Cell-to-Cell Communication in Rat Pancreatic Islet Monolayer Cultures is Modulated by Agents Affecting Islet-Cell Secretory Activity

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SUMMARY
Single islet cells in monolayer cultures of neonatal rat pancreas were microinjected with the fluorescent dye Lucifer Yellow CH and the cultures were observed by combined phase contrast and fluorescent microscopy. The dye spread from an injected cell directly into neighboring islet cells, and successive microinjections of dye into different cells defined territories comprised of 2–6 communicating cells. The number of communicating cells could be modulated by addition to the cultures of different agents known to affect islet cell secretory activity. Cell communication was significantly increased by a high (16.7 mM) glucose concentration, by the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX, 0.1 mM), and by the calcium ionophore, A23187. The effect of A23187 was transient and dose-dependent. Somatostatin (1 µg/ml) significantly inhibited cell communication. These results demonstrate that cell-to-cell communication may participate in the regulation of islet cell secretory activity.

METHODS
Islet cell cultures. Monolayer cultures of neonatal rat pancreatic islet cells were prepared by a modification of a collagenase-trypsin method. The islet cells were cultured in medium 199 with modified Earle's salts, L-glutamine, and phenol red (Gibco Laboratories, Grand Island, New York), buffered with 25 mM sodium bicarbonate, and supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), 10% fetal calf serum, and 16.7 mM glucose. The cultures were prepared on a 0.1-mm-thick coverglass cemented to a 20-mm diameter circular stainless steel ring designed for observation in an optical system for microfluorometry and microinjection. Ten-to-fourteen-day-old cultures were used in the experiments. At that time, the islet cells were well spread out in monolayer clusters of approximately 20–200 cells each. Previous electron microscopic evaluations of the islet cells in these cultures revealed that approximately 80% are B-cells, 10% are A-cells, and 10% are D-cells. Contamination with fibroblastic, endothelial, or acinar cells was negligible.

Microinjection experiments. All observations were carried out at 37°C, on a microscope heated stage. Individual islet cells were microelectrophoretically injected with the fluorescent dye, Lucifer Yellow CH (kindly provided by Dr. Walter...
W. Stewart, National Institutes of Health, Bethesda, Maryland). This dye is a low-mol-wt, highly fluorescent probe that will not permeate the nonjunctional membrane of a viable cell. The injected cell and its neighbors in the islet cell cluster were continually observed by fluorescence microscopy. Often, two or more injections could be carried out successively in different islet cells of the same cluster. After removal of the microinjector, the selected cluster was photographed under combined phase-contrast and fluorescence microscopy and identified on the cover glass with a diamond marker objective for subsequent immunohistochemical and ultrastructural studies.

Medium 199 supplemented with 10% serum and 16.7 mM glucose was used as control medium for islet cell injections of fluorescent dye. Test additions to this medium, or to medium containing lower concentrations of glucose (3.3 and 5.6 mM), included somatostatin (1 μg/ml) (Boehringer Mannheim Biochemicals, Indianapolis, Indiana), 3-isobutyl-1-methylyxanthine (IBMX, 0.1 mM) (Aldrich Chemical Co., Milwaukee, Wisconsin), and the calcium ionophore, A23187 (Calbiochem-Behring Corp., La Jolla, California).

**RESULTS**

Microinjection of Lucifer Yellow CH into individual islet cells was usually, but not always, followed by transfer of the dye from the injected cell into one or more adjacent cells in the same cluster. The number of communicating cells defined the size of a communicating microterritory, and this was determined for different culture conditions (Table 1).

In the first series of experiments, the effects of the glucose concentration in the culture medium were examined. A communicating microterritory comprised 2.2 ± 0.3 cells for cultures incubated in medium containing a high (16.7 mM) glucose concentration. There was no significant change in the size of a communicating territory after 2–5 h of incubation in a lower (3.3 mM) glucose concentration. After 24 h of incubation in 3.3 mM glucose, however, the size of the communicating territory (1.7 ± 0.4 cells) was significantly decreased compared with the value with 16.7 mM glucose (2.2 ± 0.3 cells, P < 0.01). In other experiments, addition of somatostatin (1 μg/ml) to 16.7 mM glucose also decreased cell-to-cell communication significantly, and the effect was more rapid (within 5–60 min) than that observed after decreasing the glucose concentration.

