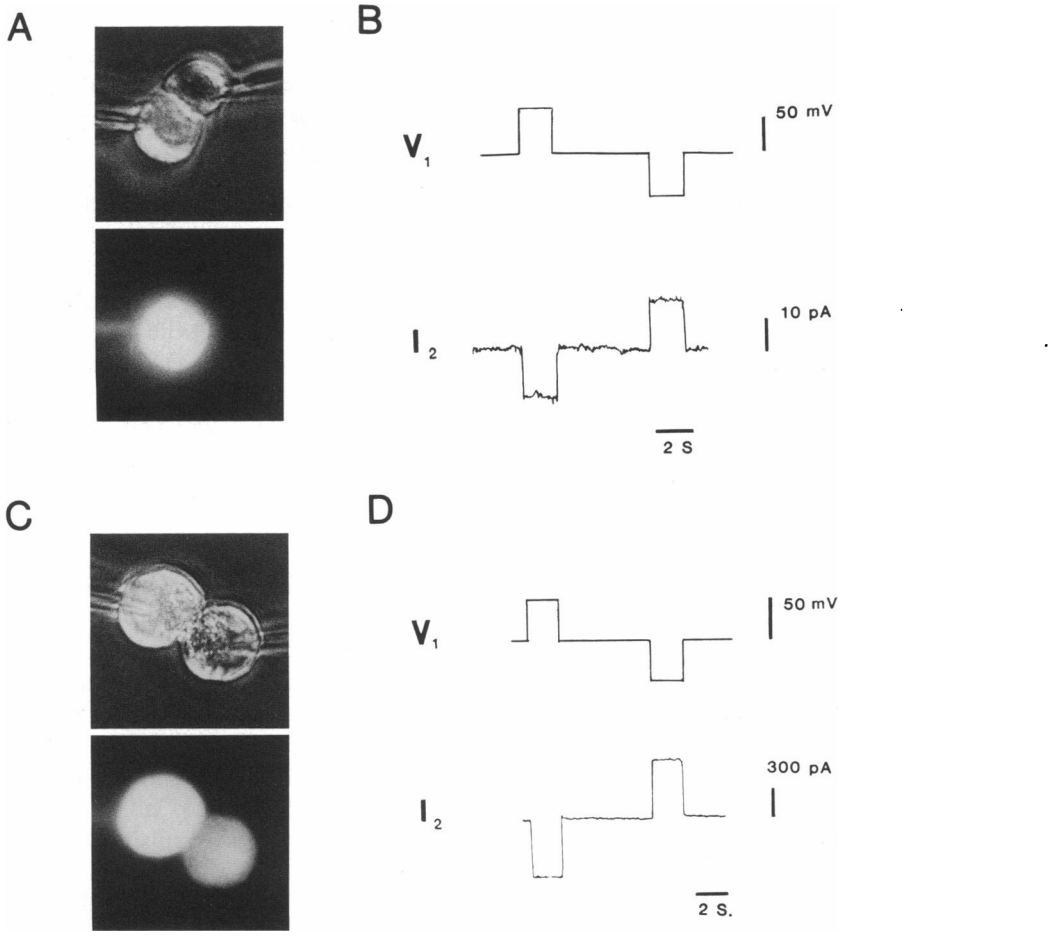


FIGURE 5 Pairs of beta cells are electrically but not dye coupled. (*A*) the right side shows phase contrast and fluorescent micrographs from a doubly voltage clamped pair of beta cells. Lucifer yellow (30 mM in internal solution) was injected through one patch pipette into the left cell, 2.5 min. before the second patch seal was opened. Voltage-dependent K^+ currents were recorded from both cells (not shown). The fluorescent micrograph was obtained 20 min after dye injection. (*B*) Junctional currents from the same cell pair recorded from for 15 min. g_j calculated for this pair was 220 pS. (*C*) As a control exocrine acinar cell pairs were studied with a similar protocol; phase contrast and fluorescent micrographs. Dye transfer was detected by 15 s after dye injection. (*D*) Corresponding junctional currents. g_j was 13 nS.

cubed, the relation between detection times of the two cell types with the same k measured under the same conditions will be:

$$t_{db} = t_{da}(r_b/r_a)^2(g_{ja}/g_{jb}),$$

where subscript letters are for the two cell types. If we use the $g_{ja} \cdot t_{da}$ value for acinar cells of 3.3×10^{-7} from the slope of the line fit by least squares to our measurements of t_{da} vs. $1/g_{ja}$, the mean g_j for beta cells 2.15×10^{-10} and the mean diameters of 16.5 μm for acinar cells and 12 μm for beta cells we have a predicted detection time for beta cells of:

$$t_{db} = 13.5 \text{ min.}$$

In eleven beta cell pairs, LY was injected through patch pipettes for 15 ($n = 6$) and 26 ($n = 5$) min. Cells were held at a potential of −70 mV and exhibited activation of inward and/or outward currents in response to depolarization during the injection time. No dye transfer was observed when fluorescent excitation was applied 26 min after rupture of patch. 12 of 14 other cell pairs from the same dish were electrically coupled with a mean g_j of 220 ± 92 pS. These results suggest that gap junctions between freshly dispersed beta cells are of lower permeability than those found between exocrine acinar cells. However, to explain our data the dye permeability only need be smaller by a factor of ~2.

