Characterization of potassium channels in pancreatic β cells from *ob/ob* mice

Manuel Kukuljan[1,2], Min Yi Li[1] and Illani Atwater[1]

[1] *Laboratory of Cell Biology and Genetics, NIDDK, National Institutes of Health, Building 8, Room 403, Bethesda, MD 20892, USA* and [2] *Faculty of Medicine, Universidad de Valparaiso, Valparaiso, Chile*

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The patch-clamp technique in the cell-attached mode was used to study the K channels present in the membrane of cultured pancreatic β cells from *ob/ob* mice. Three types of K^+ channels were regularly observed, with conductances of 64, 20 and 146 pS. The conduction and kinetic properties of the 64 pS channel were similar to those of the ATP-sensitive potassium channel from normal β cells. Furthermore, glucose blocked the activity of this channel at the same concentrations as that reported for normal cells. The 20 pS and the 146 pS were insensitive to glucose. The latter K^+ channel appears to be similar to the large conductance voltage-activated potassium channels described in normal rodent β cells. Thus, potassium channels in *ob/ob* pancreatic β cells in culture are in most respects normal. Other factors may account for the abnormal electrical response to glucose of *ob/ob* pancreatic islets, such as reversible impairment of their function in vivo or defects not related to potassium permeability.

Pancreatic β cell; Potassium channel; Diabetic mouse; Sulphonylurea

1. INTRODUCTION

The regulation of potassium permeability is a key factor in glucose-sensing and insulin secretion by the pancreatic β cell [1,2]. Defects in the potassium permeability of some diabetic mouse models have been reported [3]. Membrane potential measurements in pancreatic islets from homozygous *db/db* (diabetic) and *ob/ob* (obese) mice show abnormalities in the response to glucose consistent with a defective potassium conductance [4-6].

In order to know if the defect is expressed at the single channel level, we investigated potassium channels of cultured β cells from *ob/ob* mice. We found that those cells have channels that behave in a similar way to that described for normal mouse models.

2. MATERIALS AND METHODS

Homozygous (obese) *ob* mice, strain C57BL/6J-ob, were purchased from Jackson Laboratories (Bar Harbor, ME). Pancreatic β cells were prepared from collagenase isolated islets from 2- to 5-month-old mice [7]. Primary cultures were maintained for 2-5 days in CMRL 1066 (Gibco) medium supplemented with 5.6 mM glucose.

Patch-clamp electrodes were pulled from microhematocrit capillary glass, using a two-stage puller (Mecanex, Geneva, Switzerland). Electrode resistance ranged from 3 to 8 MΩ when filled with a 140 mM KCl, 10 mM Na-Hepes, pH 7.3, solution. Electrodes were connected to the probe of an EPC-7 patch-clamp amplifier (List, Darmstadt, FRG). Single-channel potassium currents were recorded in the cell-attached configuration of the patch-clamp [8] and stored on magnetic tape for off-line analysis. For open time probability and kinetic parameters measurement, records were filtered at 2.5 kHz, digitalized at 5 kHz and stored on the hard disk of a microcomputer. Commercially available programs (pCLAMP, Axon Instruments, Burlingame, CA) were used for analysis.

Previous to all experiments, culture medium was replaced by modified Krebs solution containing (in mM): 130 NaCl, 4 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 Na-Hepes, pH 7.4. Addition of experimental solutions was done by either direct application to the culture dish or by means of a gravity-actioned perifusion system. All experiments were conducted at room temperature (20-22°C).

Potassium glyburide was a kind gift from The Upjohn Co. Kalamazoo, MI.