The effects of agents that stimulate islet secretory activity were examined next. The phosphodiesterase inhibitor, IBMX, was added to the culture medium at a concentration (0.1 mM) that significantly increases islet cell levels of cyclic adenosine-3',5'-monophosphate (cyclic AMP) without being toxic to the cells during 48 h or more of culture. There was a gradual increase in the size of a communicating microterritory during culture in medium with 5.6 mM glucose supplemented with 0.1 mM IBMX, and this increase was significant after 48 h (2.2 ± 0.4 versus 1.2 ± 0.1 cells, P < 0.01). The stimulatory effect of IBMX on islet cell communication was smaller and not significant in medium containing a high glucose concentration.

In the final series of experiments, the effects of a calcium ionophore with stimulatory effects on insulin release were examined. The ionophore A23187 (10^4M) produced a rapid (within 10 min) significant increase in the size of a communicating territory in medium containing 16.7 mM glucose, and the maximal effect was observed between 10 and 30 min after addition of ionophore (3.6 ± 0.2 compared with

**TABLE 1**

| Culture media additions* | Number of cells communicating | Number of injections |
|--------------------------|-------------------------------|---------------------|
| 16.7 mM G                | 2.2 ± 0.3                     | 4                   |
| 3.3 mM G (2-5 h)         | 2.0 ± 0.4                     | 10                  |
| 3.3 mM G (24 h)          | 1.3 ± 0.1                     | 12                  |
| 16.7 mM G                | 2.1 ± 0.2                     | 16                  |
| 16.7 mM G + 1 μg/ml somatostatin (5-60 min) | 1.3 ± 0.2 | 15 |
| 16.7 mM G                | 1.9 ± 0.3                     | 7                   |
| 5.6 mM G (24 h)          | 1.2 ± 0.1                     | 9                   |
| 5.6 mM G (22 h) → 5.6 mM G + 0.1 mM IBMX (2-3 h) | 1.5 ± 0.2 | 14 |
| 5.6 mM G + 0.1 mM IBMX (24 h) | 1.7 ± 0.2 | 15 |
| 5.6 mM G + 0.1 mM IBMX (48 h) | 2.2 ± 0.4 | 9 |
| 16.7 mM G                | 1.7 ± 0.2                     | 17                  |
| 16.7 mM G + 0.1 mM IBMX (48 h) | 2.1 ± 0.3 | 9 |
| 16.7 mM G                | 1.7 ± 0.2                     | 4                   |
| 16.7 mM G + 10^-4 M A23187 (10 min) | 3.0 ± 0.8 | 7 |
| 16.7 mM G + 10^-4 M A23187 (10-30 min) | 3.6 ± 0.2 | 7 |
| 16.7 mM G + 10^-4 M A23187 (>30 min) | 1.8 ± 0.7 | 6 |
| 5.6 mM G (24 h)          | 1.1 ± 0.1                     | 6                   |
| 5.6 mM G (24 h) → 5.6 mM G + 10^-4 M A23187 (20 min) | 1.1 | 2 |
| 5.6 mM G (24 h) → 5.6 mM G + 10^-4 M A23187 (20 min) | 1.8 ± 0.3 | 4 |
| 5.6 mM G (24 h) → 5.6 mM G + 10^-4 M A23187 (>20 min) | 1.5 ± 0.2 | 11 |
| 5.6 mM G (24 h) → 5.6 mM G + 10^-4 M A23187 (>20 min) | 1.1 ± 0.1 | 4 |

*Islet monolayer cultures were incubated continuously in medium containing 16.7 mM glucose (control), or in test media with the additions and for the times indicated.

†Mean (±SEM) number of communicating cells. 1 = no transfer of dye from the individual cell injected.

‡Number of microinjections performed on different individual cells in one or more clusters in at least two different dishes.
FIGURE 1. Combined phase-contrast and fluorescence photomicrographs of the sequential effects of the calcium ionophore, A23187, on intercellular dye transfer in an islet cell cluster in monolayer culture. (a) The culture was maintained in control medium containing 5.6 mM glucose, and the fluorescent dye, Lucifer Yellow CH, was injected into single islet cells (asterisks) at sites 1 and 2. No dye transfer from the injected cells was observed. (b) The ionophore A23187 (10⁻⁷M) was added to the culture medium, and 5 min later, the dye was injected into a single islet cell (asterisk) at site 3. Dye transfer into one adjacent islet cell was observed at site 3. (c) Seven and ten minutes after addition of A23187, the dye was injected into single islet cells (asterisks) at sites 4 and 5, respectively. Dye transfer occurred into one adjacent islet cell at site 4, and into three adjacent islet cells at site 5. (d) Thirteen minutes after addition of A23187, the fluorochrome was injected into a single islet cell (asterisk) at site 6. No dye transfer occurred.