Short term exposure to cAMP did not increase g_j in pairs of pancreatic beta cells

Evidence for long term modulation of gap junctions in islet cells by a cAMP-dependent mechanism has been reported. The number of gap junctions was increased by raising intracellular cAMP levels in reagregated cells after 16 h of incubation with glucagon or db-cAMP (Int' Veld et al., 1985) and dye transfer was enhanced between cultured cells after 24 h of exposure of IBMX (Kohen et al., 1983). We looked for effects of cAMP on g_j. Two groups of cell pairs (n = 20) were cultured for 5–8 hr in the presence or absence of 1 mM db-cAMP. No significant difference was found in the incidence of coupling or distribution of g_j values between the groups (Fig. 6). Longer incubation times could not be explored because beta cell pairs reaggregate preventing their unequivocal identification as pairs. Short term modulation of g_j in beta cells by cAMP-dependent protein kinase was also proposed because an increase in the coupling coefficient (V_2/V_1) was recorded in pairs of islet cells 1 min after bath application of 7 μM forskolin (Santos and Rojas, 1987). We found no effect of forskolin on g_j. In six pairs, I_j was recorded and 25 μM forskolin was applied in the bath. No change in I_j was found for up to 7 min (not shown). These results suggest that gap junctions in freshly isolated pairs of beta cells are not modulated by A kinase for at least several hours (see Discussion).

Lower concentrations of octanol and heptanol than required to uncouple beta cells substantially decrease voltage-dependent potassium and calcium currents

A decrease in insulin secretion associated with a decrease in dye transfer and the number of gap junction particles has been reported to be induced by 1 mM octanol and by 3.5 mM heptanol suggesting that uncoupling might affect the glucose-induced insulin response (Meda et al., 1986*a*). To study how alkanols might affect insulin secretion, we examined the effect of octanol and heptanol on g_j and voltage sensitive K^+ and Ca^{2+} currents. In eight pairs of beta cells, 2 mM octanol reduced g_j to unmeasurable levels within 1 min; on washing g_j partially recovered within 2–4 min (Fig. 7). In three other pairs, 1 mM octanol did not reduce g_j within the next 5 min. In four cells in which potassium currents were monitored with high EGTA and ATP in the internal pipette solution (solution F) and Cd^{2+} in the external recording solution (solution C) to isolate the delayed rectifier current from the ATP-sensitive K^+ and Ca^{2+}-activated K^+ currents, 1 mM octanol reduced voltage-dependent potassium currents by 75% within seconds (Fig. 8*A*). K^+ currents completely recovered within 1 min after rinsing with normal saline. The same concentration of octanol significantly suppressed voltage-sensitive Ca^{2+} currents also within seconds (n = 3)

