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Glucose dependent K^+-channels in pancreatic β-cells are regulated by intracellular ATP

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Abstract. The resting conductance of cultured β-cells from murine pancreases was investigated using the whole-cell, cell-attached and isolated patch modes of the patch-clamp technique. Whole-cell experiments revealed a high input resistance of the cells (>20 GΩ per cell or >100 kΩ · cm^2), if the medium dialysing the cell interior contained 3 mM ATP. The absence of ATP evoked a large additional K^+ conductance. In cell-attached patches single K^+-channels were observed in the absence of glucose. Addition of glucose (20 mM) to the bath suppressed the channel activity and initiated action potentials. Similar single-channel currents were recorded from isolated patches. In this case the channels were reversibly blocked by adding ATP (3 mM) to the solution at the intracellular side of the membrane. The conductances (51 pS and 56 pS for $[K^+]_0 = 145$ mM, T = 21° C) and kinetics (at −70 mV: τ_{open} = 2.2 ms and 1.8 ms, τ_{closed} = 0.38 ms and 0.33 ms) of the glucose- and ATP-dependent channels were found to be very similar. It is concluded that both channels are identical. The result suggests that glucose could depolarize the β-cell by increasing the cytoplasmic concentration of ATP.

Key words: Pancreatic β-cell – Patch clamp – K^+-conductance – Glucose dependence – ATP dependence

Introduction

Pancreatic β-cells respond to insulin-releasing concentrations of D-glucose with a slow initial depolarization from the resting potential to a threshold, followed by a more rapid depolarization and action potentials (review: Henquin and Meissner 1984). Studies using $^{42}K^+$ or $^{86}Rb^+$ demonstrated that an early effect of exposure to glucose is a reduction of K^+ efflux, which probably causes the initial depolarization (Sehlin and Täljedal 1975; Henquin 1978). The mechanisms involved in the process could not be clarified unequivocally. Recent patch-clamp recordings from β-cells showed a K^+-channel controlled by ATP in isolated patches of the cell membrane (Cook and Hales 1984) and another K^+-channel inhibited by glucose in intact cells (Ashcroft et al. 1984). How these two channels are interrelated was not clear. The present study aims at comparing the properties of the ATP- and glucose-dependent channels. It will be shown that the conductances and kinetics of the two channels are very similar. Therefore, we suggest that glucose may depolarize the β-cell by closing a K^+-channel regulated by cytoplasmic ATP. Part of the results have been published in abstract form (Rorsman and Trube 1985).

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Methods

Preparation. Islets of Langerhans were isolated from the pancreases of NMRI-mice by collagenase digestion (Boehringer, Mannheim, FRG), and dissociated into single cells or small clusters by shaking in a solution without Ca^{2+} (Lernmark 1974). The cells were plated on Falcon Petri-dishes and maintained for 1–6 days in MEM tissue culture medium (Gibco) containing Earle's salts, 5.6 mM D-glucose, 10% (v/v) heat-inactivated horse serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. The ability of a similar preparation to respond to glucose and other secretagogues with a stimulated secretion of insulin has been reported elsewhere (Rorsman and Abrahamsson 1985). When exposed to 10 mM glucose, 80–90% of the cells produced action potentials and thus were identified as β-cells. Voltage-clamp experiments in the whole-cell configuration (see below) were only done on single cells, but small clusters of cells were preferentially used for the cell-attached patches.

Electrophysiology. Three different configurations of the patch-clamp technique were employed, which have been described previously (Hamill et al. 1981): cell-attached patches, inside-out patches and the whole-cell recording configuration.

A cell-attached patch permits the study of currents through single ion channels in a small patch of the surface membrane. In this configuration the channels are exposed to the normal intracellular environment. Consequently, effects requiring an intact cell metabolism can be studied.

When the patch in the tip of the recording pipette is ruptured, direct access to the cell interior is obtained. The cytoplasm is thereby replaced by the artificial medium in the pipette. The same pipette is used for controlling the potential and for measuring the current flowing across the membrane of the whole cell. The series resistance (3–20 MΩ) of the pipette and the cell capacitance are compensated by the patch-clamp amplifier (EPC7, List Electronic, Darmstadt, FRG, see also: Marty and Neher 1983).

