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Inhibition of the ATP-sensitive potassium channel by class I antiarrhythmic agent, cibenzoline, in rat pancreatic β-cells

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1 Cibenzoline, a class I antiarrhythmic agent, was investigated for its effect on the ATP-sensitive K^+ channel of pancreatic β-cells by the patch clamp technique.

2 In perforated patch clamp experiments, cibenzoline depolarized the membrane of single β-cells and thereafter, caused firing of action potentials in the presence of 2.8 mM glucose.

3 Cibenzoline inhibited the activity of the ATP-sensitive K^+ channel in cell-attached recordings in the presence of 2.8 mM glucose and evoked repetitive fluctuations of the baseline current, apparently reflecting the action potentials of the β-cell.

4 In whole-cell clamp experiments, time-independent outward current was induced by depleting cytoplasmic ATP with 0.1 mM ATP and 0.1 mM ADP in the solution contained in the pipette. The outward current was inhibited by cibenzoline in a dose-dependent manner in the concentration range of 1 μM to 100 μM and half maximum inhibition occurred at 1.5 μM.

5 Cibenzoline blocked substantially the ATP-sensitive K^+ channel current when applied at the inner side of the membrane in isolated inside-out membrane patches.

6 It is concluded that cibenzoline blocks the ATP-sensitive K^+ channel of pancreatic β-cells and, thereby, stimulates insulin secretion at sub-stimulatory levels of glucose.

Keywords: Cibenzoline; ATP-sensitive K^+ channel; pancreatic β-cells; insulin secretion

Introduction

Stimulation of insulin secretion from pancreatic β-cells is evoked physiologically by an elevation of the plasma glucose level, via metabolic production of ATP which then inhibits the ATP-sensitive K^+ channel (K_{ATP} channel) (Cook & Hales, 1984; Ashcroft *et al.*, 1984). It has been established that the activity of the K_{ATP} channel is a major determinant of the resting potential of β-cells. Thereby, inhibition of the channel results in membrane depolarization followed by an increase in the cytosolic Ca^{2+} concentration through Ca^{2+} entry via voltage-dependent Ca^{2+} channels (Yada *et al.*, 1992) and eventual exocytosis of insulin (for reviews see Henquin & Meissner, 1984; Ashcroft & Rorsman, 1989).

A newly-synthesized class I antiarrhythmic agent, cibenzoline, is known to have an extracardiac side-effect of hypoglycaemia (Hilleman *et al.*, 1987; Gachot *et al.*, 1988; Jeandel *et al.*, 1988; Lefort *et al.*, 1988; Houdent *et al.*, 1991). Recently Bertrand *et al.* (1992) reported that cibenzoline directly stimulates pancreatic β-cells and potentiates insulin secretion. It has been reported that other antiarrhythmic agents, such as quinidine or disopyramide, also may induce hypoglycaemia (Panisko & Keystone, 1990; Kim & Benowitz, 1990). However, the mechanism by which these drugs produce hypoglycaemia, remains to be elucidated. Sulphonylureas, which are used to increase the ability of the pancreatic β-cell to release insulin in response to glucose stimulation, are known to inhibit specifically the K_{ATP} channel (Sturgess *et al.*, 1985; Trube *et al.*, 1986). It has also been reported that the K_{ATP} channel is blocked by quinidine in cardiac myocytes (Undrovinas *et al.*, 1990) and by sparteine, an antiarrhythmic agent with oxytocic action, in the insulin-secreting β-cell line, HIT-T15 (Ashcroft *et al.*, 1991). Similarly, it is likely that the K_{ATP} channel in pancreatic β-cells may be involved in antiarrhythmic agent-induced hypoglycaemia. In the present study, we have investigated the effect of cibenzoline on the activity of the K_{ATP} channel in rat pancreatic β-cells.

