J. Membrane Biol. 129, 267–276 (1992)

The Journal of
Membrane Biology

Comparison of the Properties of the ATP-Sensitive K^+ Channels of Pancreatic β-Cells of Lean and Obese (ob/ob) C57BL/6J Mice

Linda Fournier†, Nicole Bégin-Heick†, James F. Whitfield‡, and Jean-Louis Schwartz‡
†Department of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5, and ‡Cell Systems, Institute for Biological Sciences, National Research Council, Ottawa, Ontario, Canada, K1A 0R6

Summary. Cultures of pancreatic islet cells from obese and lean mice of the C57BL/6J strain were established and their secretory response to glucose stimulation was measured. Insulin secretion (as % of total cellular insulin content) from the cells of the obese mouse cultures was significantly higher than from lean mouse cells. The properties of the glucose- and ATP-sensitive potassium channels present in these cultured β-cells were compared using the cell-attached and the inside-out configurations of the patch-clamp technique. The channels of both types of mouse were indistinguishable in terms of conductance, ionic selectivity, kinetic behavior, voltage dependence or sensitivity to glucose, ATP and ADP. It is concluded that the depolarized state and the hypersecretory response of obese mouse β-cells are not related to an altered behavior of their ATP-sensitive potassium channels.

Key Words K^+ channel · islet · lean · obese · mouse · secretion

Introduction

The obese-hyperglycemic syndrome in mice is caused by an autosomal recessive mutation (ob/ob) (Ingalls, Dickie & Snell, 1950). In C57BL/6J mice, this mutation causes extreme obesity, mild nonketotic hyperglycemia and hyperinsulinemia (Coleman, 1982). The hyperinsulinemia is believed to be due to the increased mass of β-cells and their exaggerated insulin-secretory responsiveness to glucose and other secretagogues (Coleman, 1982). While the cause of this hypersecretion is not known, it appears to be an intrinsic property of the ob/ob β-cell because it persists in isolated islets from the C57BL/6J obese (ob/ob) mouse (Loreti et al., 1974; Lavine et al., 1977).

In normal animals and humans, the process of glucose-stimulated insulin release is associated with a sequence of metabolic, electrical and mechanical events (for review *see*: Prentki & Matschinsky, 1987; Malaisse, 1988; Petersen, 1990), a change in any one of which could be responsible for the hypersecretory response of the β-cells of the ob/ob mouse. The initial response of β-cells to a glucose stimulus is believed to be the closure of an ATP-sensitive K^+ channel (Dunne & Petersen, 1991). This depolarizes the cells and thus opens voltage-dependent Ca^{2+} channels (Findlay et al., 1989) through which flows the Ca^{2+} that triggers insulin secretion. ATP-sensitive K^+ channel activity appears to be a critical factor in maintaining the resting membrane potential of the β-cell (Bokvist, Rorsman & Smith, 1990), and in modulating electrical bursts and insulin secretion (Henquin, 1988, 1990; Cook & Ikeuchi, 1989).

Insulin secretion by pancreatic islets of the C57BL/6J ob/ob mouse is less dependent on extracellular Ca^{2+} (Black, Heick & Bégin-Heick, 1986; Black et al., 1988*a*; Black, Heick & Bégin-Heick, 1988*b*), more sensitive to K^+-induced membrane depolarization, and more sensitive to quinine (a blocker of the ATP-sensitive K^+ channel) than insulin secretion by islets of the wild-type C57BL/6J lean (+/+) mouse (Fournier, Heick & Bégin-Heick, 1990). These studies suggest that an altered ionic permeability of the β-cell membrane causes the hypersecretion of insulin in the obese animal.

Islets of obese mice of the Norwich colony and of normal albino mice have been compared in electrophysiological studies, using microelectrodes. The β-cell of the Norwich obese mouse is more depolarized than that of the albino mouse, both with and without a glucose stimulus (Rosario, 1985; Scott, Dawson & Gonçalves, 1985), and its membrane potential is less sensitive to quinine and glibenclamide (Rosario, Atwater & Rojas, 1985). Although the significance of these studies was compromised by the use of mice of different genetic backgrounds, the results suggest that a defective ion channel, probably the ATP-sensitive K^+ channel, is responsible for the insulin hypersecretion in the ob/ob mouse. Recently, the patch-clamp technique (Hamill et al.,

1981) has been used to study single ion channel activity in cultured β-cells of the C57BL/6J ob/ob mouse (Kukuljan, Li & Atwater, 1990). However, the channel activity in β-cells of control C57BL/6J (+/+) mice was not measured and no data on the insulin secretory capacity of cultured ob/ob β-cells was provided. These investigators reported K^+ channels with conductances and kinetic properties similar to those described by others for normal mouse β-cells (Rorsman & Trube, 1985).

Because of the important role played by the ATP-sensitive K^+ channel in initiating insulin secretion and the evidence for an altered ionic conductance in the membranes of the β-cells of the obese mouse, we were prompted to examine the properties of the ATP-sensitive K^+ channels in the β-cells of this animal to find out whether they were affected by the ob mutation. The appropriate controls for attempting to determine differences in the behavior of channels of β-cells of the C57BL/6J ob/ob mouse are the β-cells of the C57BL/6J +/+ mouse which, except for the ob locus, have the same genetic background (Coleman, 1982). However, such genetically matched animal models have not so far been used in electrophysiological studies.

Functionally competent cultures of islet cells from +/+ and ob/ob C57BL/6J mice were used in single channel patch-clamp studies. The biophysical properties and the metabolic regulation of the ATP-sensitive K^+ channels were compared in +/+ and ob/ob β-cells.

Materials and Methods

Animals

Male C57BL/6J (ob/ob) obese mice and their homozygous (+/+) lean counterparts were obtained from Jackson Laboratory, Bar Harbor, ME. They were maintained on Purina Chow and water *ad libitum*. All animals used for this study were between 8 and 12 weeks old.

Islet Cell Culture

The culture procedure was modified from that developed by Braaten et al. (1975) and modified by Schwartz et al. (1990) for neonatal rat islet cells. Briefly, the splenic portion of the pancreas was removed and the islets were prepared by collagenase digestion and individually hand-picked. Islet cells were then dispersed by aspiration through a 20-gauge needle. They were seeded in 35-mm plastic Petri dishes (Nunc, Roskilde, Denmark) and maintained in culture, at 37°C in a humidified air incubator, in RPMI 1640 medium (GIBCO, Grand Island, NY) containing 11.1 mM glucose, buffered to pH 7.2 with 10 mM HEPES, and supplemented with 5% (vol/vol) fetal bovine serum, penicillin (100 U/l) and streptomycin (100 U/ml). For each group of experiments lean and obese mouse islet cell cultures were prepared on the same day from animals of the same age.