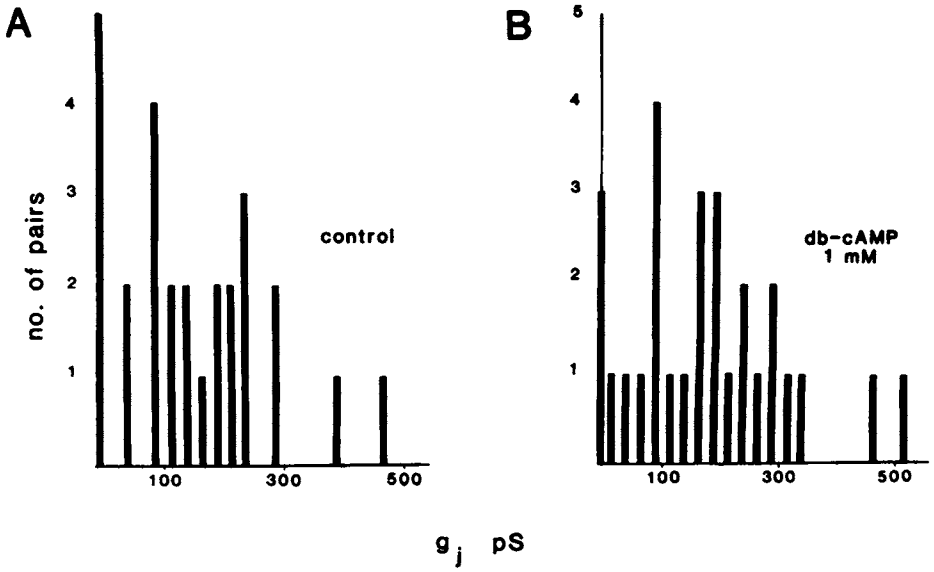


FIGURE 6 Junctional conductance between pairs of beta cells is not modulated by cAMP. Histograms of g_j values in doubly voltage clamped pairs of cells. Two groups of cell pairs were incubated for 4–6 h in culture medium with 11 mM glucose in the presence or absence (control) of 1 mM db-cAMP. No significant difference was found in the incidence of coupling or distribution of g_j values between the groups.

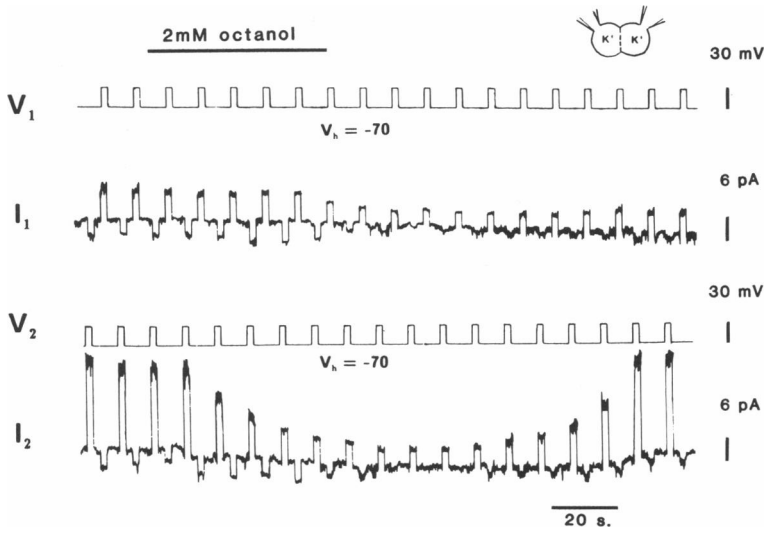


FIGURE 7 Octanol decreases junctional conductance between pairs of beta cells. Recordings as in Fig. 3 *A*. Positive steps of voltage were applied alternately to both cells from a holding potential of −70 mV. I_1 and I_2 illustrate junctional currents (downward deflections) and nonjunctional currents (upward deflections). 50 s after application of 2 mM octanol junctional currents were almost completely blocked and nonjunctional currents were also significantly reduced. Only partial recovery of junctional currents was observed after washout of octanol.

(Fig. 8 *B*). Suppression depended on voltage and was maximal at voltages at which activation was increasing. During octanol perfusion Ca^{2+} currents inactivated faster than in control solution and after octanol washout inactivation returned slowly toward its control values within 5 min. At this time partial recovery of calcium currents was obtained. Dose-response relations for octanol effects on peak Ca^{2+} and steady state K^+ currents are shown in Fig. 8 *C*. To study the effect of heptanol, voltage sensitive Ca^{2+} currents were recorded in six cells. 0.5 mM heptanol decreased Ca^{2+} currents by ~60%. In one experiment Ca^{2+} currents and g_j were monitored simultaneously. Within 1 min after 0.5 mM heptanol application, Ca^{2+} currents were decreased (Fig. 9) whereas 2 min later g_j was unaffected or even slightly increased. Remaining outward current observed during heptanol application could be due to leakage because these records were obtained without leakage correction. After 5 min of heptanol wash out g_j remained almost constant and calcium currents recovered partially. Incompleteness of recovery of calcium currents could have been a result of heptanol effects on the channel or its environment, or be due to wash out phenomena, even though an ATP regenerating system was contained in the pipette solution (solution G). To answer this question further experiments are required; however, this experiment demonstrates that beta cells do not uncouple at a concentration of heptanol that decreases Ca^{2+} currents. Because sustained glucose-induced insulin release is dependent on calcium influx through voltage-sensitive channels (Henquin et al., 1985), these findings indicate that octanol and heptanol are not ideal uncoupling agents to evaluate the role of gap junctions in insulin release.