1.7 ± 0.2 cells before ionophore addition, P < 0.001). The stimulatory effect of the calcium ionophore was transient, however, and no longer apparent after 30 min. The sequential effects of A23187 on islet cell-to-cell communication are illustrated in Figure 1. There was no dye transfer from the injected cell incubated in control medium containing 5.6 mM glucose (the mean value for the number of communicating cells incubated in control medium was only 1.1 in this series of experiments). Islet-cell communication was increased maximally at 10 min after addition of the calcium ionophore (Figure 1c), and at 13 min, the stimulatory effect was no longer evident (Figure 1d). Table 1 also shows that 10⁻⁷M A23187 was an optimal concentration for stimulating increased islet-cell communication, and that a higher concentration (10⁻⁶M) of the ionophore was less effective, the stimulation lasting less than 20 min.

DISCUSSION

The present study demonstrates that, by using the techniques of tracer fluorochrome microinjection and combined phase-contrast fluorescence microphotography, it is possible to investigate the effects of nutrients, hormones, and drugs on the size and distribution of communicating territories in monolayer cultures of islet cells. Identification of the particular islet endocrine cell types participating in the modulation of microterritory size described in the present experiments awaits completion of the immunohistochemical and ultrastructural studies to be performed on the retrieved islet clusters. Nevertheless, it is likely that a large majority of the cell-to-cell transfers of fluorochrome were between homologous insulin-containing B-cells, since we have found that homologous B-cell couplings account for approximately 90% of dye couplings between islet cells, consistent with the numerical proportion of B-cells in our islet cultures (~80%).

Overall, the numbers of communicating cells were significantly increased by insulin secretagogues and decreased by conditions or agents that inhibit insulin release (Table 1). Thus, a glucose concentration (3.3 mM) that is nonstimulatory for insulin release in islet monolayer cultures leads to a significant decrease in islet cell communication after 24 h. Somatostatin significantly and rapidly decreased islet cell communication in the presence of 16.7 mM glucose, an action which may contribute to the recognized effects of this peptide to inhibit both insulin and glucagon secretion.

The phosphodiesterase inhibitor, IBMX, leads to a gradual increase in cell communication over 48 h. This observation

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is similar to the recent demonstration that cyclic AMP promotes the formation, over 6–24 h, of junctional membrane particles and functional membrane permeability in a junction-deficient malignant fibroblast cell line. The delayed effect of IBMX to increase intercellular communication in islet cells in the present study differs from that of insulin release stimulated by IBMX, since the latter begins promptly upon addition of the phosphodiesterase inhibitor. Therefore, although an increase in intercellular communication may contribute to insulin secretion, this change does not appear to be necessary for the initiation of hormone release.

The calcium ionophore, A23187, produced a rapid, large increase in islet cell communication. This effect was transient, however, lasting less than 30 min with 10^{-8}M A23187 and less than 20 min in the presence of a higher (10^{-7}M) concentration of the ionophore. These results are similar to the biphasic effects of a calcium ionophore or increases in extracellular calcium concentration on insulin release. Stimulation of insulin release is observed at lower concentrations of ionophore or calcium, whereas the stimulatory effect is lost at higher concentrations, presumably due to excessive elevation of the cytosolic free calcium concentration. Increased cytosolic calcium has been reported to decrease cell junctional permeability in various cell types, and we have preliminary evidence that microelectrophoretic injection of calcium into single islet cells decreased and slowed cell coupling (unpublished observations).

Although the present study demonstrates that cell-to-cell communication in pancreatic islets can be modulated by agents affecting islet hormone secretion, it appears that intercellular communication is not related to the process of insulin release in a simple direct fashion. Thus, discrepancies were noted between the time course of the effects of glucose, and IBMX, on islet intercellular dye transfer and the known effects of these agents on islet hormone secretion. Also, a recent report revealed that gap junctions and dye coupling were increased in B-cells of islets isolated from rats treated with diazoxide, a potent inhibitor of insulin release.

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