Insulin Secretion

Insulin secretion was measured at 37°C in 14 day-old cultures by a modification of the procedure of Wollheim and Pozzan (1984). The culture medium was removed and the cells were rinsed and preincubated for 1 hr in a basal buffer composed of 0.1% bovine serum albumin (BSA) and the following (in mM): 140 NaCl, 5 KCl, 1.1 $CaCl_2$, 1.1 $MgCl_2$, 2.8 glucose, and 10 HEPES, adjusted to pH 7.2 with NaOH. Cells were then incubated for 1 hr in 1.0 ml of fresh basal buffer. A portion (0.4 ml) of the supernatant was removed for assay. The cells were then incubated for a second hour after addition of buffer (0.4 ml) containing enough glucose to bring the final concentration to 20 mM. The second supernatant was kept for assay. Finally, the total cellular insulin content of the cultures was extracted with acid-ethanol (ethanol : water : concentrated HCl, 140 : 57 : 3 by volume).

Insulin Determinations

Insulin was measured by radioimmunoassay (Dalpé-Scott, Heick & Bégin-Heick, 1982). The amount of insulin secreted was expressed as percentage of the total cellular insulin content.

Immunofluorescence

This was used to determine the location and the population density of insulin-containing β-cells in the islet cell cultures. It was adapted from the procedure of Salomon and Meda (1986).

Electrophysiological Recording Methods

Electrophysiological experiments were performed on 14- to 21-day-old cultures. Cells were identified as β-cells by the presence of glucose-and/or ATP-sensitive K^+ conductances (Ashcroft, Harrison & Ashcroft, 1984; Cook & Hales, 1984). Single-channel currents were recorded at room temperature (20–23°C) using the cell-attached and inside-out configurations of the patch-clamp technique. Cultures were preincubated in glucose-free bath solution for 30 min before use. During experiments, cultures were perfused at a rate of 1.5 ml/min. Fire-polished patch pipettes (1.5 mm outside diameter, 1 mm inside diameter; Pyrex 7740 Corning Glass, Corning, NY) had resistances of 2 to 4 MΩ. Seal resistances were in excess of 2 GΩ. Data was acquired with a Dagan 8900 amplifier (Dagan, Minneapolis, MN) and recorded with a CRC VR-100A Digital Recorder (bandwidth, 18 kHz; Instrutech, Mineola, NY) on VHS magnetic tape. For analysis, data was played back, filtered with an 8-pole Bessel filter (model 902, Frequency Devices, Haverhill, MA) and digitized using a Labmaster interface circuit TL-1-40 (Tecmar, Scientific Solutions, Solon, OH) connected to a personal computer using pCLAMP version 5.5.1 software (Axon Instruments, Foster City, CA).

Current-voltage relationships (*I-V* curves) were constructed by measuring single-channel current amplitudes at different membrane potentials. The percentage of open time was used to assess the level of channel activity. Channel activity A was defined as

the sum of the durations t_{ij} of all openings at each current level expressed as percentage of the total recording time T, i.e.

$$A = 100\left(\sum_{i=1}^{P}\sum_{j=1}^{M_i} t_{ij}\right)/T$$

where M_i is the total number of openings at the current level i and P is the total number of current levels. To compare individual experiments conducted in the cell-attached configuration, the activity was normalized (A_n) with respect to its control level (A_c) taken as 100%, since channel activity varied widely from experiment to experiment. Therefore $A_n = 100\ (A_t/A_c)$, where A_t is the channel activity under test conditions. Experiments conducted in the inside-out configuration were complicated by run-down of ATP-sensitive K^+ channel activity with time. To account for rundown, in the assessment of the ATP- and ADP-sensitivity of the channel, the control level of activity (A_c) was taken as the average of the pre- and post-control activities. The response to nucleotides was expressed as percent reduction (A_r) of the control activity, i.e., $A_r = 100\ (A_c - A_t)/A_c$. Open- and closed-time constants (τ_{open} and τ_{closed}, respectively) were obtained from frequency-distribution histograms constructed from records containing not more than one level of channel opening.

The voltages given in the figures are the patch pipette voltages, V_p, referenced to the bath maintained at ground. Therefore, in the cell-attached configuration the patch membrane potential, V_m, is the difference between V_p and the cell's resting membrane potential (V_r), and in the inside-out configuration $V_m = V_p$. Inward currents, i.e., positive charges flowing from the pipette to the cell (or bath) across the membrane patch, are displayed as downward deflections of the current traces. They are negative in the *I-V* curves.

Solutions

In the cell-attached configuration the bath contained (in mM): 140 NaCl, 5 KCl, 1.1 $CaCl_2$, 1.1 $MgCl_2$, and 10 HEPES buffer adjusted to pH 7.4 with NaOH. In the inside-out configuration the bath solution contained (in mM): 140 KCl, 2.0 $MgCl_2$, and 10 HEPES buffer adjusted to pH 7.4 with KOH. In both configurations, the pipette-solution contained (in mM): 140 KCl, 1.2 $MgCl_2$, 0.1 EGTA, and 10 HEPES buffer adjusted to pH 7.3 with KOH. In potassium-selectivity experiments, K^+ was replaced by an equimolar concentration of Na^+. For nucleotide-sensitivity studies, magnesium was omitted from the inside-out bath solution, because it interferes with the effects of ATP and ADP on the ATP-sensitive K^+ channel (Ashcroft & Kakei, 1989).

Statistical Analysis

Values are expressed as mean ± SEM. Differences were regarded as significant when P was less than 0.05, as determined by analysis of variance.

Results and Discussion

Islet Cell Cultures and Secretion

Lean (+/+) and ob/ob mouse islet cell cultures were established from dispersed islet fragments seeded in plastic Petri dishes. The smaller islet fragments formed monolayer cell clusters after one week, while the larger fragments had monolayer extensions of cells. After two weeks, the cultures were mainly monolayers consisting in both cases of at least 80% β-cells as determined by immunofluorescence staining (*not shown*).

There was no difference in the basal secretion of insulin by +/+ and ob/ob β-cells, but the ob/ob β-cells secreted significantly more insulin than their +/+ counterparts in response to a 20 mM glucose stimulus. Adding 20 mM glucose to the medium increased insulin secretion significantly more in ob/ob (8.5-fold) than in +/+ (5.9-fold) β-cells (Insulin secretion, Table). Thus, after two weeks in culture, the +/+ and ob/ob β-cells retained their secretory competence and the ob/ob β-cells continued to be more responsive to glucose than their +/+ counterparts, although to a lesser extent than the intact isolated islets of the same animals (Fournier et al., 1990).