Instead of breaking the patch an isolated inside-out patch can be formed by retracting the pipette from the

cell. Then the intracellular side of the plasma membrane is exposed to the bath solution, which can be changed easily. In our setup the bath had a volume of 0.6 ml and was perfused at a rate of 2 ml/min. Since the perfusion sometimes caused additional noise on the records, it was usually stopped between the solution changes. Therefore, the experiments were done at room temperature (20–22° C).

Solutions. During the experiments the cells were immersed in a solution containing 140 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$, 10 mM HEPES-NaOH (pH = 7.4) and glucose as stated in the figure legends. In the whole-cell experiments the pipettes were filled with an "intracellular" medium composed of 125 mM KCl, 30 mM KOH, 4 mM $MgCl_2$, 3 mM Na_2ATP (unless otherwise indicated), 2 mM $CaCl_2$, 10 mM EGTA and 5 mM HEPES (pH = 7.15; free $[Ca^{2+}] = 0.06$ μM). In experiments on inside-out patches, the same solution was used for filling the bath. The pipette solution (i.e. the solution at the extracellular side of the patch) in experiments on inside-out or cell-attached patches contained 140 mM KCl, 5 mM $CaCl_2$, 5 mM $MgCl_2$ and 10 mM HEPES-KOH (pH = 7.4). The elevated K^+ concentration was necessary for recording measurable K^+ currents at the cell resting potential. The osmolarity of all solutions was 300 mOsm.

Data analysis. For analysing the single-channel kinetics the current signal was filtered at 2 kHz, digitized at a rate of 20 kHz and stored in a computer (PDP 11/73). The time course of the step response of the recording system was fitted to each channel opening or closing to determine the length of the intervals between the transitions. Exponential functions were fitted to the frequency distributions of the channels' lifetimes by the method of maximum likelihood. A detailed description of the method of analysis has been given by Colquhoun and Sigworth (1983).

Results

Whole-cell current-voltage relationship

Figure 1A shows a current-voltage (I–V) relationship recorded from a single β-cell in a "whole-cell" (see Methods) voltage-clamp experiment. Action potential spikes recorded after the release of the clamp are shown in the inset. The I–V curve is almost flat at potentials more negative than −30 mV and increases steeply if the cell is depolarized to more positive potentials. The increase is due to the activation of a time- and voltage-dependent K^+ current, which will be described in a future publication. The slope of the I–V relation in the flat portion is determined by the parallel combination of the cell input resistance and the seal resistance between the pipette and the bath. The two current pathways cannot be separated, but at least a lower limit for the true input resistance is provided by the I–V curve. Displaying the I–V relations of 11 experiments at an enlarged amplitude scale (not shown), we found slope values of 3–24 GΩ (mean 14 GΩ) in the voltage range between −80 mV and −50 mV, where the curves are approximately linear. Since the lower values are probably due to a larger leak, this indicates that the input resistance of a single β-cell is at least 20 GΩ.

An estimate of the surface area is needed to calculate the specific membrane resistance. The average diameter of a cell

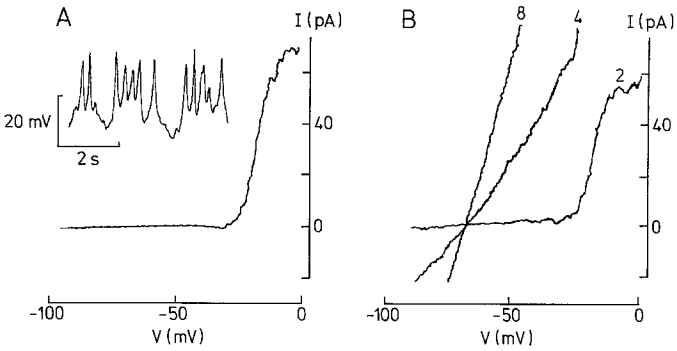

Fig. 1A, B. Whole-cell current-voltage (I–V) relationships. The I–V relationships were recorded from single cells by applying depolarizing voltage ramps of 5 mV/s. **A** The pipette was fillled with "intracellular" solution containing 3 mM ATP (see Methods). *Inset:* spontaneous action potentials after release of the voltage clamp. **B** I–V curves obtained from another single β-cell 2, 4 and 8 min (labels at the traces) after breaking the patch membrane. The pipette was filled with the same medium as in **A** but without ATP. The bath contained 10 mM glucose in both **A** and **B**

