Electrical field-induced fusion to create giant insulin-producing β -cells

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ABSTRACT

A technique for electrical field-induced cell fusion was modified to obtain a large amount of giant insulin-producing cells from normal pancreatic β -cells as well as from the clonal cell line RINm5F. The fusion was established by one or several pulses of 1.50–2.75 kV with a time constant of 175–550 μ sec. The resulting electrical field strength corresponded to $2.5 \cdot 10^5$ V/m. After fusion the giant cells (diameter $\geq 20 \mu\text{m}$) comprised as much as 60% of the cells excluding trypan blue. Except for being polynucleated, the giant cells displayed similar cytological characteristics as their normal-sized counterparts both with regard to appearance and distribution of insulin-containing secretory granules and other intracellular organelles. The fused RINm5F cells survived under tissue culture conditions, but when cultured for more than one week, division of the giant cells resulted in normal-sized cells.

The relative scarcity of information about changes in the membrane potential and intracellular ionic milieu in isolated pancreatic β -cells is not surprising in view of their limited size ($\sim 9 \mu\text{m}$) (6). Previous studies with microelectrodes have all been performed on the whole islets, where β -cells were identified on the basis of their response to D-glucose (2, 8). Also in those cases where a fluorescent dye was microinjected into clusters of pancreatic endocrine cells to study the function of junctional coupling in the secretory activity, the recognition of β -cells was possible only after characterization by immunocytochemical staining or electron microscopy (7, 9). The recording of the intracellular ionic activity by the insertion of two electrodes or a double-barrelled electrode has not been possible so far. The present study describes the technique of electrical field-induced fusion (1, 10, 14–17) applied to both normal pancreatic β -cells isolated from obese-hyperglycemic mice (5) and insulin-producing cells derived from a transplantable rat insulinoma (3, 11, 13).

Reagents of analytical grade and deionized water were used. Cells were suspended in 0.2 ml (normal β -cells) and 1.0 ml (tumour cells) of

330 mM sucrose/5 mM Tris-HCl buffer, pH 7.4, at 20°C. Suspensions contained 2×10^6 and 40×10^6 cells per ml of the buffer solution for normal and tumour cells, respectively. Suspensions were transferred to the fusion chambers and exposed to one or several high voltage pulses as described in Table 1. The pulses were generated by discharging a capacitor of either 0.25 or 0.50 μF . The fusion chambers were made of Perspex with a volume of 0.2 or 1 ml. Stainless steel plates mounted on the opposite walls of the chambers served as electrodes. After being subjected to the high voltage pulses, the cells were transferred to a 25 mM Hepes buffer containing 1.28 mM Ca^{2+} and 4 mM glucose, pH 7.4, at 20°C for 30 min to complete the fusion process. Cells were counted using a Bürker chamber and the viability was tested by trypan blue (0.1% solution) exclusion. Diameters of cells were determined using an ocular screw micrometer (Leitz, Germany). The fused cells that were cultured were exposed to normal RPMI 1640 medium (Labassco, Sweden) supplemented with 10% heat-inactivated (56°C for 30 min) fetal calf serum, 100 IU/ml penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin (Labassco, Sweden) in 50 ml

Table 1 *Fusion Conditions, Percentage of Viable β -Cells and the Mean Diameter of Fused β -Cells*

Number of experiments	Cell type	Fusion conditions	Percentage of cells excluding trypan blue after the high voltage treatment		Percentage of fused cells excluding trypan blue	Mean diameter of giant cells (μm)
			Non-fused cells	Fused cells		
7	RINm5F	2.75 kV; 1 pulse with a time constant of 350 μsec ; electrode distance, 1.1 cm	43.5 $\pm$ 8.3	95.1 $\pm$ 1.4	36.2 $\pm$ 5.9	30.4 $\pm$ 1.0
10	RINm5F	2.75 kV; 5 pulses with a time constant of 175 μsec ; electrode distance, 1.1 cm	26.5 $\pm$ 5.8	84.9 $\pm$ 3.6	59.5 $\pm$ 3.4	26.5 $\pm$ 0.5
12	Pancreatic β -cells	1.50 kV; 1 pulse with a time constant of 550 μsec ; electrode distance, 0.6 cm	23.8 $\pm$ 1.6	52.0 $\pm$ 3.6	5.8 $\pm$ 0.4	21.8 $\pm$ 0.3

The percentage was estimated by counting at least 100 cells in each experimental group. The mean diameter was calculated in each experiment in about 30 fused cells. The minimal diameter was around 20 μm . Mean values $\pm$ SE are given.

