THE ELECTRICAL ACTIVITY OF PANCREATIC β-CELLS OF DIABETIC MICE

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1. Introduction

Beta-cells of mouse pancreatic islets are electrically active in glucose-containing media [1]. Further experiments have shown that the electrical activity is usually exhibited under conditions in which insulin is released [2–4]. The close relationship between electrical activity and insulin release strongly supports the hypothesis that changes of the membrane potential of β-cells play an essential role in the mechanism of insulin release [4]. It was therefore important to investigate the electrical activity of β-cells of diabetic mice, and to see whether the correlation between insulin release and electrical activity also holds under the conditions of a severe defect of insulin secretion.

2. Materials and methods

The experiments were performed on pancreatic islets of mice of the strain C57 BL/KsJ-db/db which possesses a hereditary defect of insulin secretion [5]. The mice were bread by Dr Berglund and Prof. Hellman (Institute of Histology, University of Umeå, Sweden) until they started to lose weight, which is a characteristic sign of the developing severe diabetes [5]. Then they were flown to our laboratory and maintained on a normal diet for another 3–4 weeks, their body weight being controled twice a week. For the electrophysiological experiments we always chose the mouse which had suffered the most remarkable weight loss. The animals were killed by decapitation, and blood samples were taken. The serum glucose concentration was above 573 mg/100 ml in all animals studied. A small segment of the pancreas was placed in a plexiglas chamber (volume 1 ml) and perifused at 3 ml/min with modified Krebs-Henseleit solutions at 37°C. The islets, which were less easily detectable than in normal mice, were exposed by microdissection; β-cells were impaled with microelectrodes filled with 2 M K-citrate (tip-resistance > 200 MΩ). The membrane potential was continuously recorded on an oscilloscope (Tektronix 565) and a chart-writer (W+W 1100). The basal composition of the perifusion medium was (mM): NaCl 103, KCl 4.7, $CaCl_2$ 2.56, $MgCl_2$ 1.13, $NaHCO_3$ 25, NaH_2PO_4 1.15, glucose 2.8, sodium pyruvate 4.9, sodium fumarate 2.7, sodium glutamate 4.9. When the glucose concentration was changed, NaCl was added or removed in osmotically equivalent amounts. All solutions were equilibrated with a mixture of 95% O_2 and 5% CO_2.

3. Results

Membrane potential recordings were obtained from the pancreatic islets of nine diabetic animals. 59 impalements lasted long enough (more than 30 sec) to decide whether or not the impaled structures exhibited electrical activity. Two types of membrane potential recordings were observed. In 37 impalements the membrane potential was -42 ± 2.4 mV (mean ± SEM), but no spike activity was detected; moreover, no effect on the membrane potential was observed when the external glucose concentration was changed. Within the same islets and in the vicinity of these inactive structures 22 highly active cells were found; their membrane potentials were generally more positive (−28 ± 2 mV), and they showed typical spike activity. In nine of these cells the recording microelectrode could be maintained intracellularly for up to 104 min, and the effect of at least two

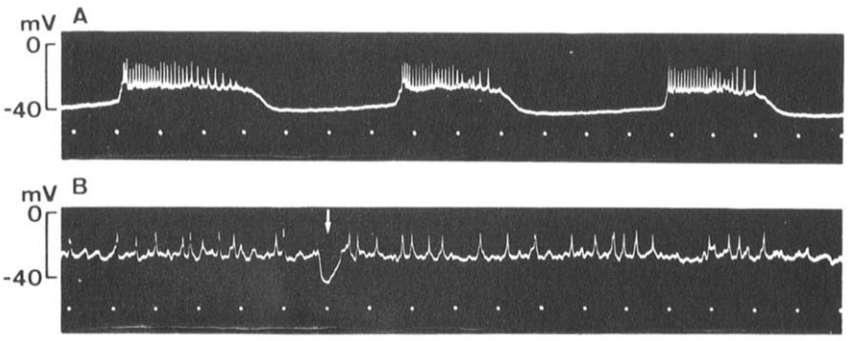

Fig.1. Oscilloscope records of the electrical activity of β-cells from a normal (A) and a diabetic mouse (B). Glucose concentration 11.1 (A) and 2.8 mM (B). Interval between dots 2 sec. Note typical burst pattern in A and continuous spike activity in B; arrow denotes a short repolarization phase.

glucose concentrations was examined. Only two out of the 22 active cells exhibited burst activity which consisted of regularly occuring slow potential waves with fast spikes superimposed on the plateau level; this type of activity was similar to that observed in normal β-cells (fig.1A). The remaining 20 cells showed the type of electrical activity demonstrated in figs.1B and 2. In general, continuous series of spikes were interrupted at irregular intervals by short repolarization phases (approx. 1 sec) of 10 to 15 mV amplitude (fig.1B). In some other cells silent periods of several seconds duration were interposed between periods of continuous spike activity which lasted 10 to 60 sec; only minor changes of the membrane potential level were observed during these phases of markedly differing spike activity (figs.2A and C). In a few cases the spike frequency periodically changed by a factor of 2 to 3 without silent periods being interposed.