was 13 μm (range 9–16 μm); from that value a surface area of about $5.3 \cdot 10^{-6}$ cm^2 is calculated assuming spherical geometry. This implies a specific membrane resistance larger than 100 kΩ · cm^2. Alternatively, the input resistance can be expressed in relation to the cell capacitance, which could be measured more accurately by using the compensation network of the patch-clamp amplifier (Marty and Neher 1983). We found an average capacitance of 5.0 ± 0.6 pF (± SE, 11 experiments) and values between 25 kΩ · μF and 150 kΩ · μF for the specific membrane resistance. Again it can be assumed that the lower values are due to a larger leak between the pipette and the bath. The specific membrane capacitance derived from the surface area calculated above is about 0.94 μF/cm^2, which is close to the value usually obtained for biological membranes.

ATP-dependence of the I–V relationship

Using the whole-cell configuration of the patch-clamp technique, the composition of the intracellular medium is determined by the pipette solution and not by the cellular metabolism. Therefore, the I–V relationships were not affected by the extracellular concentration of glucose (0–20 mM). This result was expected, because it is generally believed that the glucose dependent response of β-cells is mediated by intracellular metabolites or cofactors of glucose metabolism (Hedeskov 1980).

The I–V curves did not change during the experiments (duration up to 15 min) provided the intracellular solution contained ATP (3 mM). However, omitting ATP from the intracellular solution (i.e., the pipette) resulted in a progressive increase of the cell resting conductance. An example of such an experiment is shown in Fig. 1B, where three I–V curves were recorded 2, 4, and 8 min after breaking the patch in the pipette tip. The time-dependent variation can be explained by the diffusional exchange between the cytoplasm and the pipette solution. From comparison with the experiment described before (Fig. 1A, 3 mM ATP) we conclude that the change was induced by washing out the intracellular ATP. The I–V curves in Fig. 1B intersect at −70 mV suggesting that the conductance induced by the

lack of ATP is primarily selective for K^+ (K^+ equilibrium potential = −85 mV; calculated from intracellular $[K^+]$ = 155 mM and bath $[K^+]$ = 5.6 mM). The size of the conductance increase in four similar experiments was found to be 1.9 ± 0.1 nS/pF (normalized by the cell capacitance). The cell resting potential was measured to be −72 ± 1 mV indicating that it was determined by the ATP-dependent conductance.

Previously it has been suggested that the K^+ conductance and, consequently, the membrane potential of the β-cell might be controlled by the intracellular Ca^{2+} concentration (Atwater et al. 1983). Therefore, the experiment of Fig. 1B was repeated with pipettes containing more Ca^{2+}, but the usual ATP concentration (3 mM ATP, 8 mM $CaCl_2$ and 10 mM EGTA, free Ca^{2+} = 1 μM). Although the concentration of Ca^{2+} was higher than the values measured in intact β-cells with the fluorescent indicator quin-2 (Rorsman et al. 1984; Wollheim and Pozzan 1984), no obvious deviation from the I−V relations recorded with the usual intracellular solution (Fig. 1A, 2 mM $CaCl_2$, 10 mM EGTA, free Ca^{2+} = 0.06 μM) could be detected.

Single channel recordings

To allow a study of the effects of intracellular ATP on single K^+-channels inside-out patches of the cell membrane were formed. Numerous openings of a K^+-channel were observed following the isolation of the patch. The addition of 3 mM ATP to the bath (i.e., to the solution at the intracellular side of the membrane) rapidly and reversibly inhibited the channel activity (Fig. 2A, B, cf. Cook and Hales 1984) indicating that glucose might depolarize the β-cell by increasing cytoplasmic ATP.

