ATP-Sensitive K⁺ Channels in an Insulin-Secreting Cell Line are Inhibited by D-Glyceraldehyde and Activated by Membrane Permeabilization

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Summary. The control of K⁺ channels in the insulin-secreting cell line RINm5F has been investigated by patch-clamp single-channel current recording experiments. The unitary current events recorded from cell-attached patches are due to large and small inwardly rectifying ATP-sensitive K⁺ channels with conductance properties similar to the two channels previously identified in primary cultured rat islet cells (Findlay, I., Dunne, M.J., & Petersen, O.H. J. Membrane Biol. 88:165-172, 1985). Cell permeabilization through brief exposure to 10 μm digitonin or 0.05% saponin (outside the isolated membrane patch area) results in a dramatic increase in current through the cell-attached patch due to opening of many large and small K⁺-selective channels. These channels are inhibited in a dose-dependent manner by ATP applied to the bath (near-complete inhibition by 5 mm ATP). During prolonged ATP exposure (1-5 min) the initial inhibition is followed by partial recovery of channel activity, although further activation does occur when ATP is subsequently removed. From the maximal number of coincident channel openings in the permeabilized cells (in the absence of ATP), it is estimated that there are on average 12 large ATP-sensitive K⁺ channels per membrane patch, but in the intact cells less than 5% of the membrane patches exhibited three or more coincident K⁺ channel openings, indicating the degree to which the channels are inhibited in the resting condition by endogenous ATP. Stimulation of RINm5F cells to secrete insulin was carried out by challenging intact cells with 10 mM d-glyceraldehyde. d-glyceraldehyde induced depolarization of the membrane from about -70 to -20 mV and evoked a marked reduction in the open-state probability of both the large and small ATP-sensitive channels. d-glyceraldehyde also induced action potentials in a number of cases. All effects of stimulation were largely transient, lasting about 100 sec. The two ATP-sensitive K⁺ channels are probably responsible for the resting potential and play a crucial role in coupling metabolism to membrane depolarization.

Key Words: K⁺ channel · ATP · glyceraldehyde · RINm5F cell

Introduction

Glucose-evoked insulin secretion is associated with membrane depolarization and firing of action potentials (Dean & Matthews, 1968, 1970). The stimulant-evoked depolarization is associated with a reduction in membrane K⁺ permeability (Sehlin & Talljedal, 1975; Henquin & Meissner, 1984), and Ashcroft, Harrison and Ashcroft (1984) have with the help of the patch-clamp method identified a K⁺-selective channel in insulin-secreting pancreatic beta cells that closes when glucose is metabolized. This glucose-sensitive channel is inhibited directly by intracellular ATP (Rorsman & Trube, 1985) and is identical to the ATP-sensitive K⁺ channel in pancreatic beta cells first described by Cook and Hales (1984). However, in the absence of glucose stimulation the ATP concentration of intact beta cells is considerably greater than that required to completely inhibit the channel in excised inside-out membrane patches (Ashcroft, Ashcroft & Harrison, 1985).

Findlay, Dunne and Petersen (1985b) have shown that there are in fact two types of ATP-sensitive K⁺ channels in pancreatic islet cells, one with a conductance close to that described for the glucose-sensitive channel (Ashcroft et al., 1984; Rorsman & Trube, 1985) and another with a smaller unit conductance. Both these channels have inward rectifier properties. In intact resting islet cells both channel types are operational, although in the majority of cell-attached patches no more than one or two current levels can be observed. After excision of membrane patches into the inside-out conformation and exposure of the membrane inside to the ATP-free bathing solution there is a dramatic increase in the patch current corresponding mostly to between 10 and 20 open K⁺ channels, but thereafter run-down of channel activity occurs (Findlay et al., 1985b,c).

A number of important questions arise from these recent investigations: (1) Are the many channels that are activated in a membrane patch after excision all sensitive to ATP? (2) Does the ATP sensitivity in excised inside-out patches correspond to the situation in cell-attached patches? and (3) Does glucose or glyceraldehyde metabolism also
A few experiments were carried out using primary cultures of rat pancreatic islet cells prepared as previously described (Findlay et al., 1985b).

**Materials and Methods**

**Cell Culture**

Most experiments were carried out on the insulin-secreting cell line RINm5F (Halban, Praz & Wollheim, 1983; Praz et al., 1983). Cells were maintained in RPMI 1640 tissue culture media, containing 11 mM glucose and supplemented with 10% (vol/vol) fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 μg/ml fungizone. Cells were seeded out every two to three days onto Falcon-style 3001 type petri dishes (35 x 10 mm) and kept in a humidified atmosphere of 95% O₂ and 5% CO₂ at a temperature of 37°C. Figure 1 shows a photomicrograph of the RINm5F cells as they present themselves to the investigator in a patch-clamp experiment. Trypan Blue (0.2%) was present in the bathing solution, as seen in Fig. 1A there was no sign of dye uptake into the cells. In contrast, after a brief (20 sec) exposure of the cells to saponin (0.05%) virtually all the cells were markedly stained, indicating extensive permeabilization of the plasma membrane.

**Results**

**Single-Channel Currents Recorded from Cell-Attached Membrane Patches**

Figures 2 and 3 illustrate single-channel current-voltage (I/V) relationships as well as representative single-channel current traces from RINm5F cell-attached membrane patches, with a K⁺-rich solution in the pipette and a Na⁺-rich solution bathing the

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**Fig. 1.** (A) Photomicrograph illustrating intact RINm5F insulinoma clonal cells bathed in the Na⁺-rich solution containing 0.2% trypan blue. (B) The same dish of cells 1 min after they had been exposed to 0.05% saponin. The bar illustrated in both photomicrographs corresponds to a length of 50 μm.