Single Calcium Channel Activity in Mouse Pancreatic β-Cells

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Glucose-induced insulin secretion from pancreatic β-cells is known to involve an influx of calcium through voltage-dependent Ca channels. Recently we have recorded single-channel currents flowing through β-cell Ca channels using the outside-out configuration of the patch-clamp technique and Ba^{2+} as the charge carrier.[1] Only one type of Ca channel was observed. This had a single-channel slope conductance of 24 pS in 110 m*M* Ba^{2+}, was sensitive to dihydropyridines, and was blocked by micromolar concentrations of Cd^{2+}. These properties are consistent with the view that this Ca channel is of the L-type (for terminology see Nowycky *et al.*[2]). The outside-out patch configuration involves the replacement of the cytosol with an artificial solution and is therefore unsuitable for investigations of the metabolic regulation of the Ca channel. For these studies we have used cell-attached patches, because Ca channels are then exposed to their normal internal environment. We present here preliminary cell-attached recordings of single Ca channels in mouse pancreatic β-cells, using previously published methods.[1]

FIGURE 1 shows single-channel currents recorded from a cell-attached patch on a β-cell immersed in normal external solution containing (in m*M*): 138 NaCl, 5.6 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, and 10 HEPES-NaOH (pH 7.4). The pipette was filled with (in m*M*) 100 $BaCl_2$, 10 TEACl, 10 HEPES-$Ba(OH)_2$ (pH 7.4). In the absence of glucose, most channel openings were of brief duration and long openings were observed only rarely. One minute after the addition of 20 m*M* glucose to the bath solution, channel activity was greatly increased and long openings predominated. The associated mean current is consequently considerably larger in the presence than in the absence of glucose. The channel openings have the same amplitude in the presence and absence of the sugar, excluding the possibility that the effect is due to β-cell depolarization. In fact, the single-channel current (I)-voltage (V) relations recorded under both conditions superimpose. After three minutes in high glucose, the β-cell fired action potentials, which frequently elicited Ca-channel openings. These preliminary findings suggest that glucose might modulate Ca-channel activity in the β-cell. A related phenomenon has been described in an insulin-secreting cell line.[3]

The single-channel slope conductance was 20 ± 2 pS ($n = 6$) when measured in

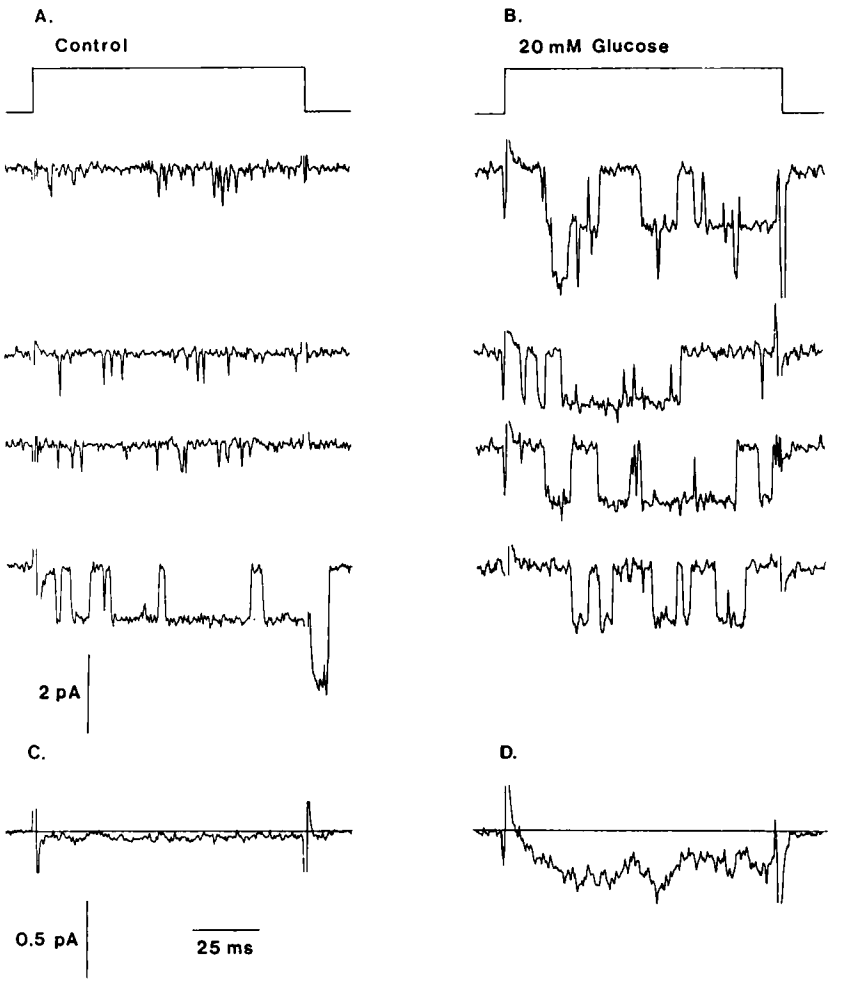

FIGURE 1. Single Ca-channel currents recorded from a cell-attached patch on a mouse β-cell immersed in normal external solution. Pulses were applied from a pipette holding potential of 0 mV to a pipette potential of −70 mV (assuming a β-cell resting potential of −70 mV, this corresponds to a membrane potential of 0 mV) at a frequency of 0.5 Hz. Records were filtered at 0.5 Hz. **(A)** Ba currents recorded in the absence of glucose. **(B)** Ba currents recorded one minute after increasing the bath glucose concentration to 20 m*M*. **(C,D)** Associated mean currents for the data in A,B obtained by averaging approximately 50 individual sweeps.

normal extracellular solution. I-V relations were also recorded in a depolarizing, high K^+ solution, which allows better control of membrane potential (in m*M*: 125 KCl, 30 KOH, 1 $MgCl_2$, 2 $CaCl_2$, 10 EGTA and 5 HEPES-KOH, pH 7.15). In this solution, the single-channel conductance was 25 ± 2 pS ($n = 4$) and the I-V relation was shifted by 70 mV with respect to that recorded in normal external solution, consistent with the usual β-cell membrane potential of around −70mV. Ca-channel activity was markedly increased by 5 μM BAY K 8644 and inhibited by 25 μM D600.

Our preliminary studies suggest that the use of cell-attached patches will be valuable in future studies concerning the regulation of Ca-channels in β-cells, and corroborate the view that, at least in mouse β-cells, L-type Ca channels contribute to most of the Ca current involved in the process of insulin release.

REFERENCES

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