One of the most important observations was that the β-cells of diabetic mice were electrically active in glucose concentrations below 4 mM (fig.1B). Firing did not stop even after perifusion with a *glucose-free* medium for 2.5 to 20 min (8 experiments on 5 β-cells). Raising the glucose content to high levels (figs.2A – D) did neither change appreciably the pattern of activity nor the membrane potential level from which the spikes arose. The obvious insensitivity towards glucose of these β-cells is diagrammatically shown in fig.3. The dashed lines demonstrate that there were marked differences as to the level of electrical activity in the β-cells of diabetic mice; however, these cells did not react to alterations of the glucose concentration. This observation applies also to the two cells with regular burst activity mentioned above. Thus, the behaviour of β-cells of diabetic mice is in sharp contrast to that of normal β-cells, which are silent

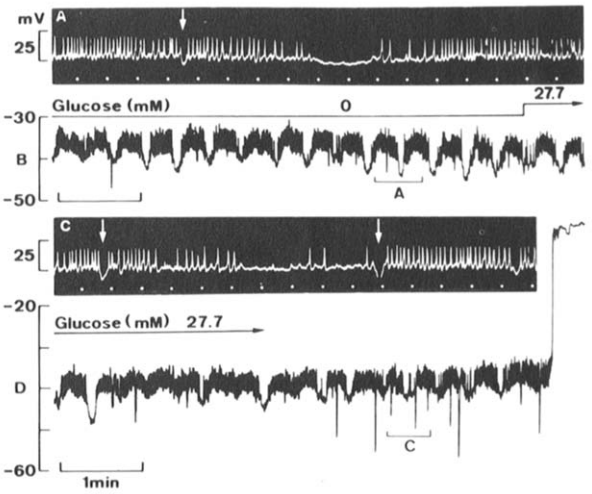

Fig.2. Electrical activity of a β-cell of a diabetic mouse in zero and 27.7 mM glucose. Oscilloscope records A and C correspond to the sections marked A and C in the chart recordings B and D. At beginning of B the islet was already perifused with zero glucose for 5.4 min. Note that addition of glucose does not appreciably alter the activity pattern. The marked downward deflections in B and D represent short hyperpolarization phases also seen in A and C (white arrows). At the end of D the microelectrode was withdrawn from the cell to obtain the zero potential level. In B and D the spike amplitudes are reduced because of the inertia of the ink-writer.

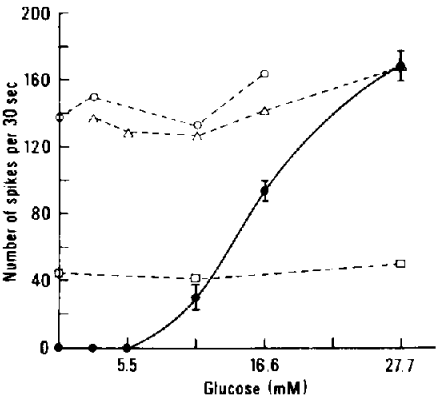

Fig.3. Relationship between glucose concentration (abscissa) and number of spikes per 30 sec period (ordinate) in β-cells of normal and diabetic mice. Continuous line, mean ± S.E.M. of 4 normal β-cells. Dashed lines, results from individual β-cells of three diabetic mice. Note sigmoid curve for normal β-cells, and loss of glucose-dependence as well as marked differences in spike frequency of the 'diabetic' cells.

in low glucose media [1,4] and exhibit a sigmoid relationship between spike frequency and glucose concentration (fig.3, continuous line; cf. [4]).

4. Discussion

The experiments have provided two major results. Firstly, in the islets of diabetic mice there was an appreciable percentage of impalements into structures which did not show electrical activity. This finding is likely to be a reflection of the observation that the islets of KsJ-db/db mice frequently contain ductlike structures lined with cuboidal epithelial cells [5]. The presence of similar structures could be confirmed in the islets examined in the present study. Secondly, the functional properties of electrically active cells differed markedly from those of normal β-cells. Only two cells showed regular burst activity, while in 20 cells the typical slow potential waves were absent. The small differences in the pattern of electrical activity described above probably correspond to developmental stages of the 'diabetic' cells. The fact that all β-cells were electrically active in zero glucose is in good agreement with the finding that pancreatic islets of the strain used in the present experiments have a high basal insulin release (Berglund, Frankel and Hellman, personal communication). In addition, Boquist et al. [5] showed, that the insulin secretion of the islets of most diabetic mice could not be influenced by the glucose concentration. This observation is paralleled by our finding that neither the spike frequency nor the membrane potential of the β-cells were altered measurably when glucose was added to the medium. Thus, insulin release and electrical activity seem to be as closely correlated in the β-cells of diabetic mice as they are in normal mice. This finding lends further support to the hypothesis that the release of insulin is regulated by a mechanism in which the membrane potential of the β-cell plays an important role [3,4,6].

Two of the β-cells exhibited burst activity similar to that of normal β-cells in spite of being insensitive to glucose. It is therefore possible that glucose-insensitivity represents one of the earliest and pathophysiologically important alterations in 'diabetic' β-cells. The precise nature of the genetic defect in KsJ-db/db mice is not known. On the background of the present electrophysiological results it may, however, well be that it is a defect in the membrane of the β-cell. It could be the loss of an ionic permeability which is normally turned on or off according to the glucose concentration of the medium; alternatively, the activity of the Na–K-pump which seems to contribute substantially to the pattern of electrical activity in normal β-cells [6,7] could be reduced in the 'diabetic' cell; both mechanisms could result in a continuous depolarization thereby eliciting the steady firing observed in the present study. In this respect it is interesting to note that the firing level of the 'diabetic' cells (-28 mV) corresponds well to the plateau potential (-33 mV) from which the spikes are fired off in normal β-cells [7]. This indicates that in 'diabetic' cells the spike mechanism is virtually intact, while the process is inactive which normally repolarizes the membrane at the end of a burst.

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