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OXYGEN CONSUMPTION RATES OF FREE AND ALGINATE-ENTRAPPED βTC3 MOUSE INSULINOMA CELLS

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SUMMARY. Oxygen consumption rates of βTC3 mouse insulinoma cells were measured in three different cell culture systems: monolayer, freshly trypsinized cell suspension, and trypsinized cells entrapped in alginate-poly-L-lysine-alginate (APA) polymer beads. The oxygen consumption rate for cells in the APA beads was similar to the rate for freshly trypsinized cells, indicating that cells can be entrapped without affecting their oxygen consumption rate. The cells in monolayers consumed oxygen at a higher rate than freshly trypsinized cells or cells in APA beads, suggesting that trypsinization may lower oxygen consumption capability. In addition, the oxygen consumption of βTC3s during glucose-induced insulin secretion was not significantly different from the basal rate, in contrast to the increased oxygen demand of actively secreting islets. Our results suggest that βTC3s may be better suited than islets for use in a bioartificial pancreas due to their stable oxygen requirements.

Transplantation of immunoisolated pancreatic islets has been shown to be effective in restoring normoglycemia in diabetic animals (1-3). However, the potential wide-spread use of this procedure in humans would be severely limited by the availability of mammalian islets, since islets exhibit little growth and lose their differentiated properties in culture. Alternatively, transformed cell lines can be propagated in culture while maintaining their ability to secrete insulin, and therefore may provide a renewable source of glucose-responsive, insulin-secreting cells for implantation. The βTC family of mouse insulinomas shows promise in the development of an implantable bioartificial pancreas for the long-term treatment of diabetes mellitus (4). The βTC3 line is a member of the βTC family that has been shown to exhibit glucose-responsive, although glucose-hypersensitive, insulin secretion (5).

In the implantation of xenogeneic tissue, it is important to prevent the possible destruction of the graft by the host immune system. For cell implants, the method of microentrapment in a biocompatible polymer has been developed for the immunoprotection of the cells. The molecular weight cut-off of the polymer can be controlled in the preparation of the beads, so that small molecules such as most metabolites can penetrate the bead but larger destructive antibodies and

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cytotoxic cells are excluded (6). Islets and transformed cells are often entrapped in beads composed of a calcium alginate core, with a poly-L-lysine layer and an outer calcium alginate coating (APA) (7, 8, 11).

The entrapment procedure places the cells in an alginate environment which may have significant effects on the metabolism of the cells. For example, the high concentration of free calcium to which the cells are exposed during entrapment may cause damage to membranes, affecting insulin secretion and other metabolic pathways. Such changes in metabolism have not been observed when comparing glucose consumption rates and ATP levels of entrapped versus freshly trypsinized βTC3 cells (8). The rate of oxygen consumption exhibited by the cells is likely to be a more sensitive metabolic indicator of the cells' proper function.

In studies of rat pancreatic islets, oxygen consumption rates have been observed to increase in response to glucose stimulation (9,10). However, islets entrapped in APA beads may have limited oxygen availability due to diffusion limitations through the bead. Therefore, in the characterization of APA beads as a device for implantation, it is important to determine if the bead environment limits the oxygen available to the cells under conditions of higher demand.

In our laboratory, we are investigating the possibility of using transformed, insulin-secreting cells in developing a stable bioartificial pancreatic tissue. With their introduction, the metabolism of such cells must be characterized to determine similarities and differences with islets. In this work, we have investigated the oxygen consumption rates of βTC3 cells cultured as monolayers, freshly trypsinized cells, and entrapped cells to determine the effect of the environment on oxygen consumption. Measurements of oxygen consumption rates have been performed under basal and glucose-stimulated conditions for insulin secretion.

MATERIALS AND METHODS

Cells and cell culture

βTC3 cells were obtained from the laboratory of Dr. Shimon Efrat, Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY. The cells were cultivated in supplemented Dulbecco's Modified Eagle's Medium (DMEM) (Sigma) as described previously (11,12). Cells were propagated as monolayers in plastic cell culture T-flasks (Falcon), and all cultures were maintained in an incubator at 37 °C in a humidified 5% CO_2/95% air atmosphere.

To prepare a suspension of freshly trypsinized βTC3 cells, cells were removed from T-flasks using trypsin/EDTA (Sigma) and the trypsin was neutralized by addition of serum-containing DMEM. Care was taken to gently disaggregate the cells with repeated pipetting.