Correspondence address: Manuel Kukuljan, Laboratory of Cell Biology and Genetics, NIDDK, National Institutes of Health, Building 8, Room 403, Bethesda, MD 20892, USA

3. RESULTS

Single K^+ channel records were made from cell-attached patches using high K^+ solution in the pipet. In the absence of extracellular glucose and at resting membrane potential, the most frequently observed inward current corresponded to a 64 ± 8 pS ($n = 27$) channel. 1-3 levels of channel openings were regularly detected. The current-voltage relationship for this channel showed a marked inward rectification, with an outward conductance of 23 ± 3 pS ($n = 8$) (Fig. 1). When the modified Krebs solution was used both in the bath and in the recording electrode, no openings of this channel were seen at positive pipet potentials. At negative pipet potentials outward single channel currents were observed, with a slope conductance of 21 ± 2 pS ($n = 5$).

The openings of this channel occurred in bursts separated by long-lived closed intervals. The open times distributed following a single exponential, with a time

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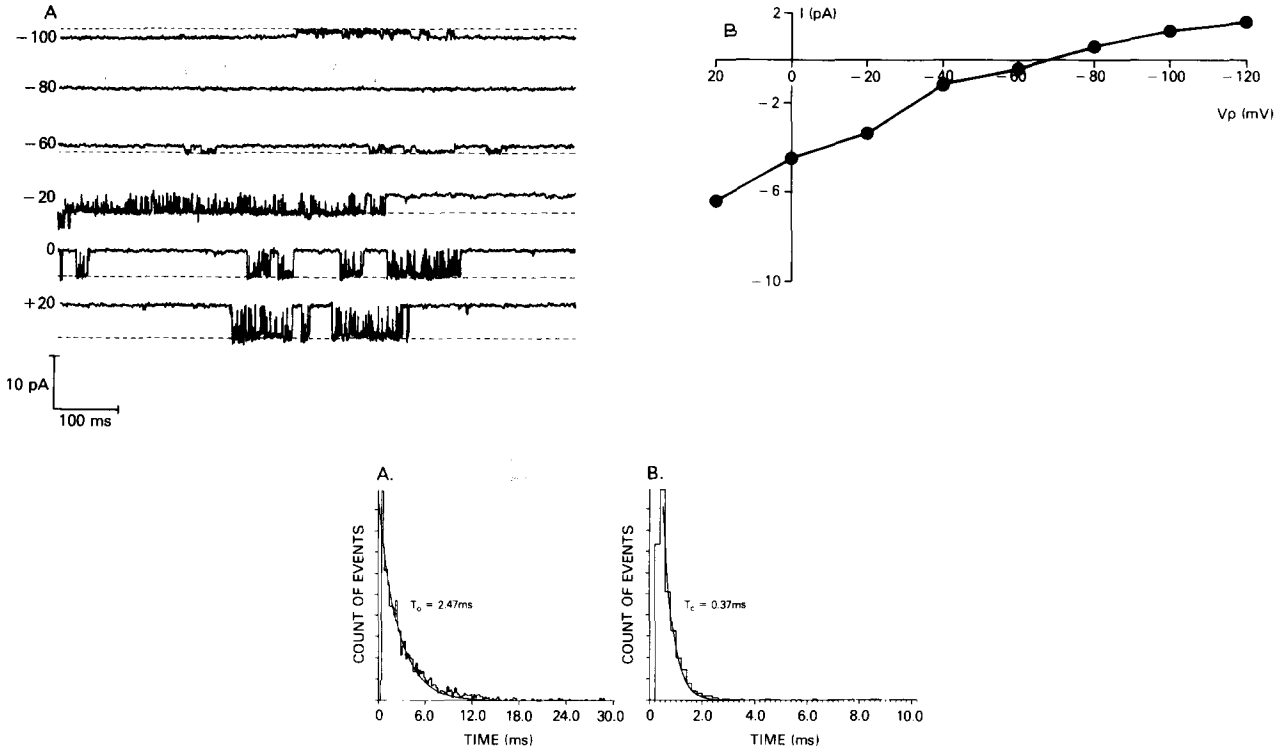

Fig. 1. (A) Single channel currents recorded from a cell-attached patch on a isolated *ob/ob* mouse pancreatic β cell. The potential applied to the pipet solution is given to the left of each trace. Dashed lines represent the open state level. It is possible to observe the bursting kinetics, with groups of rapid openings and closings separated for long closed intervals. The insert shows the histograms for open (A) and closed times (B). (B) Current-voltage relationship for the same experiment depicted in (A). The conductance for the inward current is 66 pS, and 20 pS for the outward current.

constant of 2.52 ± 23 ms ($n = 4$). The closed times within the burst also distributed monoexponentially, with a time constant of 0.39 ± 17 ms ($n = 4$). The fractional open time was insensitive to changes in membrane potential (pipet potentials from +40 to −100 mV).