Nunclon flasks (Nunc, Denmark) for 7 days with the medium changed every 48 h. The preparation for electron microscopy was performed after fixation of cells with 2.5% glutaraldehyde.

A prerequisite for electrofusion is a close contact between cells in the suspension. To overcome the effects of Brownian motion and repulsive electrostatic forces arising from the net negative charge on the outer membrane surface, both the high density cell suspension and/or dielectrophoresis have been used (1, 10, 14–17). By exposing the high density suspension of insulin-producing cells to electrical fields it was possible to obtain a large amount of giant cells. Table 1 demonstrates how variations in pulse duration and in number affected the recovery and size of the fused cells as well as their viability. A single pulse with a time constant of 350 μsec was as effective as five pulses of 175 μsec in creating giant RINm5F cells, although in the former case there was a lower yield of viable fused cells. The proportion of fused cells excluding trypan blue was always more than twice as high as that in non-fused cells. Furthermore, yield of giant cells was up to 60% of the total number of living cells which is nearly as high a value as that reported for dielectrophoretically collected cells (15–17). Although a high density of the cell suspension

seems to be a prerequisite for a successful fusion process, the field strength and pulse duration are also important and must be optimized in each specific experimental situation. In the present work it was found that the same field strength was optimal for both the tumour and the normal pancreatic β -cells. The fact that fewer normal β -cells were available made it necessary to use a smaller Perspex chamber with a shorter distance between the electrodes. When the 0.50 μF capacitor was used in combination with the smaller chamber, increased resistance in the circuit was the cause of the prolongation in pulse duration. In this context it should be noted that despite the relatively high time constants used for decaying of the electrical fields, the overall temperature rise of the cell suspension did not exceed 2°C. Whereas studies with microelectrodes need only a limited number of viable giant cells, the large numbers obtained under the present conditions make various biochemical analyses possible. The diameter of the largest cells obtained under the present conditions was about 30 μm , which implies the fusion of approximately 30 cells with the diameter of 10 μm . The smaller size of the giant cells derived from normal pancreatic β -cells as compared to those from tumour cells is probably due in part to a lower density of the

Fig. 1 A: Light micrograph of tumour β -cells fused after 1 pulse with a time constant of $350 \mu\text{sec}$. $\times 340$. B: Light micrograph of fused normal β -cells. $\times 340$. C: Light micrograph of fused tumour β -cells (1 pulse with a time constant of $350 \mu\text{sec}$) cultured for one week. $\times 420$

Fig. 2 A and B: Electron micrograph of tumour β -cells fused after 1 pulse with a time constant of 350 μ sec. A, $\times 1,800$; B, $\times 30,000$. C and D: Electron micrograph of fused normal β -cells. C, $\times 4,500$; D, $\times 30,000$. In both B and D mitochondria and secretory granules are indicated by arrow and arrowhead, respectively.

cell suspension used for the electrofusion process. But in addition, it may also reflect a smaller radius of the normal β -cells and/or different properties of the cell membrane or of the cytoskeleton.

Fig. 1 shows giant cells obtained either by fusion of RINm5F cells (A, C) or of normal β -cells (B). Giant cells from normal cells were fairly round in shape, whereas the fused RINm5F cells maintained the characteristic feature of neoplastic cells in being more irregular. As judged from the trypan blue exclusion test, most of the fused cells were morphologically intact. The long-term viability of the giant cells was also established from their ability to survive under tissue culture conditions. When defined giant RINm5F cells were examined repeatedly by phase contrast microscopy, these cells seemed to start to proliferate yielding normal-sized cells during one week of culture (Fig. 1C). Such a division is surprising when taking into account the fact that the giant cells were polynucleated. However, there is so far no evidence that the nuclei themselves are subjected either to fusion or any other change, which may result in alterations of the genetic information. Except for the number of nuclei and size, it is evident from Fig. 2 that the fused cells displayed similar ultrastructural characteristics as their small counterparts both with regard to the appearance and distribution of the insulin-containing secretory granules and other intracellular organelles (4, 12). Preliminary studies with microelectrodes indicate that giant cells display a membrane potential that is of the same order of magnitude as that obtained when the whole islets are impaled (Berggren, Sohtell and Hellman, unpublished data).

By providing a large number of giant cells capable of multiplying during culture, electrofusion can be regarded as a convenient technique for hybridization of normal β -cells with rapidly dividing and low differentiated tumour cells of β -cell origin. If such heterokaryons can be maintained in tissue culture, they must be most useful for diabetes research in providing hybrids with the properties of both parents, *i.e.* a high proliferative rate and an intact capacity to synthesize and release insulin.

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