The method of entrapment of βTC3 cells in alginate-poly-L-lysine-alginate (APA) beads was based on the work of Sun *et al.* (7,13), and has been described previously (11). For this preparation, the poly-L-lysine (MW = 25,000 - 25,700, Sigma) layer on the beads provided a molecular weight cut-off of 40 - 70 kDaltons (8). The beads were prepared with a 1 mm diameter.

Oxygen consumption measurements

To measure the oxygen consumption rates of monolayers, a perfusion system was designed consisting of a 25 cm^2 T-flask confluent with βTC3 cells (~7 x 10^5 cells/cm^2) and a screw-cap glass adapter (Lillie Glassblowers, Smyrna, GA). Serum-free DMEM was circulated through the flask and across a dissolved oxygen (DO) probe (Ingold, Wilmington, MA) housed in a flow-through chamber; the total volume of DMEM in the system was 83 mL. In preliminary tests, the dynamics of the probe were found to be much faster than the dynamics of the system (data not shown). In addition, the system was air-tight within the 85 - 100% DO saturation range, as evidenced in tests with deoxygenated DMEM in the absence of cells. The entire system was operated at 37 °C in incubator atmosphere. For each run, the system was filled with DMEM

equilibrated with incubator air; no air pockets remained in the system The medium was circulated through the apparatus while recording the drop in % DO saturation levels.

To measure the oxygen consumption rates of freshly trypsinized βTC3 cells, the trypsinized cell suspension was added to a glass bottle containing a stir bar and DMEM equilibrated with incubator air. The bottle was sealed using a custom machined Delrin cap which housed a DO probe; no air pockets remained in the system. The system was air-tight within 0 - 100% DO saturation. The entire apparatus was placed in an incubator at 37 °C, and the suspension was stirred at ~200 RPM. The volume of the sealed system was 88 mL, and the cell density ranged from 5.4×10^5 to 1.5×10^6 cells/mL.

For the APA entrapped cells, the same apparatus that is described above for the freshly trypsinized cells was used. The cell density within beads was approximately 7×10^7 cells/mL alginate, with 1.7×10^6 cells/mL medium.

Oxygen consumption rates were calculated using the following equation:

$$\text{oxygen consumption rate} = -\frac{mVk}{b}$$

where m is the slope of the output of the DO probe (% DO saturation/min); V is the total volume of medium in the system (L); k is the oxygen concentration in medium saturated with incubator air (194 μM) (14); and b is the total number of cells in the system. Oxygen consumption rates were calculated from data over the 88 - 95% DO saturation range.

Assays

Total cell numbers were obtained by lysing the cells and staining the nuclei with a crystal violet/citric acid (CVCA) solution, and then counting the nuclei in a hemacytometer. Cell viability for the freshly trypsinized cells and the cells in the APA beads was estimated using the trypan blue exclusion method. Cell viability was maintained at 94 - 98% for monolayers and freshly trypsinized cells and at 88% or higher for entrapped cells. Insulin concentrations were measured with a double-antibody radioimmunoassay kit (Binax, South Portland, ME); measurements were indicative of total insulin related peptides (IRP), including both insulin and proinsulin, measured according to their respective reactivities against the antibody.

RESULTS

The oxygen consumption rate of monolayers was measured in the absence (basal) and the presence (stimulated) of glucose. At 0 mM glucose, the rate was measured to be 1.52 ± 0.24 μmol/min•10^9 cells (mean ± standard deviation) (Table 1). At higher glucose concentrations, the oxygen consumption rate did not change significantly, with values of 1.55 ± 0.23 μmol/min•10^9 cells at 5.5 mM glucose and 1.51 ± 0.23 at 25 mM glucose. Even though oxygen consumption rates were not observed to change, the higher glucose concentrations caused insulin secretion rates to increase from 6.4 ± 3.5 μU/hr•10^5 cells under basal conditions to 85.2 ± 8.3 μU/hr•10^5 cells at 5.5 mM glucose and 65.5 ± 4.4 μU/hr•10^5 cells at 25 mM glucose. The difference in insulin secretion rates under basal and stimulated conditions is statistically significant. However, the secretion rates determined at 5.5 and 25 mM glucose are not significantly different when taking into account the reproducibility of cell culturing, sampling procedure, cell counting, and radioimmunoassay technique.

Freshly trypsinized cells consumed oxygen under basal conditions at a rate of 0.94 ± 0.03 μmol/min•10^9 cells. Insulin secretion rates averaged 6.2 ± 2.2 μU/hr•10^5 cells. Freshly trypsinized βTC3 cells consumed oxygen at a rate of 1.02 ± 0.07 μmol/min•10^9 cells under induced conditions at 5.5 mM glucose and 1.05 ± 0.12 μmol/min•10^9 cells at 25 mM glucose. Insulin secretion rates under induced conditions averaged 64.3 ± 9.7 μU/hr•10^5 cells.