Increasing the concentration of glucose to 5 mM caused a progressive reduction of open time probability. Fig. 2 shows the time course of that inhibition. The inhibition was the result of a marked increase in number and duration of the interburst closed states, without changes in intraburst kinetics. The inhibition was reversible upon the removal of glucose. The glucose-induced channel activity inhibition was dose dependent with the half-maximal response at 2.2 ± 0.2 mM glucose ($n = 4$),

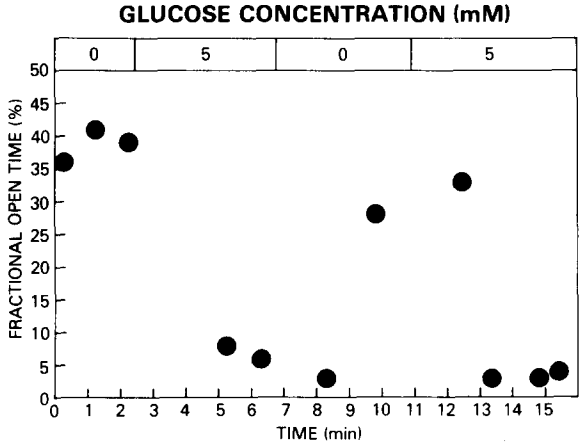

Fig. 2. Time course of glucose-induced inhibition of the 64 pS channel. Each point represents the fractional open time of one channel during a 30 s recording segment. The solutions were exchanged in a time shorter than 40 s. When glucose was removed shortly after the channel inhibition a rapid reversion of this effect was observed, as shown in the figure.

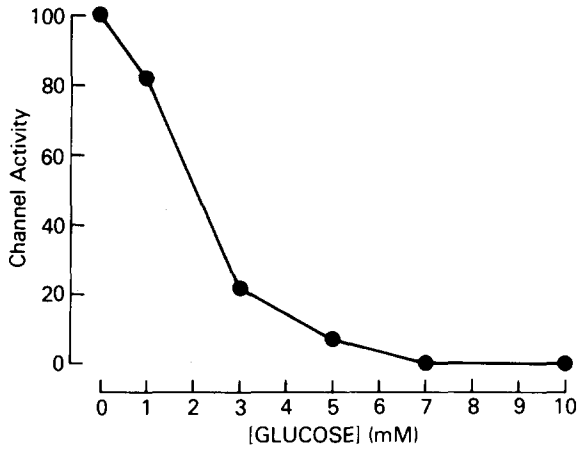

Fig. 3. Dose-dependence of glucose-induced channel inhibition. Each point represents the fractional open time of one channel during a 90 s recording segment, after 5 min in each glucose concentration. Data from 1 of 4 representative experiments.

