Microelectrode studies of D-glucose- and K^+-induced changes in membrane potential of electrofused insulin-producing cells

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In the present study electrical field-induced fusion has been applied to both normal pancreatic islet cells isolated from obese hyperglycemic mice and clonal insulin-producing cells (RINm5F) derived from a transplantable rat insulinoma. The fused cells were then punctured with microelectrodes to measure changes in membrane potential after exposure to stimulatory concentrations of D-glucose or K^+. Fused cells of normal islet cellular origin revealed a resting membrane potential of -60 mV and were depolarized by 24 or 27 mV after exposure to 11 mM D-glucose or 30 mM K^+. Although D-glucose induced depolarization, it was not possible to establish the existence of an oscillating burst pattern superimposed by action potentials. The resting membrane potential of the fused RINm5F cells was also -60 mV and decreased to -30 mV after exposure to 30 mM K^+. As judged from the membrane potential measurements, the reconstitution of the plasma membrane subsequent to electrical breakdown is essentially the same whether the giant cells originated from normal islet cells or RINm5F cells.

Microelectrode *Electrofusion* *Insulin-producing cell* *Membrane potential*

1. INTRODUCTION

In view of their limited size ($\approx 9\ \mu$m in diameter) [1] microelectrode recordings of membrane potential and intracellular ionic activities in isolated pancreatic β-cells have so far not been successful. Previous studies with microelectrodes have all been performed on entire islets, the β-cells being putatively identified on the basis of the response to D-glucose [2,3]. Fusion of several cells into giant cells should improve the conditions for microelectrode insertions and even make it possible to perform conventional two-electrode voltage-clamp studies. In this case the loss of cell constituents, a problem when applying the patch-electrode voltage-clamp technique [4], could be avoided.

Here, normal islet cells isolated from obese hyperglycemic mice [5] and clonal insulin-producing RINm5F cells derived from a transplantable rat insulinoma [6–8] were subjected to electrical field-induced fusion [9]. Microelectrodes were then inserted into the electrofused cells and changes in membrane potential were measured after exposure to stimulatory concentrations of D-glucose or K^+.

2. MATERIALS AND METHODS

Reagents of analytical grade and deionized water were used. Normal pancreatic islet cells were isolated essentially as described [10]. Cells were suspended in 0.2 ml (normal islet cells) and 1.0 ml (RINm5F 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 RINm5F cells, respectively. Suspensions were transferred to the fusion chambers and exposed to a single high-voltage

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pulse as described in fig.1. The pulses were generated by discharging a capacitor of either 0.25 (normal cells) or 0.50 μF (RINm5F cells). The fusion chambers were made of perspex with a volume of 0.2 (normal cells) or 1 ml (RINm5F cells). Stainless-steel plates mounted on the opposite walls of the chambers served as electrodes. After being subjected to the high-voltage pulse, the cells were transferred to 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 allowed to attach to 3 × 10 mm rectangular plastic plates cut from the bottom of Nunclon petri dishes (A/S Nunc, Kamstrup, Denmark) during 24 h of culture in RPMI 1640 medium (Statens Veterinärmedicinska anstalt, Uppsala) supplemented with 10% heat-inactivated (56°C for 30 min) fetal calf serum, 1% L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin (Flow Laboratories, Irvine, Scotland) at 37°C and pH 7.4 in an atmosphere saturated with water and equilibrated with 5% CO_2 in air. After culture the plate with the attached cells was placed in the perifusion chamber making it possible to measure the membrane potential from single cells. The membrane potential was measured during subsequent perifusion of the fused cells with a Krebs-Ringer bicarbonate medium (the various additions given in the figure and table legends) equilibrated with 5% CO_2 in O_2 (pH 7.4) at 37°C. Both the test solution flowing through the chamber (7 ml/min) and the test solution reservoirs were thermostatted at 37°C. The system allowed bath exchange in approx. 3–5 s. The electrophysiological procedure for recording the membrane potential was essentially that described by Biagi et al. [11]. Microelectrodes (tip diameter <0.5 μm) were pulled from thick-walled capillary tubing containing an extruded fiber for easy filling (Frederick Haer, Brunswick, ME; OD = 1.2 mm). Electrodes were filled with 1 M KCl and had a resistance in the range of 80–150 MΩ. Punctures were obtained with nonbeveled electrodes connected via an Ag-AgCl half-cell to the input of a high-impedance electrometer (KS 700, W-P Instruments, New Haven, CT). The electrometer probe was held by a hydraulic micromanipulator (M 102 R, Narishige Instrument Laboratory, Tokyo). A second Ag-AgCl half-cell filled with 0.16 M NaCl was used as the reference electrode and connected to the bath via a 0.16 M NaCl–3% agar bridge. In order to puncture a cell the microelectrode tip was placed on the plasma membrane at an angle of 30–40°. Gentle tapping of the micromanipulator support moved the microelectrode tip through the plasma membrane. During this procedure the cells were viewed in an inverted microscope with fixed stage (Leitz, Wetzlar, FRG). The experimental equipment was mounted on an air suspension table (Micro-G, Backer-Loring, Peabody, MA) to reduce vibrations.