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Methods

Islets of Langerhans were isolated from Wistar rats weighing 200–300 g by collagenase digestion (Type 4, Funakoshi Co. Ltd., Japan) as described elsewhere (Sutton *et al.*, 1986). The animal was anaesthetized by an intraperitoneal injection of pentobarbitone (80 mg kg^{-1}). The abdomen was opened and collagenase (3 mg ml^{-1}) dissolved in 5 ml of 5 mM Ca^{2+}-containing Krebs-Ringer bicarbonate buffer (KRB) solution was injected into the common bile duct at the distal end after ligation of the duct proximal to the pancreas. Then, the pancreas was dissected out and incubated for 17 min at 37°C. The islets of Langerhans were examined under the biological microscope and were dispersed mechanically into single cells in Ca^{2+}-free KRB solution. The isolated islet cells were plated on coverslips (18 × 24 mm, 0.12 mm in thickness) in 35 mm tissue culture dishes and cultured for 1–4 days in Eagle's MEM medium containing 5.6 mM glucose supplemented with 10% foetal bovine serum, 100 μg ml^{-1} streptomycin and 100 u ml^{-1} penicillin at 37°C in an atmosphere of 95% air plus 5% CO_2. The coverslip was cut into small pieces, placed in the recording chamber (0.3 ml) and superfused with the KRB solution containing 2.8 mM glucose at least 20 min before either whole-cell voltage clamp, perforated current clamp or cell-attached patch recordings, or superfused with the standard internal solution in the inside-out membrane patch experiments.

Standard whole-cell or patch-clamp techniques (Hamill *et al.*, 1981) were used to record the K_{ATP} channel currents. Patch pipettes were pulled from hard glass tubing (Narishige Scientific Inst. Lab., Tokyo, Japan), coated with silicon resin to reduce their electrical capacitance and fire-polished just before use. The pipettes had resistances between 5 and 10 MΩ when filled with the solution (140 mM KCl) in either the cell-attached patch or inside-out patch configuration and resistances less than 3 MΩ with the internal solution in the perforated or ordinary whole-cell clamp configuration, respectively. The current was recorded by an amplifier (List, EPC7) and stored on a PCM digital data recorder (TEAC, RD-1117Z). Replayed data were then low-pass filtered

(24 dB/octave. NF, E-3201A) at a cut-off frequency indicated in the figure legends and digitized by a 12-bit AD converter to be analysed with a computer (Hewlett Packard, HP-9816).

The perforated whole-cell current clamp mode was used to record the membrane potential by using 0.01% nystatin dissolved in 0.2% dimethylsulphoxide in the pipette (Horn & Marty, 1988).

In whole-cell clamp experiments, the pipette solution contained 0.1 mM ATP and 0.1 mM ADP in order to evoke the ATP-sensitive K^+ channel current (Trube *et al.*, 1986). After formation of the whole-cell clamp configuration, the resting membrane conductance progressively increased due to the reduction of cytoplasmic ATP concentration by dialysis between the cell interior and the pipette solution and reached a steady state level within 10 min. To construct the dose-response relations between cibenzoline and the K_{ATP} channel current, the membrane potential was voltage-clamped to a holding potential of − 70 mV and depolarized by 10 mV with 100 ms duration every 5 s throughout the experiment. The current through the K_{ATP} channels was determined by its time- and voltage-independent kinetics and sensitivity to the sulphonylurea, 0.1 mM tolbutamide. We then tested effects of cibenzoline on this tolbutamide-sensitive current fraction. The current amplitude during exposure to cibenzoline was normalized with respect to that of the tolbutamide-sensitive current fraction, and plotted as a function of cibenzoline concentration. Relative current was fitted to the Hill equation to obtain the half maximal inhibition (IC_{50}) of the K_{ATP} channel activity by a least squares method:

$$\text{relative current} = 1/\{1 + ([L]/K_{1/2})^n\}$$

where L is a ligand concentration (cibenzoline). $K_{1/2}$ is the half-maximal concentration of cibenzoline on the channel activity and n is the Hill coefficient.

To calculate the mean K_{ATP} current we constructed an amplitude histogram with a current level of 0.04 pA/bin on the abscissae during superfusion with each test solution for at least 2 min. The mean current was obtained by integrating open channel distributions on the histogram and by averaging over time. The mean amplitude of the single channel event was determined as the difference between the current level of closed channel distribution and that of the first-step channel events.