The whole-cell and isolated-patch modes of the patch-clamp technique involve not only the clamping of the membrane potential, but also a "chemical clamping" of the intracellular solution, and consequently are unsuitable when looking for effects requiring an intact metabolism. Therefore, single K^+-channels were also studied in cell-attached patches without affecting the intracellular milieu. Under these conditions, openings of a channel were observed in the absence of D-glucose (Fig. 2D). When 20 mM glucose was added to the solution superfusing the cells, channel activity stopped within 4−6 min and action potentials appeared (Fig. 2E, cf. Ashcroft et al. 1984). These effects were reversed with a similar time course upon removal of glucose. The time course is comparable with that reported for the glucose-induced reduction of $^{86}Rb^+$ efflux taking into account that the metabolic rate at 21° C is only one third of that at 37° C (Q_{10} = 2.05; Atwater et al. 1984).

K^+-channels of higher conductance similar to the Ca^{2+}-dependent channels described recently (Cook et al. 1984; Findlay et al. 1985) were only occasionally seen in cell-attached patches. Furthermore, these channel displayed no obvious glucose sensitivity. This in agreement with recent results on isolated patches (Cook et al. 1984; Findlay et al. 1985) showing only weak activation of the Ca^{2+}-dependent K^+-channel in the physiological range of membrane potential and intracellular Ca^{2+}.

Analysis of single channel currents

As shown in the expanded traces (Fig. 2C, F), the ATP- and glucose-sensitive single-channel currents had a similar appearance suggesting that the same type of channel could be active. To test this possibility we analysed the conductances and kinetics of the channels. Figure 3A shows the I−V relationships of the ATP- and glucose-regulated

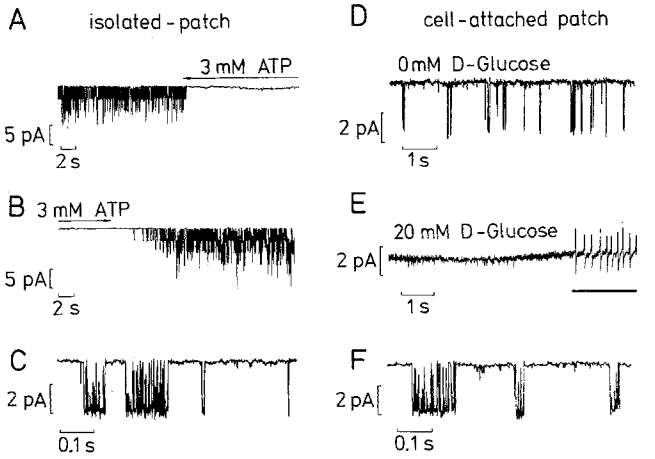

Fig. 2A−F. Effects of ATP or glucose on single-channel currents. **A, B** The membrane potential was held at −70 V. Currents during channel openings were inwardly directed, because the K^+ concentrations on both sides of the membrane were approximately equal (see Methods: Solutions), and were plotted as downward deflections. The intracellular side of the patch membrane was initially exposed to the "intracellular" medium without ATP. 3 mM ATP were added to the perfusion medium during the period indicated. Note suppression of channel activity. **D** Channel activity recorded from a cell-attached patch at the resting potential in the absence of glucose. **E** Continuation of the record 6 min after adding 20 mM glucose. The channel activity has ceased and biphasic current deflections (underlined) due to action potentials are observed. Note difference of time scales in traces **A−B** and **D−E**. **C, F** Examples of the single channel openings in **A** and **D** respectively, at expanded time base. Many transitions to a closed state of short lifetime are truncated due to the low filter frequency (1 kHz) and rate of digitization (5 kHz)