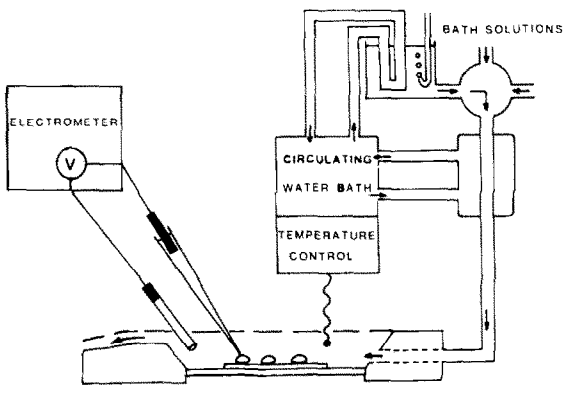

Fig.1. Perifusion chamber of perspex for recording the membrane potential in isolated cells.

3. RESULTS AND DISCUSSION

Fig.2 shows fused insulin-producing cells. When derived from normal islet cells (B) the fused cells were fairly round in shape, whereas the fused RINm5F cells maintained the characteristic feature of neoplastic cells in being more irregular (A). It has previously been demonstrated by trypan blue exclusion that most of the fused cells were viable and that the fused RINm5F cells survived under tissue culture conditions [9]. Furthermore, the yield of giant cells was up to 60% of the total number of living cells. The diameter of the cells corresponded to about 20 and 30 μm for the fused normal islet cells and RINm5F cells, which implies the fusion of approx. 10–30 cells, respectively.

To test the perifusion system and electrical set-up for the membrane potential measurements, aggregated pancreatic islet cells were exposed to D-glucose and tetraethylammonium (TEA). It is clearly demonstrated in fig.3 that the aggregated islet cells, under the present experimental condi-

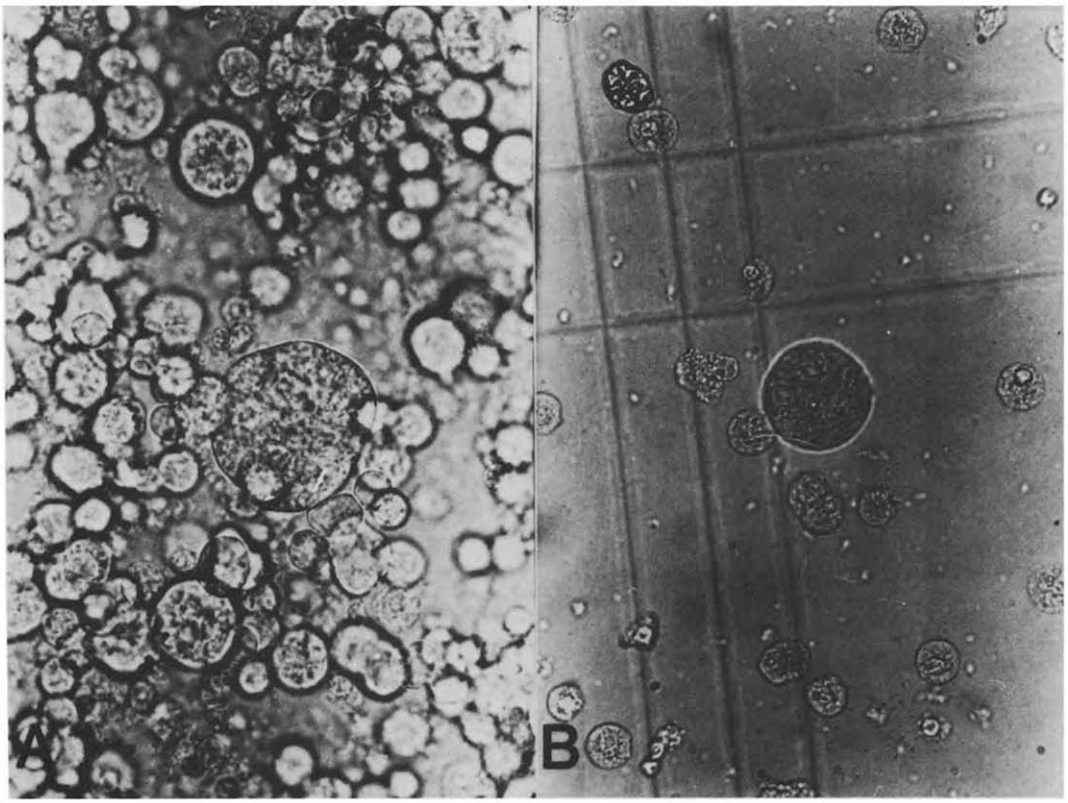

Fig.2. Light microscopic picture of fused cells (× 340). (A) Continuously growing RINm5F cells subjected to a single pulse of 2.75 kV, with a time constant of 350 μs and an electrode distance of 1.1 cm. (B) Normal pancreatic islet cells subjected to a single pulse of 1.50 kV, with a time constant of 550 μs and an electrode distance of 0.6 cm.