Channel Activity in Cultured β-Cells

In the cell-attached configuration, in the absence of glucose and at resting membrane potential ($V_p = 0$), the currents through the most frequently observed channel had the same amplitude for both types of cell (*C/A* current, Table). Figures 1*A* and *B* illustrate single-channel current records and the current-voltage relationships of these channels. The conductances in the linear portion of the plot were not significantly different (*C/A* conductance, Table). In both cases, the currents displayed pronounced inward rectification. Adding glucose to the bath reversibly blocked channel activity (Fig. 1*A*). The conductances and responses to glucose were the same as those of the glucose-sensitive K^+ channels reported previously (Ashcroft et al., 1984; Rorsman & Trube, 1985; Misler et al., 1986; Ribalet & Ciani, 1987; Kukuljan et al., 1990; Schwartz et al., 1990).

The number of channels increased upon excision of the membrane patch to the inside-out configuration with $V_p = 40$ mV. The conductance of the channels was similar in +/+ and ob/ob β-cell membranes (*I/O* conductance, Table). Figure 1*C* and *D* show typical current records and the current-voltage relationships of these channels. Inward rectification was less pronounced without Mg^{2+} in the bath (Fig. 1*D*), an effect reported earlier in insulin-secreting cell lines (Findlay, 1987; Kozlowski & Ashford, 1990). Exposing the cytoplasmic side of the membrane patch to 100 μM ATP completely, but reversibly, blocked channel activity (Fig. 1*C*). These data, together with those from the cell-attached experiments, demonstrate that cultured β-cells from+/+

Table. Summary of the insulin secretory response to glucose and of the properties of the ATP-sensitive K^+ channel in cultured β-cells of +/+ and ob/ob mice

	+/+	ob/ob
Insulin secretion (%)		
2.8 mM glucose	0.54 ± 0.07 (11)	0.63 ± 0.05 (11)
20 mM glucose	3.18 ± 0.32 (11)[a]	5.35 ± 0.75 (11)[a]
Channel properties		
C/A current (pA)		
at V_p = 0 mV	4.4 ± 0.1 (15)	4.0 ± 0.1 (9)
C/A conductance (pS)	58.5 ± 2.0 (15)	54.7 ± 3.6 (9)
C/A activity, A_c(%)	0.73 ± 0.10 (12)	0.60 ± 0.16 (6)
I/O conductance (pS)	57.4 ± 1.8 (5)	58.0 ± 2.8 (8)
C/A intraburst closures:		
% of total closures	84 ± 2 (5)	83 ± 3 (5)
C/A τ_{open} (msec)	1.6 ± 0.1 (5)	1.9 ± 0.1 (5)
C/A τ_{closed} (msec)	0.42 ± 0.01 (5)	0.46 ± 0.02 (5)
C/A voltage dependence		
Normalized % activity, A_n:		
−40 mV	77.5 ± 13.7 (8)	96.0 ± 15.5 (8)
−20 mV	78.7 ± 9.2 (10)	106.9 ± 12.8 (9)
40 mV	114.4 ± 9.8 (11)	98.4 ± 18.3 (11)
60 mV	86.9 ± 16.7 (7)	87.2 ± 20.8 (8)
I/O nucleotide sensitivity		
% reduction in activity, A_r		
15 μM ATP	84.6 ± 2.9 (10)	79.8 ± 4.7 (8)
30 μM ATP	95.2 ± 2.5 (6)	94.3 ± 2.6 (6)
100 μM ADP	62.2 ± 5.0 (10)	60.7 ± 8.1 (8)
C/A glucose sensitivity		
Normalized % activity, A_n:		
2.8 mM glucose	32.6 ± 6.7 (9)	23.3 ± 9.2 (5)
5.6 mM glucose	11.4 ± 4.4 (7)	13.2 ± 6.2 (5)

[a] Indicates a significant difference ($P \leq 0.05$) between +/+ and ob/ob mouse β-cells.
Data are mean ± SEM for n observations (in parentheses).
C/A: cell-attached configuration. *I/O*: inside-out configuration.

and ob/ob mice possess glucose- and ATP-sensitive K^+ channels with identical conductances. Furthermore, these channels are similar to the ATP-sensitive K^+ channels found in other rodent pancreatic β-cells (Cook & Hales, 1984; Findlay, Dunne & Petersen, 1985; Rorsman & Trube, 1985; Misler et al., 1986; Schwartz et al., 1990).

Having established that the channel under study was an ATP-sensitive K^+ channel, it was important to determine whether it was altered by the ob mutation. Thus, the biophysical properties of the channel, its sensitivity to ADP and ATP, and its modulation by glucose metabolism were investigated.

Kinetics, Voltage-Dependence and Selectivity Properties of the ATP-Sensitive K^+ Channel

In the cell-attached configuration, without glucose and at $V_p = 0$, both types of cell usually had between 1 and 3 active channels in membrane patches, with no obvious difference in channel density. Variable channel activity was observed, but there was no significant difference between the activities recorded in +/+ and ob/ob β-cell membrane patches displaying only one level of opening (*C/A* activity, A_c, Table). This variable behavior may be due to the presence of more than one active channel under the pipette, variable occurrence and duration of interburst intervals and/or variation in the metabolic state of the cell under study.

In both types of cultured β-cell, channel openings in the cell-attached and inside-out configuration occurred in bursts separated by relatively long and variable closed intervals. Brief closures were observed within the bursts (Fig. 2, upper part). This behavior suggests that, similar to other ATP-sensitive K^+ channels in muscle and other β-cells (Kakei & Noma, 1984; Trube & Hescheler, 1984; Rorsman & Trube, 1985; Spruce, Standen & Stanfield, 1985; Misler et al., 1986; Ashcroft, Ashcroft & Harrison,