Octanol reduced g_j without apparent single gap junction channel fluctuations

Spontaneous uncoupling was not common between pairs of beta cells, but observation of single channel fluctuations was expected because mean g_j was very low, 215 pS, and input conductance was also low (0.2–1 nS). However, even during uncoupling induced with octanol no obvious single channel currents were observed. To reduce interference by channels in nonjunctional membranes we made ionic substitutions in the pipette and external recording solution (solutions G and E). Under these conditions input conductances of 0.02–0.4 nS were obtained and Ca^{2+} currents were initially recorded in both cells. After 100 μM Cd^{2+} was added to the external solution to further reduce nonjunctional membrane activity, the noise level decreased to <1 pA. One of these experiments is shown in Fig. 10. To enhance the probability of seeing single channel fluctuations a transjunctional potential of 60 mV was applied, which at the recorded noise level should have made 20 pS events detectable. 2 mM octanol was applied to the bath to induce uncoupling. g_j was initially 130 pS; after octanol application g_j progressively decreased to <30 pS in the

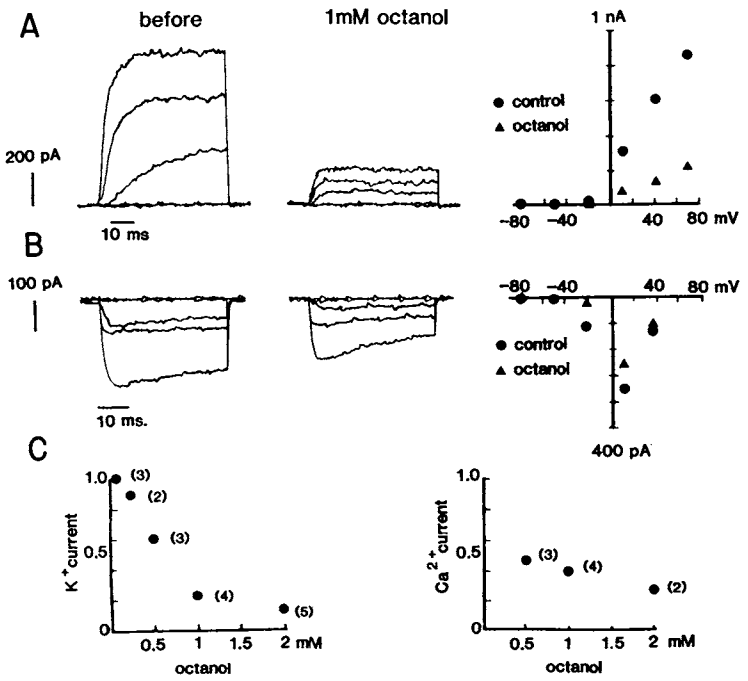


FIGURE 8 Octanol suppresses calcium and potassium currents at lower concentrations than required to uncouple beta cells. (*A*) Families of leakage corrected potassium currents recorded in control medium from a single beta cell before and in the presence of 1 mM octanol. The external solution contained 100 μM Cd^+ (solution C) to block Ca^{2+} currents, and the pipette contained solution F. The membrane potential was stepped in 30 mV increments starting from a holding potential of −80; on the right are the corresponding I–V curves. Control currents were obtained during the second minute after opening the seal and 2 min later the effect of octanol was evaluated. (*B*) Families of leakage corrected Ca^{2+} currents recorded as in *A*, except that the external solution contained 10 mM $CaCl_2$ and 200 nM TTX (solution D) and Cs^+ replaced K^+ in the internal pipette solution (solution G). (*C*) Dose response relations for octanol's effect on peak Ca^{2+} and steady state K^+ currents. Ordinates shows values normalized to those in control saline. Numbers in parenthesis indicate number of experiments.

absence of apparent decremental steps (Fig. 10 *B*). Washout of octanol was followed by a partial recovery of I_j without incremental steps in g_j. Recovery could usually be followed for only a limited time (2–3 min) because one of the very high resistance seals usually became leaky after the solution changes associated with octanol perfusion. In five experiments similar results were obtained.

DISCUSSION

This work utilized isolated pairs of pancreatic islet cells, which allowed for the first time direct measurement of gap junctional conductances between beta cells.

Cell identification

Most of the freshly dispersed cells (>95%) were identified by immunofluorescence as beta cells, and all of the cells studied exhibited voltage-activated Ca^{2+} and/or K^+ currents with characteristics similar to those previously reported in cultured beta cells. Given that islets represent only 1% of the total pancreas the main possible source of contamination should be exocrine acinar pancreatic cells; however, acinar cells do not attach to coverslips when obtained with protocols used for beta cells, are usually larger, and do not exhibit voltage-dependent currents. Alpha cells are the other endocrine cell type which also exhibits voltage sensitive Ca^{2+} and K^+ currents (Rorsman and Hellman, 1988). However, the low abundance of alpha cells in the islet (<8%) and the high percentage of insulin positive cells in our cultures indicate that most g_j records represent beta cell pairs.