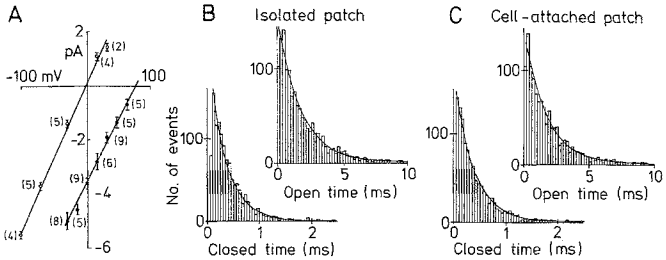

Fig. 3 A−C. Current voltage (I−V) relationships and kinetics of single channels. **A** I−V relationships of the single-channel currents recorded from isolated (○) and cell attached (●) patches. The data are presented as mean values ± SE of indicated number of experiments. The *straight lines* were fitted to the individual experimental values by regression analysis. **B, C** Frequency-versus-duration histograms of channel openings (*top*) and closures (*bottom*) in an isolated (**B**) and a cell-attached (**C**) patch. The time constants of the exponential fits to the open times were 1.8 ms in both examples shown. The time constants of the fast component of the closed times were 0.33 ms (**B**) and 0.37 ms (**C**), respectively. Number of closed times longer than 2.5 ms, i.e., outside the time range of the histograms: 180 out of 1361 events in **B** and 77 out of 1203 events in **C**

single-channel currents recorded from isolated and cell-attached patches, respectively. The curves were linear in the voltage ranges tested with similar slope conductances of 56 ± 1 pS and 50 ± 2 pS (mean values $\pm$ SE of 9 experiments). These values are similar to those found recently by other investigatiors in rat β-cells (Cook and Hales 1984; Ashcroft et al. 1984). The I – V curves of the glucose-dependent currents in the cell-attached patches intersect the voltage axis at 68 ± 4 mV as expected for a cell with a resting potential between -60 mV and -70 mV and an intracellular K^+ concentration of 120 mM (Meissner et al. 1978). Frequency-versus-duration histograms of the channel openings and closures are shown in Fig. 3B, C. The distributions of the openings could be fitted by single exponentials of time constants 1.84 ± 0.01 ms (ATP-sensitive channel, 3 experiments) or 2.18 ± 0.15 ms (glucose-dependent channel, 4 experiments). At least two exponentials were needed to fit the distributions of the closed times; this was expected, because the channel openings were grouped into bursts (Fig. 2C, F). Again, the time constants of the fast component, i.e., the mean lifetimes of the closures within the bursts, were similar: 0.33 ± 0.01 ms for the ATP-sensitive channels and 0.38 ± 0.02 ms for the glucose-dependent channels. The fast components comprised about 90% of the observed closures irrespective of whether it was an isolated or a cell-attached patch. The time constants of the slow components were in the order of some hundred milliseconds but were quite variable from patch to patch. A similar variability of an ATP-regulated channel in heart cells has been described previously (Trube and Hescheler 1984).

Discussion

Considering the similarities of the conductances and the kinetics we conclude that the ATP- and glucose-regulated channels are identical. The slight differences of the values could be explained by the influence of some intracellular factor, which is lost when the cytoplasmic side of an isolated patch is exposed to an artificial medium. The kinetic parameters of the ATP- and glucose-dependent K^+-channels in the β-cell are also similar to those recently reported for an ATP-sensitive K^+-channel in heart cells (Trube and Hescheler 1984).

An important question is to what extent changes in the cytoplasmic concentration of ATP can regulate the K^+ conductance of the β-cells. Circumstantial evidence for a relation between intracellular ATP levels and K^+ permeability is provided by observations that the efflux of $^{86}Rb^+$ (tracer for K^+) is affected by glucose in a range of concentrations similar to that where changes in ATP content occur (Malaisse and Herchuelz 1982). However, Cook and Hales (1984) have recently demonstrated that the ATP-sensitive K^+ channel is half-inactivated by a concentration of ATP as low as 15 µM. The average ATP levels of β-cells are generally believed to be much higher (about 4 mM) and increase only moderately when the cells are exposed to glucose (Hellman et al. 1969; Ashcroft et al. 1973). Therefore, it was important to show that the same K^+-channel is indeed active in intact β-cells under basal conditions and that it can be inhibited by increasing extracellular glucose (Fig. 2). The result suggests that the regulation of the K^+ conductance by ATP constitutes a link between the intracellular metabolism and the membrane potential changes involved in the process of glucose-stimulated insulin release. Several explanations for the discrepancy between the concentrations seem possible.