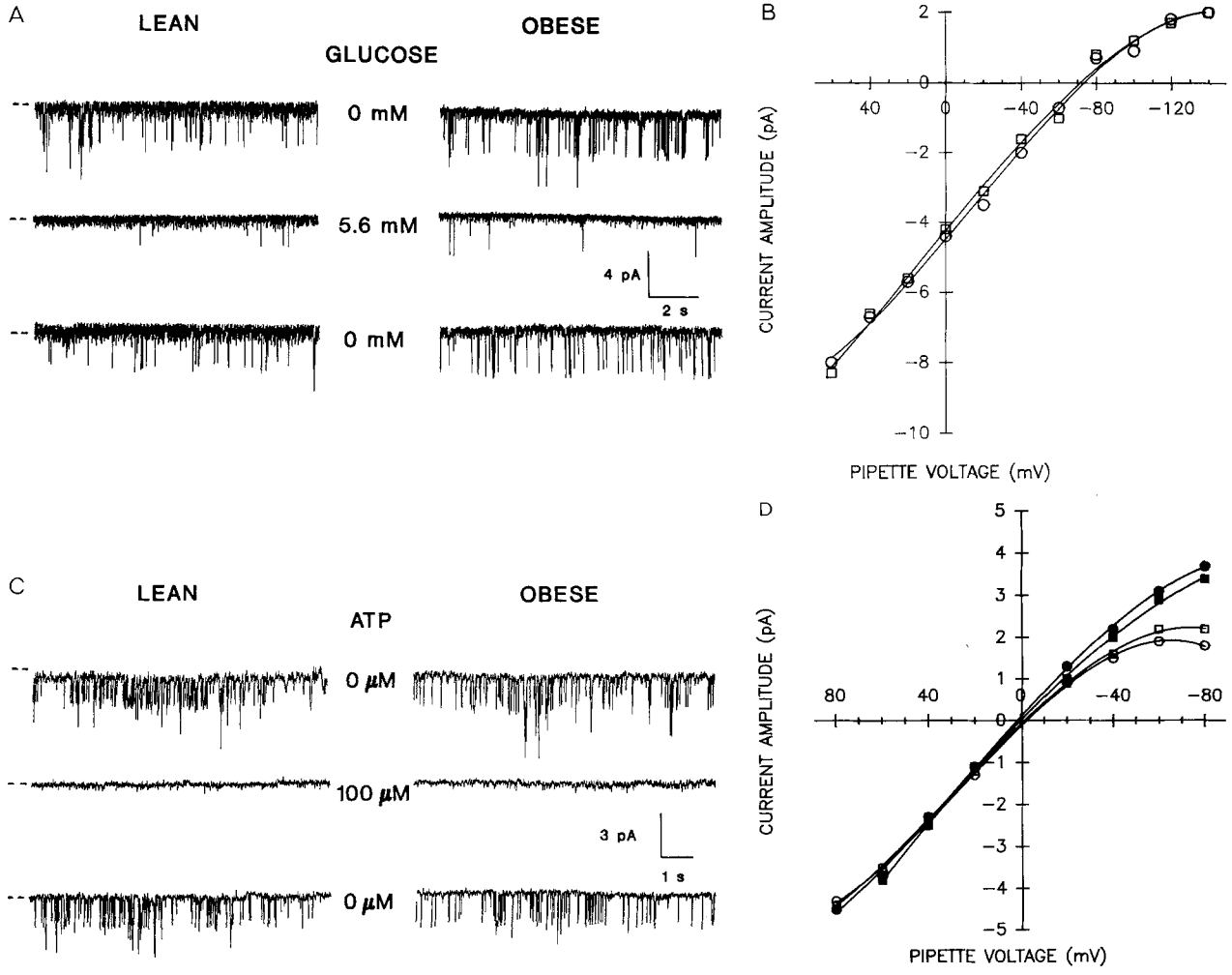

Fig. 1. The effect of glucose and ATP on the activity of a K^+ channel in cultured β-cells of +/+ and ob/ob mice. (*A*) Channel activity recorded in the cell-attached configuration in the +/+ (left) and ob/ob (right) models. Cells were initially exposed to glucose-free solution and then to the glucose concentration indicated, with V_p = 0 mV. The dotted lines indicate the closed state of the channels. (*B*) Current-voltage relations for the same K^+ channel recorded from cell-attached patches of the +/+ (squares) and ob/ob (circles) models. (*C*) Channel activity recorded in the inside-out configuration in the +/+ (left) and ob/ob (right) models. The cytoplasmic side of the membrane patch was initially exposed to ATP-free solution and then to the ATP concentration indicated. V_p = +40 mV. The dotted lines indicate the closed state of the channels. (*D*) Current-voltage relations for the same K^+ channel recorded from inside-out patches of the +/+ (squares) and ob/ob (circles) models in the presence (open symbols) and in the absence (filled symbols) of Mg^{2+} in the bath.

1988; Ribalet, Eddlestone & Ciani, 1988; Kukuljan et al., 1990), the ATP-sensitive K^+ channel may have at least one open state and two closed states. The brief intraburst closures comprised about 85% of the observed closed events (*C/A* intraburst closures, Table). The interburst closures had a mean closed time of the order of several hundred milliseconds and varied from patch to patch. Therefore, kinetic analysis of the ATP-sensitive K^+ channel in +/+ and ob/ob β-cells was limited to the fast events within bursts. Representative open- and closed-time histograms obtained in +/+ and ob/ob β-cells in the cell-attached configuration (V_p = 0, no glucose) are shown in Fig. 2 (lower part). In both types of β-cell, open- and closed-time histograms could be fitted best to single exponential functions with time constants that were not different for +/+ and ob/ob β-cells (*C/A* τ_{open} and τ_{closed}, Table). Similar open- and closed-time constants for the β-cell ATP-sensitive K^+ channel have been reported in other studies (Rorsman & Trube, 1985; Misler et al., 1986; Ashcroft et al., 1988; Kukuljan et al., 1990). Within the limitations due to the variability in channel activity and the fact that we excluded interburst intervals from our analysis, we conclude that the ATP-sensitive K^+ channels in the +/+ and ob/ob β-cells dis-

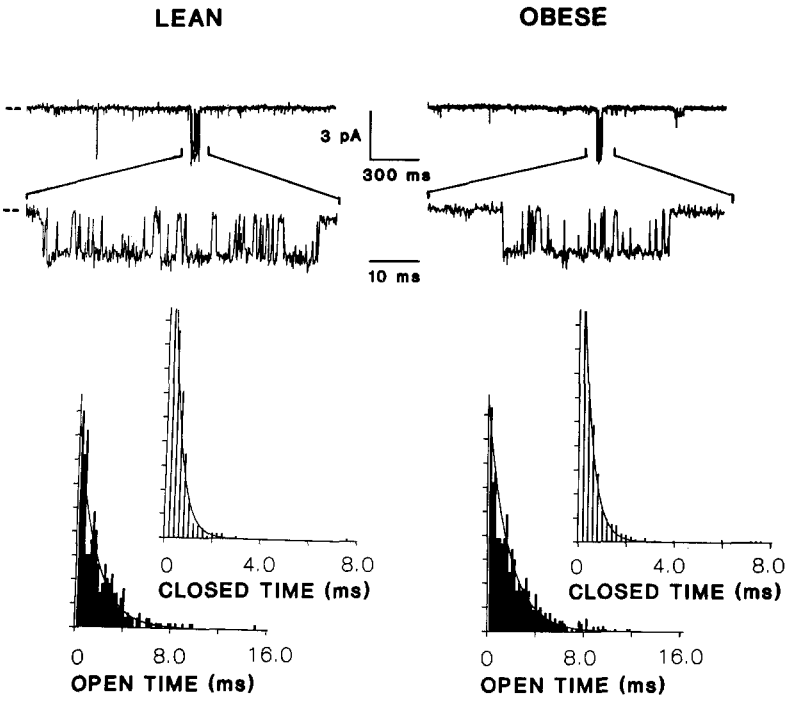

Fig. 2. Kinetics of the ATP-sensitive K^+ channel in cultured β-cells of +/+ and ob/ob mice. Single-channel currents were recorded in the cell-attached configuration, in the absence of glucose and with V_p = 0 mV. The upper part of the figure shows single-channel currents demonstrating the short closures occurring during a burst of the ATP-sensitive K^+ channel of the +/+ (left) and ob/ob (right) models. The dotted lines indicate the closed state of the channels. The lower part of the figure shows the open and closed time histograms of the ATP-sensitive K^+ channels from the +/+ (left) and ob/ob (right) models. Histograms were constructed from periods of recording (data filtered at 2 kHz) of at least 60 sec during which only one level of opening was observed. The distributions were fitted to a single exponential. For the +/+ model, τ_{open} = 1.6 msec and τ_{closed}= 0.4 msec. For the ob/ob model, τ_{open}= 1.70 msec and τ_{closed} = 0.4 msec. The proportion of closed events outside the histogram range, i.e., longer than 10 msec, and excluded from the analysis was 30/347 and 71/613 for the +/+ and ob/ob models, respectively.

play the same kinetic behavior as those described in other β-cell models, and that, as far as intraburst kinetics are concerned, the β-cells of +/+ and ob/ob mice are not different.