tions, revealed a similar continuous spike activity as has been described when entire islets are impaled in the presence of D-glucose and TEA [12,13].

Pancreatic islet cells in situ are known to be coupled through gap junctions [14]. When these junctions were functionally investigated electrical coupling was found to occur over several β-cell diameters [15–17] and the injection of a low-M_r fluorescent dye into one cell led to spread of the fluorescence to several adjacent cells [14]. Although it has been demonstrated that D-glucose increases the degree of electrical coupling and

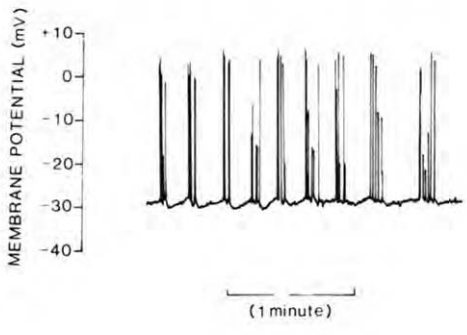

Fig.3. Changes in membrane potential of aggregated normal islet cells after stimulation by D-glucose and TEA. Normal pancreatic islet cells were allowed to aggregate during 24 h of culture. To prevent cell attachment to the bottom of the culture flasks the cell suspension was shaken for 1–2 s every 10 s at a frequency of 2–3 Hz using an MTS shaker (IKA-Werke, Staufen, FRG). The aggregated cells were mounted as previously described and the membrane potential recorded in the presence of 11 mM D-glucose and 20 mM TEA. The figure shows a representative experiment which was repeated 3 times.

mitochondrial inhibitors reduce it [17], it is still a matter of debate as to whether such a coupling is a prerequisite for the β-cells to possess an oscillating burst pattern superimposed by action potentials. With access to giant islet cells, it should be possible to perform more extended electrophysiological studies and thereby gain further information about the basic mechanisms regulating electrical activity in insulin-secreting cells. In fig.4A and B as well as in table 1A the effects of D-glucose (11 mM) or high concentrations of K^+ (30 mM) on the membrane potential of fused normal pancreatic islet cells are demonstrated. Pulses of D-glucose caused a slow membrane depolarization of about 20 mV, that was reversible upon withdrawal of the sugar. Despite the D-glucose response, it was not possible to establish the existence of an oscillating burst pattern superimposed by action potentials. To what extent this is due to the fact that the fused cells were cultured for 24 h is not yet known. Fusion of a large number of cells implies that most of the membrane area is subjected to electrical breakdown [18]. Since the exact molecular mechanisms behind electrofusion are unknown, it cannot be excluded that changes in the distribution of ionic channels are involved in the mechanisms responsible for the defective sugar response. Although the cell suspension of normal pancreatic islet cells is obtained from islets of Langerhans containing approx. 90% β-cells [5], there are still about 10% other cells present. At the moment of electrofusion