i) Probably only a small fraction of the ATP-regulated channels has to be activated to increase the low input conductance (~ 50 pS $= 1/20$ GΩ) of the β-cells. Measurements of the input resistance of cells in whole islets by conventional microelectrodes (which do not change the cytoplasmic solution) or $^{42}K^+$ flux measurements suggest that the input conductance is increased about 1.5-fold when extracellular glucose is removed from the bath (Atwater et al. 1978; Henquin 1978). The single-channel conductance of the ATP-dependent channel in β-cells has not yet been measured for normal extracellular $[K^+]$, but presumably it is close to 20 pS, which is the value found in heart cells (Noma 1983). That means that the simultaneous opening of about two channels per cell (note that β-cells are electrically coupled) would almost double the input conductance. On the other hand, the conductance increased almost 200-fold (from ~ 50 pS to ~ 9.5 nS) if the intracellular ATP was washed out by the pipette solution in the whole-cell experiments (Fig. 1B). This implies that the degree of activation of the channels in intact cells is probably less than 1% ($\simeq 1.5/200$) if glucose is removed from the extracellular solution. The ATP-concentration corresponding to 1% activation on the dose-response curve shown by Cook and Hales (1984) is 0.7 mM and, of course, this value is much closer to physiological ATP levels than the concentration at 50% activation (15 µM). A total number of about 500 ATP-dependent channels per cell is estimated from the maximum conductance after washing out the intracellular ATP (~ 9.5 nS) and a single-channel conductance of 20 pS.

ii) A major fraction of the cellular ATP is stored in subcellular organelles such as the secretory granules (Leitner et al. 1975) and, therefore, the cytosolic concentration could be much lower. It should be noted that many other enzymes have K_m-values for ATP in the same order of magnitude as the concentration causing 50% inhibition of the channels, e.g. the Ca^{2+}-ATPase (Colca et al. 1983) and adenylate cyclase (Davis and Lazarus 1972) of the plasma membrane as well as the cytoplasmic enzymes glucokinase (Meglasson et al. 1983) and cyclic AMP dependent protein kinase (Sugden et al. 1979).

iii) Another possibility to consider is that the channel in isolated patches displays an altered sensitivity to ATP due to the loss of a unknown intracellular modulator. Future experiments are required to test the role of intracellular enzymes or products of glucose metabolism in the control of the ATP-dependent K^+-channels.

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References

Ashcroft SJH, Weerasinghe LCC, Randle PJ (1973) Interrelationship of islet metabolism, adenosine triphosphate content and insulin release. Biochem J 132:223–231

Ashcroft FM, Harrison DE, Ascroft SJH (1984) Glucose induces closure of signgle potassium channels in isolated rat pancreatic β-cells. Nature 312:446–448

Atwater I, Ribalet B, Rojas E (1978) Cyclic changes in potential and resistance of the β-cell membrane induced by glucose in islets of Langerhans from mouse. J Physiol 278:117–139

Atwater I, Rosario L, Rojas E (1983) Properties of the Ca-activated K^+-channel in pancreatic β-cells. Cell Calcium 4:451–461

Atwater I, Goncalves A, Herchuelz A, Lebrun P, Malaisse WJ, Rojas E, Scott A (1984) Cooling dissociates glucose-induced insulin release from electrical activity and cation fluxes in rodent pancreatic islets. J Physiol 348:615–627

Colca JC, Kotagal N, Lacy PE, McDaniel ML (1983) Comparison of the properties of active Ca^{2+} transport by islet-cell endoplasmic reticulum and plasma membrane. Biochim Biophys Acta 729:176–184