The ATP-sensitive K^+ channels in intact β-cells (Cook & Hales, 1984; Findlay et al., 1985; Misler et al., 1986; Ashcroft et al., 1988; Kukuljan et al., 1990; Schwartz et al., 1990) and neoplastic insulin-secreting RINm5F cells (Findlay & Dunne, 1986; Ribalet et al., 1988) are voltage independent, unlike those of skeletal muscle (Spruce et al., 1985) and, in most cases, those of cardiac muscle (Kakei & Noma, 1984; Trube & Hescheler, 1984). We examined the possibility that the activity of the ATP-sensitive K^+ channel of the ob/ob β-cell, in contrast to normal β-cells, is voltage dependent. A decrease in channel activity in response to membrane depolarization would provide an explanation for the anomalous electrical activity reported for the ob/ob β-cell (Rosario, 1985; Rosario et al., 1985; Scott et al., 1985). To test this, the activity of the ATP-sensitive K^+ channel in both +/+ and ob/ob β-cells was measured at different pipette potentials in the cell-attached configuration. The normalized activity A_n was obtained from the test activity, A_t, measured at different V_p values and the control activity, A_c, at V_p= 0. In both types of β-cell the ATP-sensitive K^+ channel displayed voltage independence within the range of voltages tested (*C/A* voltage dependence, Table).

The possibility of differences in ion selectivity of the ATP-sensitive K^+ channel in +/+ and ob/ob β-cells was investigated. Figure 3 shows the current-voltage relationship obtained in the inside-out configuration for three different K^+ concentrations in the bath. For both +/+ and ob/ob β-cells, reducing the bath K^+ concentration progressively shifted the zero-current potential towards less negative pipette potentials. A Nernst plot of the zero-current potential (Fig. 3, inset) shows that the data could be fitted by straight lines with slopes of 53.3 and 49.4 mV/decade for the +/+ and ob/ob β-cells, respectively. Thus, the ATP-sensitive K^+ channel of both +/+ and ob/ob β-cells behaves like a potassium electrode.

Sensitivity to ATP and ADP of the ATP-Sensitive K^+ Channel

The concentration of ATP and/or the ATP/ADP ratio is believed to be the primary intracellular regulator of ATP-sensitive K^+ channel activity in the intact β-cell (Misler et al., 1986; Ohno-Shosaku, Zünkler & Trube, 1987; Ribalet & Ciani, 1987; Cook et al., 1988; Dunne et al., 1988; Ashcroft & Kakei, 1989; Bokvist et al., 1991). Reports that the ob/ob β-cell membrane is in a depolarized state (Rosario, 1985; Rosario et al., 1985; Scott et al., 1985) suggested that the increased responsiveness of the insulin secretory apparatus to glucose may be due to an enhanced sensitivity of the ATP-sensitive K^+ channels to ATP

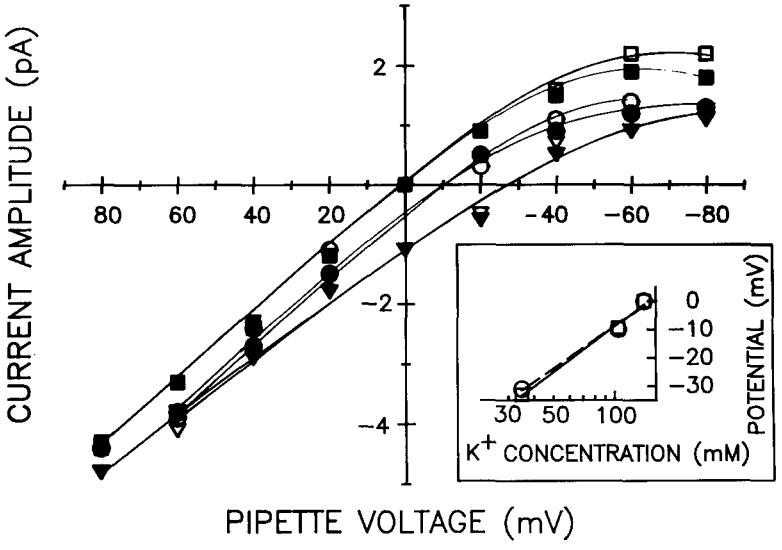

Fig. 3. Current-voltage relation for the ATP-sensitive K^+ channel in asymmetrical potassium conditions. Currents were recorded in the inside-out configuration in the +/+ (open symbols) and ob/ob (filled symbols) models in the presence of 140 mM (squares), 105 mM (circles), and 35 mM (triangles) potassium in the bath. *Inset:* Nernst plot of the zero-current membrane voltage *versus* potassium concentration in the bath. The data points were fitted by linear regression (53.3 mV/decade for the +/+ model (solid line), and 49.4 mV/decade for the ob/ob model (dashed line). Each data point is the mean ± SEM of five determinations at each potassium concentration for each model.

and/or a diminished sensitivity to ADP compared to the +/+ β-cells. The effects of ATP and ADP were therefore tested in the inside-out configuration.

An increase in channel activity was observed after excision of the membrane patch. This is typical of the ATP-sensitive K^+ channel (Rorsman & Trube, 1985; Findlay et al., 1985; Ribalet & Ciani, 1987). This enhanced channel activity diminished with time, which is consistent with the characteristic rundown of ATP-sensitive K^+ channels (Findlay et al., 1985; Findlay & Dunne, 1986; Misler et al., 1986). The time course of rundown was the same in membranes of +/+ and ob/ob β-cells. It was generally faster during the first 1–2 min and varied from patch to patch for β-cells of the same type of mouse. Rundown may be reduced or eliminated by adding to the bath substances such as Mg^{2+} (1 mM) and/or low concentrations of ATP (10 μM) (Findlay & Dunne, 1986; Misler et al., 1986; Ohno-Shosaku et al., 1987; Ribalet & Ciani, 1987). However, rundown has been shown to be promoted by Mg^{2+} (Kozlowski & Ashford, 1990). Furthermore, interaction between Mg^{2+}, the nucleotides and the channels might have complicated the interpretation of the results. The data were rather corrected for rundown following the procedure described in Materials and Methods.