Incidence and strength of coupling

Most freshly dispersed pairs of cells (67%) were coupled but g_j was smaller than measured in other tissues. Coupling was mediated by gap junctions because (*a*) gap junctions were observed in freeze fracture replicas in cell preparations similar to ones used for recording; (*b*) g_j measurements were similar when Cs^+ replaced K^+ in

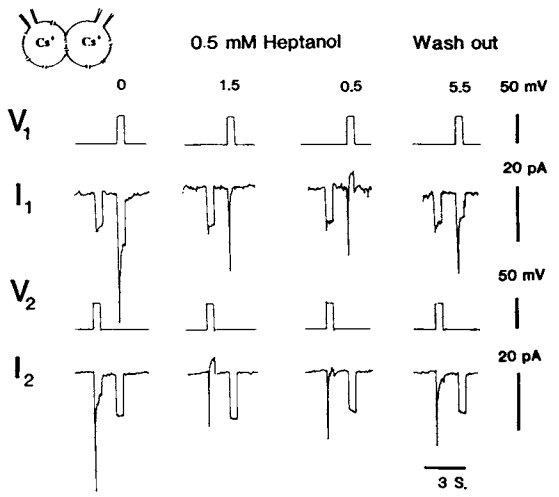


FIGURE 9 Heptanol reduces calcium currents at lower concentrations than required to uncouple beta cells. Simultaneous recording of voltage sensitive Ca^{2+} currents (without leakage correction) and g_j from a doubly voltage clamped pair of cells. Ca^{2+} currents were activated by alternate depolarizing steps applied in each cell (V_1 and V_2) from a membrane potential of −60 mV, and recorded with solutions G and D in the internal pipette and external solution. Decreasing inward currents shown in I_1 and I_2 in the polarized cell represent the sum of Ca^{2+} currents, leakage, other nonjunctional and junctional currents, I_j. Net nonjunctional current can be obtained by subtracting I_j recorded in the other cell. The more rectangular current deflections in the nonpolarized cell represent I_j. Numbers on the top of traces represents time (in min) during application (0, 1.5) and after washout (0.5, 5.5) of 0.5 mM heptanol in the bath solution. Inward Ca^{2+} currents decreased and net nonjunctional current became positive during heptanol perfusion, whereas I_j was little affected. 5.5 min after heptanol washout, Ca^{2+} currents recovered only partially.

the internal pipette solution, demonstrating that coupling of cell pairs was not mediated by accumulation of K^+ between them (Pérez-Armendariz et al., 1985) or by apposition of high conductance membranes permeable to K^+; (*c*) coupling between cell pairs could not have been due to cytoplasmic bridges because Lucifer yellow did not pass between coupled cells; (*d*) g_j was reduced rapidly and reversibly by octanol which is known to decrease g_j in other systems; and (*e*) octanol and heptanol at concentrations that had little effect on g_j substantially decreased nonjunctional conductance and voltage sensitive K^+ and Ca^{2+} conductances; thus nonjunctional and coupling conductances are not mediated by the same kinds of channel.

The low g_j values between freshly dispersed pairs of cells is unlikely to result from enzymatic alteration of gap junctions during islet cell dispersion because a similar range of g_j values was recorded from islet cells isolated with and without papain. These g_j values correlate with the small size of gap junctions in islets where an unusually small median number of 11 gap junction particles per array is found in 43% of the polygonally packed junctions and median number of eight particles is found in linearly arrayed junctions (In't Veld et al., 1986; Meda et al., 1983). Given the high input resistance measured in beta cells, a small amount of intercellular current flow should effectively synchronize electrical activity in its neighbors. The mean junctional resistance that we measured between isolated pairs of beta cells (4.6 GΩ) is close to that calculated from the spatial decay of current measured within the islet using microelectrodes (7.7 GΩ) (Eddlestone et al., 1984). Also, in a recent mathematical model of a cubic array of cells electrically coupled by a junctional conductance in the range that we report, spatial decay is similar to that recorded in the islet and synchronization of electrical activity occurs. In fact, fitting with a junctional conductance close to ours produces a more stable burst pattern than using infinite conductance (Sherman and Rinzel, 1991).

The high incidence of coupling recorded between isolated pairs suggests that most of the islet is electrically coupled in situ and agrees with previous studies of the intact islet showing that: (*a*) membrane potential oscillations induced by glucose concentrations higher than 5 mM are almost synchronous when recorded in cells separated by as much as 400 μm (Meda et al., 1984*a*); (*b*) oscillations in intercellular potassium concentration recorded with K^+ sensitive microelectrodes are synchronous at sites separated by at least 40 μm (Pérez-Armendariz and Atwater, 1986); and (*c*) oscillations in the intracellular calcium concentration between islet cells, measured with the fluorescent indicator Indo-1, are synchronous throughout the islet (Valdeolmillos et., 1989). Our results suggest that electrical coupling is the main mechanism for synchronizing electrical activity within the islet. Accumulations in interstitial $[K^+]$ could reinforce the effect of electrical coupling and synchronize cells that were marginally coupled or not coupled at all (Pérez Armendariz et al., 1985).