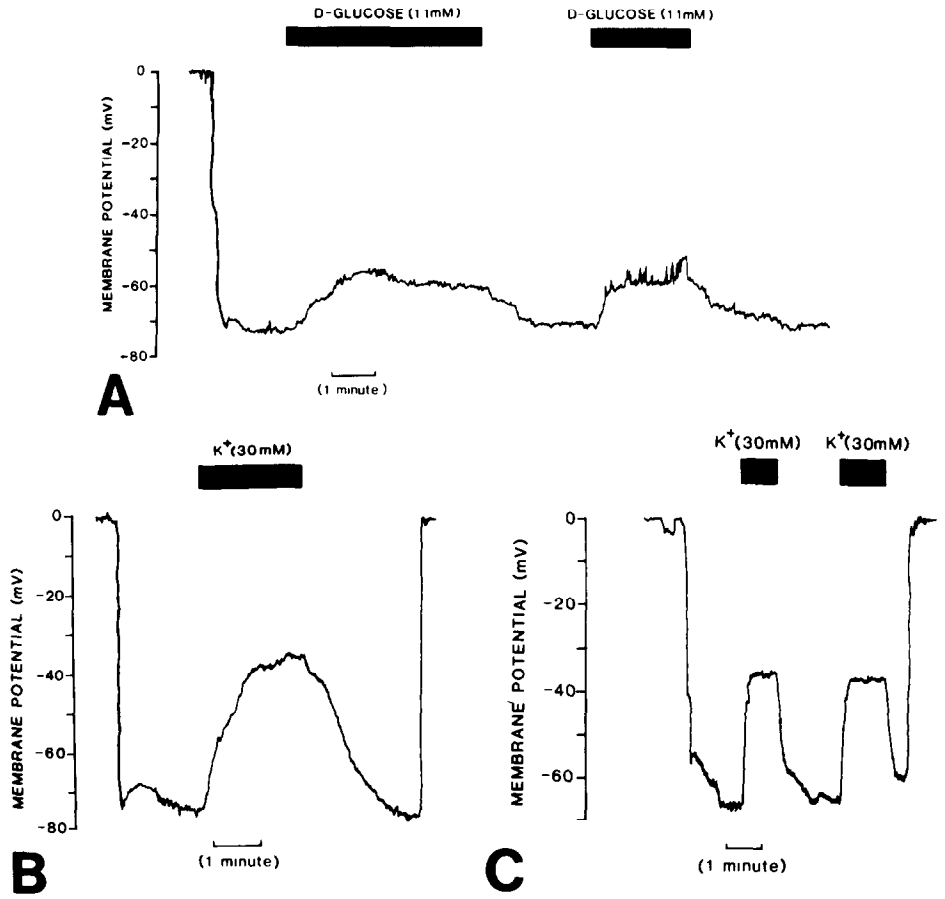

Fig.4. Changes in membrane potential of fused cells. Recording of membrane potential of fused normal islet cells stimulated by two pulses of 11 mM D-glucose (A) or by one pulse of 30 mM K^+ (B). (C) Recording of membrane potential of fused RINm5F cells stimulated by two pulses of 30 mM K^+.

Table 1

(A) Effects of D-glucose and K^+ on membrane potential of normal fused cells
(B) Effects of high K^+ on membrane potential of fused RINm5F cells

(A) Additives	Membrane potential (mV)	Effects of additives
None	-60.0 ± 3.9 ($n = 12$)	–
D-Glucose (11 mM)	-42.5 ± 8.9 ($n = 4$)	24.0 ± 4.7[a] ($n = 4$)
K^+ (30 mM)	-27.2 ± 3.0 ($n = 5$)	26.6 ± 5.0[b] ($n = 5$)
(B) $[K^+]$ (mM)	**Membrane potential (mV)**	**Effect of high K^+**
5.9	-57.3 ± 3.7 ($n = 4$)	–
30.9	-31.0 ± 3.8 ($n = 4$)	25.3 ± 3.7[b] ($n = 4$)

The means ± SE for indicated numbers of individual membrane potential measurements are shown. The data shown in fig.4 are part of these experiments. [a] $P < 0.025$, [b] $P < 0.010$ as judged from the differences between paired test and control data using Student's t-test

these cells may also be incorporated within the giant cells and consequently change the responsiveness to D-glucose. By systematically varying the percentage of non-β-cells in the suspension subjected to electrofusion, it should be possible to obtain giant cells whose plasma membranes more or less represent multicellularity. Detailed investigations of such cells might yield information about the interplay between islet cells in the physiological regulation of the electrical activity in response to D-glucose. The possible reorganization of plasma membrane does not change the ability of the giant cells to possess a resting membrane potential comparable to that of normal pancreatic β-cells in situ and changing accordingly after stimulation by 30 mM K^+ [19]. Both the resting membrane potential and the change evoked by K^+ indicate that the plasma membrane of the giant cells maintains the same electrochemical gradients as that of non-fused cells.

Since the clonal insulin-producing cell line RINm5F [6–8] has some properties in common with those of normal β-cells it was of interest to clarify whether cells of this type, that are kept in tissue culture, can effectively reconstitute their plasma membrane after electrofusion. The resting membrane potential of RINm5F cells has been reported to be about -50 mV [20] and they supposedly depolarize in response to high concentrations of K^+ but not to D-glucose [21]. Although the electrofused RINm5F cells comprised a larger number of cells, the resting membrane potential and response to K^+ did not differ to any great extent from the fused normal islet cells (fig.4B,C and table 1A,B).

With the application of electrical field-induced fusion on secretory cells, there have emerged new possibilities for investigating the mechanisms involved in the regulation of stimulus-secretion coupling. Here, the fused secretory cells have been successfully investigated with conventional electrophysiological methods. The fact that these cells seem to maintain normal electrochemical gradients should make them very useful not only for more detailed studies of membrane potential but also for recording with ion-sensitive intracellular microelectrodes.

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