The percent reduction in activity, A_r, of the ATP-sensitive K^+ channel in response to exposure of the cytoplasmic side of patches from +/+ and ob/ob β-cells to 15 and 30 μM ATP or 100 μM ADP is shown in Fig. 4*A*. There was no significant difference in the effect of ATP or ADP in the +/+ and ob/ob cell membranes (*I/O* nucleotide sensitivity, Table), showing that the sensitivity to these substances was not different. The results obtained in these experiments are consistent with those reported for other rodent β-cells where half-maximal inhibitory concentrations for ATP range from 10–20 μM and for ADP from 0.5–2.0 mM (Cook & Hales, 1984; Misler et al., 1986; Ohno-Shosaku et al., 1987; Ribalet & Ciani, 1987; Bokvist et al., 1991).

Sensitivity to Glucose of the ATP-Sensitive K^+ Channel

Although the ATP-sensitive K^+ channel of +/+ and ob/ob β-cells responded similarly to ADP or ATP, the different responsiveness of insulin secretion to glucose in the two types of cell might still be due to a difference in ATP-sensitive K^+ channel regulation. Ob/ob β-cells might be more efficient producers of ATP, for example by having a greater rate of glucose uptake via the glucose transporter (Johnson et al., 1990). In this case ATP-sensitive K^+ channel activity would decrease more in ob/ob than in +/+ β-cells upon adding glucose.

To test this, +/+ and ob/ob β-cells were exposed to 2.8 or 5.6 mM glucose in the cell-attached configuration. Channel activity began falling 2 to 3 min after adding glucose and reached its minimum within 5 to 10 min. Activity resumed within 5 to 30 min after removing glucose. The steady-state channel activity (A_t) was measured and normalized by comparing it to the channel activity (A_c) in the absence of glucose. These data show that glucose caused the same reduction in the activity of the ATP-sensitive K^+ channel in +/+ and ob/ob β-cells (Fig. 4*B* and *C/A* glucose sensitivity, Table), indicating that the rate of glucose uptake and its

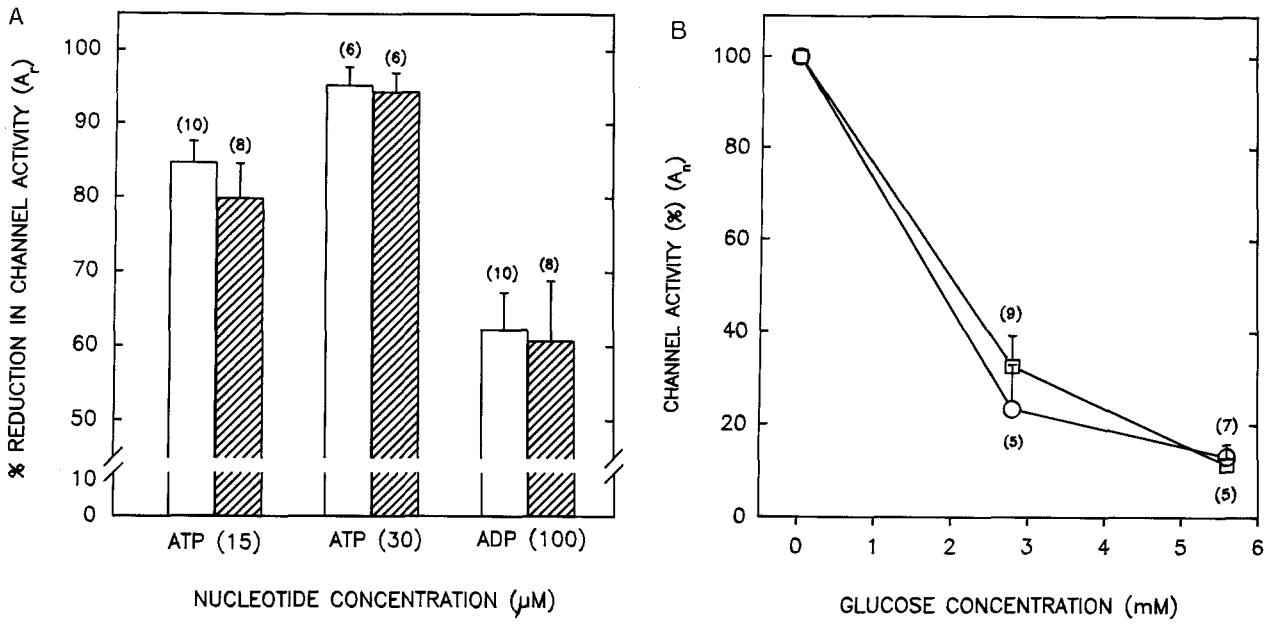

Fig. 4. Sensitivity to ATP, ADP and glucose of the ATP-sensitive K^+ channel in cultured β-cells of +/+ and ob/ob mice. (*A*) Percentage reduction in ATP-sensitive K^+ channel activity (A_r) in response to the indicated nucleotide concentration for the +/+ (open bar) and ob/ob (hatched bar) models. There was initially no nucleotide on the cytoplasmic side of the inside-out membrane patch and V_p= +40 mV. Recordings were at least for 4 min at each condition. Exposure to each nucleotide concentration (test channel activity, A_t) was preceeded (pre-control) and followed (post-control) by incubation in nucleotide-free solution (control channel activity, A_c). The percentage reduction in channel activity was obtained from the formula $A_r = 100(A_c - A_t)/A_c$. Each bar represents the mean ± SEM for n observations indicated above the bar. (*B*) ATP-sensitive K^+ channel activity (A_n) in response to glucose for the +/+ (squares) and ob/ob (circles) models. Experiments were conducted in the cell-attached configuration with V_p = 0 mV and no glucose in the bath. Records were for at least 2 min. Channel activity A_t recorded at each glucose concentration was normalized with the control activity A_c recorded in the absence of glucose (i.e., $A_n = 100(A_t/A_c)$). Data are presented as mean ± SEM for the n observations indicated above (+/+) and below (ob/ob) each data point.

metabolism do not differ in β-cells of +/+ and ob/ob mice.