Table 1: Oxygen Consumption Rate of βTC3 Cells under Basal and Stimulated Conditions

	Oxygen Consumption Rate (μmol /min•10^9 cells)	Insulin Secretion Rate (μU/hr•10^5 cells)
Monolayers		
Basal (0 mM glucose)	1.52 ± 0.24 * (n = 9)	6.4 ± 3.5 † (n = 4)
5.5 mM glucose	1.55 ± 0.23 * (n = 6)	85.2 ± 8.3 (n = 2)
25 mM glucose	1.51 ± 0.23 * (n = 5)	65.5 ± 4.4 (n = 2)
Freshly Trypsinized Cells		
Basal (0 mM glucose)	0.94 ± 0.03 (n = 4)	6.2 ± 2.2 † (n = 2)
5.5 mM glucose	1.02 ± 0.07 (n = 4)	64.3 ± 9.7 (n = 2)
25 mM glucose	1.05 ± 0.12 (n = 2)	-----
Entrapped Cells		
Basal (0 mM glucose)	1.11 ± 0.03 (n = 2)	5.3 ± 1.1 † (n = 2)
25 mM glucose	1.13 ± 0.08 (n = 4)	58.2 ± 6.3 (n = 4)

* identifies statistically significant difference in oxygen consumption rate between monolayers and corresponding freshly trypsinized cells or entrapped cells at $p < 0.002$.

† identifies statistically significant difference in insulin secretion rate against stimulated conditions within the same culture mode at $p < 0.001$.

The oxygen consumption rates of βTC3 cells entrapped in APA beads were 1.11 ± 0.03 μmol/min•10^9 cells at 0 mM glucose and 1.13 ± 0.08 μmol/min•10^9 cells at 25 mM glucose. The insulin secretion rates at 0 mM and 25 mM glucose were 5.3 ± 1.1 μU/hr•10^5 cells and 58.2 ± 6.3 μU/hr•10^5 cells, respectively.

DISCUSSION

The oxygen consumption rate exhibited by freshly trypsinized βTC3 cells is approximately 30% less than that exhibited by βTC3 monolayers under both basal and glucose-induced conditions. The primary purpose of trypsinization in this study was to remove the cells from the culture flask surface, but the polypeptide-cleavage action of the trypsin enzyme may inflict damage to the cell membrane, reducing the ability of the cells to consume oxygen. It may be that the cell damage and the reduced oxygen consumption rates are only transient and that the cells recover after a certain time period. However, since the freshly trypsinized cell suspension is difficult to maintain for extended experiments due to cell aggregation or cell death, we were not able to measure rates of the same culture after a long recovery time. Although oxygen consumption rates

of monolayers are greater than the rates of the other two systems (increase is statistically significant at $p < 0.002$), the insulin secretion rates remain relatively unaffected. Therefore, oxygen consumption rate appears to be a more sensitive indicator of changes in cell function.

While contained within the APA beads, βTC3 cells consume oxygen at a rate very similar to the rate exhibited by freshly trypsinized cells. In other studies, the issue has been raised as to whether the entrapment of cells may create hypoxia that is detrimental to cell function (15, 16). The environment of the APA bead may limit oxygen diffusion to the cells, and therefore some fraction of the cells may experience hypoxia. However, our results suggest that for these 1 mm APA beads, any possible diffusion limitation does not significantly decrease the rate of oxygen consumption of βTC3 cells for the range of DO concentrations studied.

In studies of rat pancreatic islets, secretagogue-induced insulin secretion has caused an immediate increase in the islet oxygen consumption (9). However, βTC3 cells do not exhibit this increased oxygen consumption during stimulated insulin secretion; overall, within each culture mode, statistically significant changes in oxygen consumption rate between basal and stimulated conditions were not observed, although insulin secretion increased during stimulation.

CONCLUSIONS

βTC3 cells entrapped in APA beads consume oxygen at a similar rate to that exhibited by freshly trypsinized cells, indicating that the alginate environment does not inhibit oxygen consumption. In addition, the oxygen consumption rate for monolayers is higher than that for freshly trypsinized or entrapped cells, perhaps because of cell membrane damage incurred during the trypsinization procedure. During glucose-induced insulin secretion, the oxygen consumption rate is not statistically different from the rate observed under basal conditions; with islets, increased oxygen consumption has been linked to insulin secretion. The stable demand for oxygen exhibited by βTC3 preparations may prove to be advantageous for their use in a bioartificial pancreas.

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