The substantial fraction of pairs that were not electrically coupled could have (*a*) resulted from reaggregated cells that had not yet formed active gap junctions, (*b*) been due to injury during isolation, or (*c*) represented a fraction of cells that were not coupled in the intact islet (Meda et al., 1984*a*).

Electrically coupled domains are larger than dye coupled ones

Extensive dye coupling was detected between almost all cells within one acinus from the exocrine pancreas and other glands studied (e.g., Itwatsuki and Petersen, 1978). Dye coupling within the islet (Michaels and Sheridan, 1981; Meda et al., 1986*b*) and monolayers

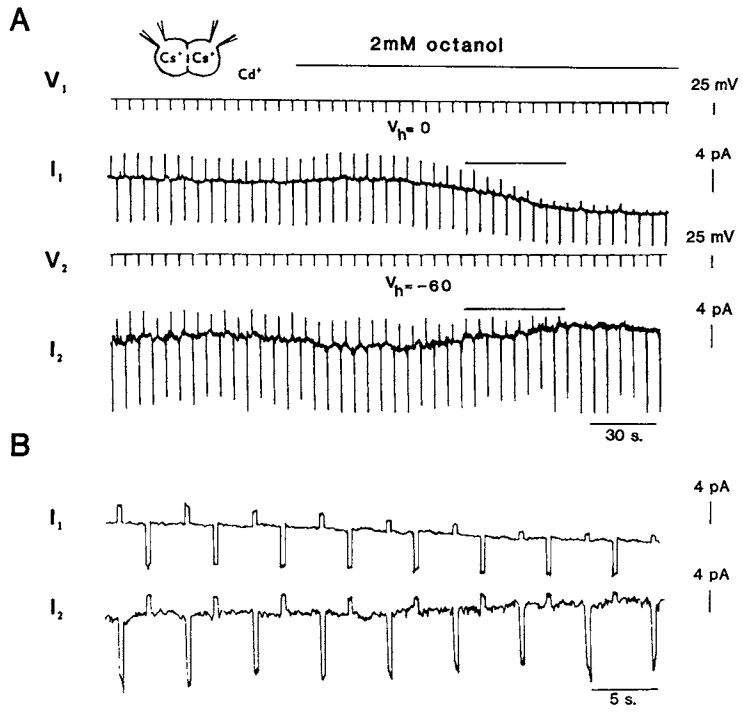


FIGURE 10 Octanol reduces g_j without the appearance of unitary fluctuations in junctional current. (*A*) Recordings as in Fig. 3 *A* from a doubly voltage-clamped pair of cells. Holding potentials for cells 1 and 2 were 0 and −60 mV, respectively. Brief hyperpolarizing pulses were applied alternately in the two cells to monitor g_j. Calcium currents were recorded in both cells before 100 μm Cd^+ was applied in the bath solution. Membrane noise level was < 1 pA which should have allowed detection of fluctuations as small as 20 pS. At 60 mV transjunctional voltage the 4 pA calibrations correspond to 67 pS. 1 min after 2 mM octanol was applied to the bath g_j decreased gradually from 130 to 30 pS, without evidence of single channel fluctuations. (*B*) Expanded records from the period in A indicated by horizontal lines.

(Meda et al., 1982; Kohen et al., 1983) is restricted to small groups of cells (2–5), and about one third of the cells are not dye coupled to any others (Meda et al., 1982, 1986*b*). Communicating and noncommunicating cells can be adjacent and when there is dye coupling it occurs within seconds after which there is no further extension of the dye coupled domain (Meda et al., 1982, 1984*b*). This result suggests that there is a qualitative change in permeability at the border of the domain and not simply a reduced number of junctional channels.