Conclusion

We established islet cell cultures from +/+ and ob/ob mice of the C57BL/6J strain and demonstrated that they retain a significant insulin secretory difference in response to glucose. This is the first electrophysiological study comparing the properties of the ATP-sensitive K^+ channel in pancreatic β-cells of the obese mouse to those of its lean counterpart. The behavior of this channel does not differ beween lean and obese mice. Since this channel is believed to play a critical role in the maintenance of the resting membrane potential (Bokvist et al., 1990) and in the modulation of electrical activity and insulin secretion (Henquin, 1988, 1990; Cook & Ikeuchi, 1989), our results do not explain why the membrane is more depolarized in the β-cell of the ob/ob mouse compared to nonobese mouse of a different strain (Rosario, 1985; Scott et al., 1985). Although we have demonstrated that the biophysical properties and the metabolic regulation of the channel by ATP, ADP and glucose are not different in the two types of mouse and are consistent with those described in other β-cell models (Dunne & Petersen, 1991), we cannot at present exclude the possibility of differential modulatory effects of pH (Misler, Gillis and Tabcharani, 1989), hormones or other metabolites on the ATP-sensitive K^+ channel itself (De Weille et al., 1989; Dunne et al., 1989), or the possibility that other elements of the insulin secretory pathway are involved (Zawalich & Rasmussen, 1990), including other ion channels (Rosario et al., 1985, Hiriart & Matteson, 1988; Plant, 1988; Rorsman, Ashcroft & Trube, 1988; De Weille & Lazdunski, 1990; Rojas et al., 1990).

This research was supported through a grant from the Canadian Diabetes Association. LAF is a recipient of a studentship from the Medical Research Council of Canada. We wish to thank R. Jefferey who prepared the cultures and developed the experimental protocol for the immunofluorescence technique. We are very

grateful to G.A.R. Mealing for his criticisms of the manuscript and for being a resource person for LAF in conducting the electrophysiological experiments.

References

Ashcroft, F.M., Ashcroft, S.J.H., Harrison, D.E. 1988. Properties of single potassium channels modulated by glucose in rat pancreatic β-cells. *J. Physiol.* **400:**501–527

Ashcroft, F.M., Harrison, D.E., Ashcroft, S.J.H. 1984. Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. *Nature* **312:**446–448

Ashcroft, F.M., Kakei, M. 1989. ATP-sensitive K^+ channels in rat pancreatic β-cells: Modulation by ATP and Mg^{2+} ions. *J. Physiol.* **416:**349–367

Black, M.A., Fournier, L.A., Heick, H.M., Bégin-Heick, N. 1988*a*. Different insulin-secretory responses to calcium-channel blockers in islets of lean and obese (ob/ob) mice. *Biochem. J.* **249:**401–407

Black, M., Heick, H.M., Bégin-Heick, N. 1986. Abnormal regulation of insulin secretion in the genetically obese (ob/ob) mouse. *Biochem. J.* **238:**863–869

Black, M.A., Heick, H.M.C., Bégin-Heick, N. 1988*b*. Abnormal regulation of cAMP accumulation in pancreatic islets of obese mice. *Am. J. Physiol.* **255:**E833–E838

Bokvist, K., Ämmälä, C., Ashcroft, F.M., Berggren, P.-O., Larsson, O., Rorsman, P. 1991. Separate processes mediate nucleotide-induced inhibition and stimulation of the ATP-regulated K^+-channels in mouse pancreatic β-cells. *Proc. R. Soc. London B* **243:**139–144

Bokvist, K., Rorsman, P., Smith, P.A. 1990. Block of ATP-regulated and Ca^{2+}-activated K^+ channels in mouse pancreatic β-cells by external tetraethylammonium and quinine. *J. Physiol.* **423:**327–342

Braaten, J.T., Järlfors, U., Smith, D.S., Mintz, D.H. 1975. Purification of monolayer cell cultures of the endocrine pancreas. *Tissue Cell* **7:**747–762

Coleman, D.L. 1982. Diabetes-obesity syndromes in mice. *Diabetes* **31 (Suppl. 1):**1–6

Cook, D.L., Hales, C.N. 1984. Intracellular ATP directly blocks K^+ channels in pancreatic B-cells. *Nature* **311:**271–273

Cook, D.L., Ikeuchi, M. 1989. Tolbutamide as mimic of glucose on β-cell electrical activity. ATP-sensitive K^+ channels as common pathway for both stimuli. *Diabetes* **38:**416–421

Cook, D.L., Satin, L.S., Ashford, M.L.J., Hales, C.N. 1988. ATP-sensitive K^+ channels in pancreatic β-cells. Spare-channel hypothesis. *Diabetes* **37:**495–498

Dalpé-Scott, M., Heick, H.M.C., Bégin-Heick, N. 1982. An improved double antibody radioimmunoassay for the determination of insulin in serum, plasma, and tissue incubation media. *Can. J. Biochem.* **60:**962–966

De Weille, J.R., Lazdunski, M. 1990. ATP-sensitive K^+ channels reveal the effects of intracellular chloride variations on cytoplasmic ATP concentrations and mitochondrial function. *Biochem. Biophys. Res. Commun.* **168:**1137–1142

De Weille, J.R., Schmid-Antomarchi, H., Fosset, M., Lazdunski, M. 1989. Regulation of ATP-sensitive K^+ channels in insulinoma cells: Activation by somatostatin and protein kinase C and the role of cAMP. *Proc. Natl. Acad. Sci. USA* **86:**2971–2975

Dunne, M.J., Bullett, M.J., Li, G., Wollheim, C.B., Petersen, O.H. 1989. Galanin activates nucleotide-dependent K^+ channels in insulin-secreting cells via a pertussis toxin-sensitive G-protein. *EMBO J.* **8:**413–420

Dunne, M.J., Petersen, O.H. 1986. Intracellular ADP activated K^+ channels that are inhibited by ATP in an insulin-secreting cell line. *FEBS Lett.* **208:**59–62

Dunne, M.J., Petersen, O.H. 1991. Potassium selective ion channels in insulin-secreting cells: Physiology, pharmacology and their role in stimulus-secretion coupling. *Biochim. Biophys. Acta* **1071:**67–82

Dunne, M.J., West-Jordan, J.A., Abraham, R.J., Edwards, R.H.T., Petersen, O.H. 1988. The gating of nucleotide-sensitive K^+ channels in insulin-secreting cells can be modulated by changes in the ratio of ATP^{4-}/ADP^{3-} and by nonhydrolyzable derivatives of both ATP and ADP. *J. Membrane Biol.* **104:**165–177

Findlay, I. 1987. The effects of magnesium upon adenosine triphosphate-sensitive potassium channels in a rat insulin-secreting cell line. *J. Physiol.* **391:**611–629

Findlay, I., Ashcroft, F.M., Kelly, R.P., Rorsman, P., Petersen, O.H., Trube, G. 1989. Calcium currents in insulin-secreting β-cells. *Ann. NY Acad. Sci.* **560:**403–409

Findlay, I., Dunne, M.J. 1986. ATP maintains ATP-inhibited K^+ channels in an operational state. *Pfluegers Arch.* **407:**238–240

Findlay, I., Dunne, M.J., Petersen, O.H. 1985. ATP-sensitive inward rectifier and voltage- and calcium-activated K^+ channels in cultured pancreatic islet cells. *J. Membrane Biol.* **88:**165–172