Colquhoun D, Sigworth F (1983) Fitting and statistical analysis of single-channel records. In: Sakmann B, Neher E (eds) Single channel recording, Cht 11. Plenum Press, New York, pp 191–263

Cook DL, Hales N (1984) Intracellular ATP directly blocks K^+ channels in pancreatic B-cells. Nature 311:271–273

Cook DL, Ikeuchi M, Fujimoto WY (1984) Lowering of pH_i inhibits Ca^{2+}-activated K^+ channels in pancreatic B-cells. Nature 311:269–271

Davis B, Lazarus NR (1972) Insulin release from mouse islets. Effects of glucose and hormones on adenylate cyclase. Biochem J 129:373–379

Findlay I, Dunne MJ, Petersen OH (1985) High conductance K^+ channel in pancreatic islet cells can be activated and inactivated by internal calcium. J Membr Biol 83:169–175

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recordings from cells and cell-free membrane patches. Pflügers Arch 391:85–100

Hedeskov CJ (1980) Mechanisms of glucose-induced insulin secretion. Physiol Rev 60:442–509

Hellman B, Idahl LA, Danielsson A (1969) Adenosine triphosphate levels in mammalian pancreatic β-cells after stimulation with glucose and hypoglycemic sulfonylureas. Diabetes 18:509–516

Henquin JC (1978) D-glucose inhibits potassium efflux from pancreatic islet cells. Nature 271:271–273

Henquin JC, Meissner HP (1984) Significance of ionic fluxes and changes in membrane potential for stimulus-secretion coupling in pancreatic B-cells. Experientia 40:1043–1052

Leitner JW, Sussman KE, Vatter AE, Schneider FH (1975) Adenosine nucleotides in secretory granule fraction of rat islets. Endocrinology 96:662–677

Lernmark A (1974) The preparation of, and studies on, free cell suspensions from mouse pancreatic islets. Diabetologia 10:431–438

Malaisse WJ, Herchuelz A (1982) Nutritional regulation of K^+ conductance: An unsettled aspect of pancreatic B cell physiology. In: Litwack G (ed) Biochemical actions of hormones, vol IX, Cht 3. Academic Press, New York, pp 69–92

Marty A, Neher E (1983) Tight-seal whole-cell recording. In: Sakmann B, Neher E (eds) Single channel recording, Cht 7. Plenum Press, New York, pp 107–122

Meglasson MD, Burch PT, Berner DK, Najafi H, Vogin AP, Matschinsky FM (1983) Chromatographic resolution and kinetic characterization of glucokinase from islets of Langerhans. Proc Natl Acad Sci USA 80:85–89

Meissner HP (1976) Electrophysiological evidence for coupling between β-cells of pancreatic islets. Nature 262:502–504

Meissner HP, Henquin JC, Preissler M (1978) Potassium dependence of the membrane potential of pancreatic B-cells. FEBS Lett 94:87–89

Noma A (1983) ATP-regulated K^+ channels in cardiac muscle. Nature 305:147–148

Rorsman P, Abrahamsson H, Gylfe E, Hellman B (1984) Dual effects of glucose on the cytosolic Ca^{2+} activity of mouse pancreatic β-cells. FEBS Lett 170:196–200

Rorsman P, Abrahamsson H (1985) Cyclic AMP potentiates glucose-induced insulin release from mouse pancreatic β-cells without increasing cytosolic free Ca^{2+}. Acta Physiol Scand (in press)

Rorsman P, Trube G (1985) Evidence that glucose-induced depolarization in the pancreatic β-cells involves the closure of an ATP-dependent K^+ channel. Acta Physiol Scand 124 (Suppl 542):147 (Abstract)

Sehlin J, Täljedal IB (1975) Glucose-induced decrease in Rb^+ permeability in pancreatic β-cells. Nature 253:635–636

Sugden MC, Ashcroft SJH, Sugden PH (1979) Protein kinase activities in rat pancreatic islets of Langerhans. Biochem J 180:219–229

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

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

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