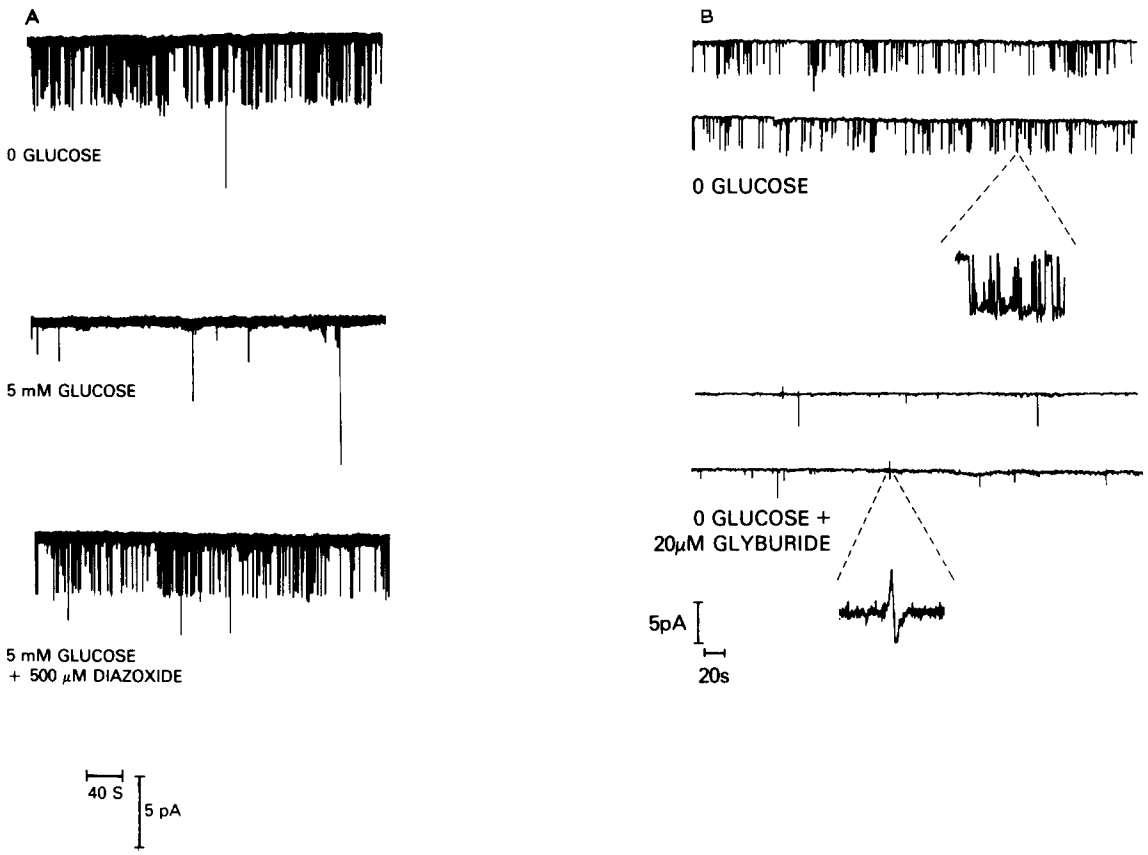

Fig. 4. (A) Effect of glucose and diazoxide on the 64 pS channel activity. 5 mM glucose inhibited almost completely the channel openings. In the presence of glucose, 500 μM diazoxide reverted the effect, reaching the original open time probability. (B) Effect of glyburide on the 64 pS channel. 20 μM glyburide induced a rapid closure of the channel. Some action currents were observed after the blockade by glyburide. Some openings of the higher conductance channel are observed.

as shown in Fig. 3. With glucose concentrations above 5 mM, action potentials were regularly seen.

Fig. 4 shows that the addition of 500 μM diazoxide reversed the glucose effect, reopening the 64 pS K^+ channel. In the absence of glucose, addition of 20 μM glyburide rapidly induced an irreversible blockade of the channel.

A smaller, 20±3 pS conductance channel ($n=7$), which was voltage-insensitive, was observed upon formation of the seal in about 25% of the patches. Its ac-