In contrast with the restricted dye coupling between islet cells is the widespread synchrony in electrical activity. These results raised the possibility that small compartments of coupled cells could exist within the islet (Orci, 1982) that were synchronized through other mechanisms such as accumulation of interstitial potassium (Pérez-Armendariz et al., 1985). However, it was not determined if cells within the islet or monolayer could be electrically coupled but not dye coupled. Gap junctions had been found between noncommunicating beta cells (Meda et al., 1982) and electrical coupling without dye coupling has been reported during embryonic development (Lo and Gilula, 1979; Serras et al., 1988), although junctional conductances were not measured. By injecting Lucifer yellow through patch pipettes we were able to monitor g_j and dye coupling simultaneously. Our technique demonstrated electrical and dye coupling between pairs of exocrine cells. However, we failed to see dye coupling between pairs of electrically coupled beta cells. We asked whether the much lower g_j values between beta cells accounted for the failure to see dye coupling or whether the beta cell junctions were less permeable to the dye molecules. Beta cell junctions did indeed appear to be less permeable, but the P_j/g_j ratio needs to be smaller for beta cells by a factor of ~2 to explain our data, and we cannot conclude that these junctions are completely impermeant to the dye. The apparently more restrictive permeability of beta cell junctions correlates with their estimated smaller single channel conductance compared with acinar cells where their single channel conductance ranges from 22–120 pS (Somogyi and Kolb, 1988).

The frequent observation of dye coupling occurring in islets and monolayers can be reconciled with our failure to see coupling in pairs by larger g_j's in the former preparations. Alternative possibilities are that in there is

expression of a less abundant gap junction protein which is missed during preparation of pairs or that gap junction channels are modified to a lower conductance state. In preliminary experiments with the same methods we have observed dye transfer in some beta cell clumps cultured for 24 h; however, to explain this observation a detailed quantification of dye coupled cells and days of culture will be required. In situ hybridization or immunohistochemistry should also resolve the issue of whether additional connexins are expressed. In any case these experiments show that beta cells are extensively coupled by gap junction channels with a small unitary conductance (<20 pS) apparently less permeable to LY. These channels may be present between islet cells as well as in dispersed preparations and could explain the apparent discrepancy between the restricted dye transfer and widespread electrical synchrony observed between islet cells. Edges of dye coupled domains could be borders where specific permeability or junctional conductance is decreased but where there is still sufficient conductance to assure electrical synchrony. Transfer of large molecules could be more restricted than that of small ions allowing differential compartmentalization, in agreement with the functional heterogeneity of beta cells in terms of their biosynthetic (Schuit et al., 1988) and insulin release capability (Bosco et al., 1989).

Junctional conductance between beta cells is not voltage gated

In agreement with previous studies where linearity in V_1/V_2 was recorded between pairs of islet cells (Eddlestone et al., 1984), g_j between isolated pairs of beta cells did not show transjunctional voltage dependence between -80 and 80 mV, nor was there inside–outside voltage dependence when the nonjunctional membrane potential of both cells was changed to different values between -80 and 0 mV.

Short term exposure to cAMP does not affect g_j in pairs of pancreatic beta cells

Our results suggest that g_j between beta cells is not modulated by increments in $[cAMP]_i$ for at least 8 h. Although we detected no short term effect of forskolin on g_j, forskolin was reported to enhance the coupling coefficient between intact islet cells within seconds (Santos and Rojas, 1987). This apparent contradiction could be explained if forskolin affected nonjunctional conductances through a cAMP-independent mechanism, e.g., blocking of the delayed rectifier channels (Zünckler et al., 1988), which would enhance V_2/V_1. This explanation is supported by the reversal of forskolin effects on V_2/V_1 within seconds of removal, whereas elevations in $[cAMP]_i$ induced by forskolin require minutes to dissipate (Flamm et al., 1987). Another possibility is that in our experiments exchange of intracellular medium in cell pairs with the pipette solution could have prevented cAMP-induced modulation of conductances by dilution or inactivation of intracellular components, e.g., adenylyl cyclase or A kinase. cAMP reagents increase g_j in hepatocytes and neonatal heart cells recorded from with patch electrodes, although in both of these cases the cells are larger than beta cells (Saez et al., 1986; Burt and Spray, 1988*b*). In islet cells pretreated with db-cAMP for 5–8 h measured g_j values were the same as in controls. It is possible that a modest effect on g_j after 5–8 h of incubation with dibutyryl cAMP was not detected because of the large variability in our measurements. An increase in gap junction particles between islet cells was observed after 24 h of treatments which enhance $[cAMP]_i$ (In't Veld et al., 1985). Modulation at longer times has been observed in other tissues (e.g., Saez et al., 1989), intervals which could not be followed in beta cells because of reaggregation.