Fournier, L.A., Heick, H.M.C., Bégin-Heick, N. 1990. The influence of K^+-induced membrane depolarization on insulin secretion in islets of lean and obese (ob/ob) mice. *Biochem. Cell Biol.* **68:**243–248

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Henquin, J.-C. 1988. ATP-sensitive K^+ channels may control glucose-induced electrical activity in pancreatic B-cells. *Biochem. Biophys. Res. Commun.* **156:**769–775

Henquin, J.-C. 1990. Glucose-induced electrical activity in β-cells. Feedback control of ATP-sensitive K^+ channels by Ca^{2+}. *Diabetes* **39:**1457–1460

Hiriart, M., Matteson, D.R. 1988. Na channels and two types of Ca channels in rat pancreatic B cells identified with the reverse hemolytic plaque assay. *J. Gen. Physiol.* **91:**617–639

Ingalls, A.M., Dickie, M.M., Snell, G.D. 1950. Obese, a new mutation in the house mouse. *J. Hered.* **41:**317–318

Johnson, J.H., Newgard, C.B., Milburn, J.L., Lodish, H.F., Thorens, B. 1990. The high K_m glucose transporter of islets of Langerhans is functionally similar to the low affinity transporter of liver and has an identical primary sequence. *J. Biol. Biochem.* **265:**6548–6551

Kakei, M., Noma, A. 1984. Adenosine-5′-triphosphate-sensitive single potassium channel in the atrioventricular node cell of the rabbit heart. *J. Physiol.* **352:**265–284

Kozlowski, R.Z., Ashford, M.L.J. 1990. ATP-sensitive K^+-channel run-down is Mg^{2+} dependent. *Proc. R. Soc. London B* **240:**397–410

Kukuljan, M., Li, M.Y., Atwater, I. 1990. Characterization of potassium channels in pancreatic β cells from ob/ob mice. *FEBS Lett.* **266:**105–108

Lavine, R.L., Voyles, N., Perrino, P.V., Recant, L. 1977. Functional abnormalities of islets of Langerhans of obese hyperglycemic mouse. *Am. J. Physiol.* **233:**E86–E90

Loreti, L., Dunbar, J.C., Chen, S., Foà, P.P. 1974. The autoregu-

lation of insulin secretion in the isolated pancreatic islets of lean (obOB) and obese-hyperglycemic (obob) mice. *Diabetologia* **10:**309–315

Malaisse, W.J. 1988. Stimulus-secretion coupling in the pancreatic B cell. *In:* Current Topics in Neuroendocrinology. Vol. 9, pp. 231–251. D. Ganten and D. Pfaff, editors. Springer-Verlag, Berlin

Misler, S., Falke, L.C., Gillis, K., McDaniel, M.L. 1986. A metabolite-regulated potassium channel in rat pancreatic B cells. *Proc. Natl. Acad. Sci. USA* **83:**7119–7123

Misler, S., Gillis, K., Tabcharani, J. 1989. Modulation of gating of a metabolically regulated, ATP-dependent K^+ channel by intracellular pH in B cells of the pancreatic islet. *J. Membrane Biol.* **109:**135–143

Ohno-Shosaku, T., Zünkler, B.J., Trube, G. 1987. Dual effects of ATP on K^+ currents of mouse pancreatic β-cells. *Pfluegers Arch.* **408:**133–138

Petersen, O.H. 1990. Control of insulin secretion in pancreatic β-cells. *News Physiol. Sci.* **5:**254–258

Plant, T.D. 1988. Na^+ currents in cultured mouse pancreatic B-cells. *Pfluegers Arch.* **411:**429–435

Prentki, M., Matschinsky, F.M. 1987. Ca^{2+}, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol. Rev.* **67:**1185–1248

Ribalet, B., Ciani, S. 1987. Regulation by cell metabolism and adenine nucleotides of a K channel in insulin-secreting B cells (RINm5F). *Proc. Natl. Acad. Sci. USA* **84:**1721–1725

Ribalet, B., Eddlestone, G.T., Ciani, S. 1988. Metabolic regulation of the K(ATP) and a Maxi-K(V) channel in the insulin-secreting RINm5F cell. *J. Gen. Physiol.* **92:**219–237

Rojas, E., Hidalgo, J., Carroll, P.B., Li, M.X., Atwater, I. 1990. A new class of calcium channels activated by glucose in human pancreatic β-cells. *FEBS Lett.* **261:**265–270

Rorsman, P., Ashcroft, R.M., Trube, G. 1988. Single Ca channel currents in mouse pancreatic B-cells. *Pfluegers Arch.* **412:**597–603

Rorsman, P., Trube, G. 1985. Glucose dependent K^+-channels in pancreatic β-cells are regulated by intracellular ATP. *Pfluegers Arch.* **405:**305–309

Rosario, L.M. 1985. Differential effects of the K^+ channel blockers apamin and quinine on glucose-induced electrical activity in pancreatic β-cells from a strain of ob/ob (obese) mice. *FEBS Lett.* **188:**302–306

Rosario, L.M., Atwater, I., Rojas, E. 1985. Membrane potential measurements in islets of Langerhans from *ob/ob* obese mice suggest an alteration in $[Ca^{2+}]_i$-activated K^+ permeability. *Q. J. Exp.Physiol.* **70:**137–150

Salomon, D., Meda, P. 1986. Heterogeneity and contact-dependent regulation of hormone secretion by individual B cells. *Exp. Cell Res.* **162:**507–520

Schwartz, J.-L., Mealing, G.A.R., Whitfield, J.F., Braaten, J.T. 1990. Long-term culture of neonatal rat pancreatic endocrine cells as model for insulin-secretion and ion-channel studies. *Diabetes* **39:**1353–1360

Scott, A.M., Dawson, C.M., Gonçalves, A.A. 1985. Comparison of glucose-induced changes in electrical activity, insulin release, lactate output and potassium permeability between normal and *ob/ob* mouse islets: Effects of cooling. *J. Endocrinol.* **107:**265–273

Spruce, A.E., Standen, N.B., Stanfield, P.R. 1985. Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. *Nature* **316:**736–738

Trube, G., Hescheler, J. 1984. Inward-rectifying channels in isolated patches of the heart cell membrane: ATP-dependence and comparison with cell-attached patches. *Pfluegers Arch.* **401:**178–184

Wollheim, C.B., Pozzan, T. 1984. Correction between cytosolic free Ca^{2+} and insulin release in an insulin-secreting cell line. *J. Biol. Chem.* **259:**2262–2267

Zawalich, W.S., Rasmussen, H. 1990. Control of insulin secretion: A model involving Ca^{2+}, cAMP and diacylglycerol. *Mol. Cell. Endocrinol.* **70:**119–137

Received 9 January 1992; revised 26 March 1992