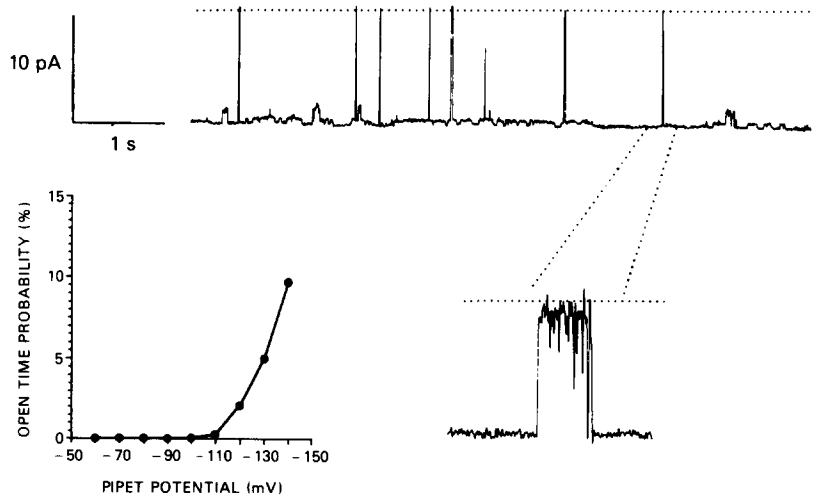

Fig. 5. Openings of the high conductance channel in a cell-attached patch. Pipet potential was held at −140 mV (this gives an estimated membrane potential of +70 mV). The insert shows the voltage dependence of this channel open time probability.

tivity was not modified by changes in extracellular glucose concentration (data not shown).

When depolarized to pipet potentials beyond −100 mV (estimated membrane potential about +30 mV), most of the seals showed openings of a 146 ± 11 pS channel as shown in Fig. 5 ($n = 12$). Its open time probability was strongly voltage-dependent, increasing with membrane depolarization. 5 and 10 mM glucose did not change the activity of this channel (data not shown).

4. DISCUSSION

Generation of electrical activity by the membrane of pancreatic β cells is closely related to metabolic control of the potassium permeability. The link between cell metabolism and ionic permeability is provided by the ATP-sensitive potassium channel characterized in normal rodents [9,10], humans [11] and in some islet-derived cell lines [12]. This channel decreases its open time probability upon elevation of extracellular glucose concentration, in a dose-dependent manner [13]. The resulting depolarization allows calcium entry through voltage-activated calcium channels.

The pancreatic islets from the *ob/ob* strain of mice (Norwich colony, UK) have been shown to display subtle abnormalities in the membrane potential fluctuations stimulated by glucose and to be relatively insensitive to sulfonylureas [5,6]. We found that the pancreatic β cells from the same *ob/ob* strain of mice (Jackson Laboratory colony) have the 3 kinds of potassium channels characterized in normal rats, mice, humans and islet-derived cell lines. The 64 pS channel appears to be the same described as the 'G-channel' by Ashcroft [13]. The values obtained for conductance, the inward rectification and kinetic properties are in close agreement with those described in the normal models. We also observed that this channel activity is inhibited in a reversible way by glucose at similar concentrations to those documented in rats. Although the membrane potential of beta cells from the *ob/ob* mouse has been shown to be relatively insensitive to sulfonylureas, in the patch-clamp study presented here, glyburide blocked the G-channel and its related compound, diazoxide, reversed the blocking effect of glucose. We also observed the existence of other two channels described in rat pancreatic β cells. The 20 pS channel is insensitive to glucose and changes in membrane potential. The 146 pS channel behaves as the large conductance, calcium-activated and voltage-gated channel described in rats and mice [14,15], being barely observed at membrane potentials within the physiological range.

A number of possible explanations can be postulated for the discrepancies between the single channel recording results and the intracellular potential measurement. First, it is possible that the abnormalities observed by previous workers are not related to a potassium channel dysfunction. The possible participation of other ionic currents in the control of the burst pattern (i.e. activation and inactivation of calcium currents) [16] offers an alternative field of study. Second, the defect characterized in whole islets could be reverted by the cell isolation and culture procedures. For example, the defective β-cells are isolated from hyperglycemic animals and maintained for several days at a basal glucose level before experimentation. It has been shown that hyperglycemia per se may be reponsible for the impaired β cell secretory response to glucose in glucose-infused normal rats and in a genetic model of type II diabetes [17,18]. The alternative explanation of differences in the electrical abnormalities existing between the Jackson Laboratory colony and the Norwich colony is discarded by unpublished results from this laboratory showing similar patterns of electrical activity in both colonies.

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