Lower concentrations of octanol and heptanol than required to uncouple beta cells decrease voltage-activated calcium and potassium currents

A higher concentration of octanol, 2 mM, was required to uncouple beta cells than in other systems (see below). Unitary fluctuations were not observed between beta cells even during uncoupling induced by octanol. The mechanism of action of alkanols on gap junctions and other membrane channels is not known. Octanol (0.4 mM) also induced a progressive decrease in g_j between pairs of exocrine acinar cells without the appearance of single channel events (Somogyi and Kolb, 1988), whereas in these cells unitary currents could be recorded when uncoupling was induced by activators of kinase C (Somogyi et al., 1989; but see also Chanson et al., 1988). In contrast gap junctions between neonatal heart myocytes, single channel conductances of 50 pS were recorded (Veenstra and DeHaan, 1986; Burt and Spray, 1988*a*; Rook et al., 1988) which were similar when uncoupling was induced by heptanol (1 mM) and when low conductance pairs allowed their resolution without treatment (Burt and Spray, 1988*b*).

Alkanols have been reported to affect various voltage sensitive currents (Paternostre and Pichon, 1987; for review: Carlen, 1987; Pozos and Oakes, 1987; Niggli et al., 1989). A concentration of octanol of 2 mM was required to decrease g_j between cell pairs. Monitoring of junctional and nonjunctional currents showed that this concentration considerably depressed Ca^{2+} and K^+ cur-

rents. Concentrations of octanol as low as 0.5 mM did not affect g_j within 5 min whereas they induced a substantial decrease in both voltage activated currents. Also we found that the lowest concentration of heptanol (0.5 mM) reported to affect glucose-induced insulin secretion between cell pairs (Meda, P., personal communication) suppressed Ca^{2+} currents in beta cells, and in cells from a pair where g_j remained unaffected. These studies demonstrate that the threshold concentration of heptanol and octanol to depress Ca^{2+} and K^+ currents is lower than required to suppress g_j. These agents may first decrease insulin secretion by affecting calcium fluxes and therefore they may not be the best uncoupling agents for study of functional roles of gap junctions in secretion (but see Meda et al., 1987).

What is the protein forming gap junctions between beta cells?

Possibly as a result of the unusually small size of gap junctions between islet cells, immunofluorescent studies remain controversial. Antibodies against connexin 32 do not label islets of control rats but do label islets obtained from glibenclamide treated and lactating rats (Dermietzel et al., 1984; Hertzberg et al., 1988) in which dye transfer and the number of gap junction particles are enhanced (Meda et al., 1983; Michaels et al., 1987). Recently, punctate labeling with antibodies against connexin 43 was observed between rat islet cells, but connexin 32 immunoreactivity was not observed. In addition mRNA from isolated rat islets was found to hybridize with cDNAs for connexin 43, but not for connexin 32 (Meda et al., 1991). Using different antibodies against anticonnexin 26, 32, and 43 we did not find with immunofluorescence typical punctate labeling of cell interfaces between mouse islets, whereas exocrine pancreas was well labeled with antibodies against connexin 26 and 32. The same results were obtained in islets from lactating mice (unpublished results). Further studies using Western and Northern blot analysis in pure preparations of beta cells should help to clarify this issue.

Electrophysiological studies reveal that gap junctions between beta cell pairs display characteristics unlike those observed in other tissues. Although mean junctional conductance is the smallest reported (215 pS), no spontaneous openings or closings were observed during long periods of stable recording. Spontaneous uncoupling during which single channel junctional currents were seen was reported for lacrimal and pancreatic acinar cells (Neyton and Trautman, 1985; Somogyi and Kolb, 1988), but was not common in pairs of beta cells, even in the absence of ATP in the internal pipette. Gap junctions between neonatal heart myocytes, which are labeled by antibodies against connexin 43, exhibit single channel conductances of 50 pS (Burt and Spray, 1988*a*; Rook et al., 1988). The smallest gap junction channel transitions reported previously correspond to unitary conductances on the order of 20–30 pS (fibroblasts: Rook et al., 1989; hepatoma cell lines: Moreno et al., 1989; Spray et al., 1991). Events of this magnitude would have been detected under our recording conditions. Unitary fluctuations were not observed even during uncoupling induced by octanol, suggesting either that the individual channel conductances are smaller than 20 pS, that the mean conductance is due to fast flickering between open and closed states, or that the transitions occur very slowly. The lower dye permeability inferred for beta cells is consistent with, although not necessary for, a smaller unitary conductance.

Since the first single channel records from gap junctions were obtained, (Neyton and Trautman, 1985), different unitary conductances of gap junction channels have been reported. More than one type of connexin can coexist in the same gap junction plaque (Nicholson et al., 1987), multiple channel sizes can be recorded between clonal cells (Spray et al., 1991) and unitary junctional conductance can be modified in heterotypic junctions that presumably have two different kinds of hemichannels (Rook et al., 1989). The unitary conductance between beta cells may be smaller than any other reported to date (<20 pS). This small unitary conductance could be a result of modification of a known gap junction protein or expression of a different connexin type.

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