Solutions

The composition of the pipette solution used in both cell-attached and inside-out membrane patch experiments was (in mM): KCl 140, $CaCl_2$ 2 and HEPES 5 (pH 7.2 adjusted with KOH). The KRB solution contained (in mM): NaCl 129, KCl 4.7, $CaCl_2$ 2.0, $MgCl_2$ 1.2, KH_2PO_4 1.2 and $NaHCO_3$ 5.0; the pH was ajusted to 7.4 with 10 mM HEPES-NaOH. Glucose was added to the KRB solution as required. The pipette solution used in perforated whole-cell clamp contained (in mM): K_2SO_4 40, KCl 50, HEPES 10, $MgCl_2$ 2, EGTA 0.5 (pH 7.2 adjusted with KOH). The standard internal solution for whole-cell clamp experiments contained (in mM): KCl 50, K_2SO_4 35, $MgCl_2$ 2.0, EGTA 11, $CaCl_2$ 1, HEPES 11 (pH 7.2 adjusted with KOH; final K^+ concentration 140 mM). The internal solution used for the inside-out patch experiments contained (in mM): KCl 113, $MgCl_2$ 2.0, EGTA 11, $CaCl_2$ 1.0, and HEPES 11 (pH 7.2 adjusted with KOH). Adenosine-5′-triphosphate (ATP-2Na) and adenosine-5′-diphosphate (ADP-K) were purchased from Boehringer and added to intracellular solutions as required. Cibenzoline, a gift from Fujisawa Pharmaceutical Co., Ltd. (Tokyo, Japan), was dissolved in distilled water as a 10 mM stock solution and diluted to the required concentration in the bathing solution before use. All the experiments were performed at room temperature (22–25°C).

Results

The effect of cibenzoline on the membrane potential of a single pancreatic β-cell superfused with the KRB solution containing 2.8 mM glucose, recorded by the perforated whole-cell current clamp technique is shown in Figure 1. Initially in formation of the whole-cell current clamp, the membrane potential was − 70 mV (control) at 2.8 mM glucose. The membrane potential was stable as long as the 2.8 mM glucose superfusion continued and this gradually depolarized approximately 2 min after the start of exposure to 100 μM cibenzoline, and eventually evoked action potentials. The membrane potential was then partially hyperpolarized after washout of the agent with the KRB solution containing 2.8 mM glucose but depolarization was sustained for further 30 min without returning to the control level. We observed cibenzoline-induced depolarization and poor reversibility on washout in all of 16 cells at concentrations exceeding 30 μM.

We then performed cell-attached experiments to record the K_{ATP} channel current and explored the effect of cibenzoline on channel activity (Figure 2). In the presence of 2.8 mM glucose, we observed openings of a K^+ channel which had a unit amplitude of 4.3 pA (Figure 2, upper trace). The single channel conductance was estimated to be 61 pS, assuming the resting membrane potential to be − 70 mV and intracellular potassium concentration to be 140 mM.

The channel openings were characterized by rapid open-close transitions separated with long closed intervals. These characteristics agreed well with those of the ATP-sensitive K^+ channel previously described in pancreatic β-cells (Cook & Hales, 1984; Ashcroft *et al.*, 1984; Rorsman & Trube, 1985).

The K_{ATP} channel was substantially inhibited by 100 μM cibenzoline within 3 min of exposure of the cell to this agent, following which biphasic deflections of the base-line current were observed (Figure 2, middle trace). It has been suggested that this phenomenon is caused by the action potential of the β-cell (Ashcroft *et al.*, 1984; Rorsman & Trube, 1985). Thus

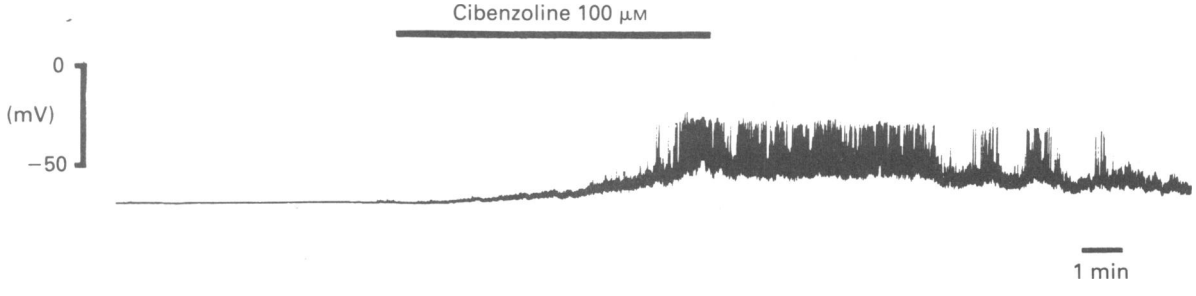

Figure 1 The effect of 100 μM cibenzoline on the membrane potential of a single pancreatic β-cell. The cell was superfused with the KRB solution containing the agent during the period indicated by the bar above the trace. The perforated current clamp technique was used. The glucose concentration in the KRB solution was maintained at 2.8 mM throughout this experiment.

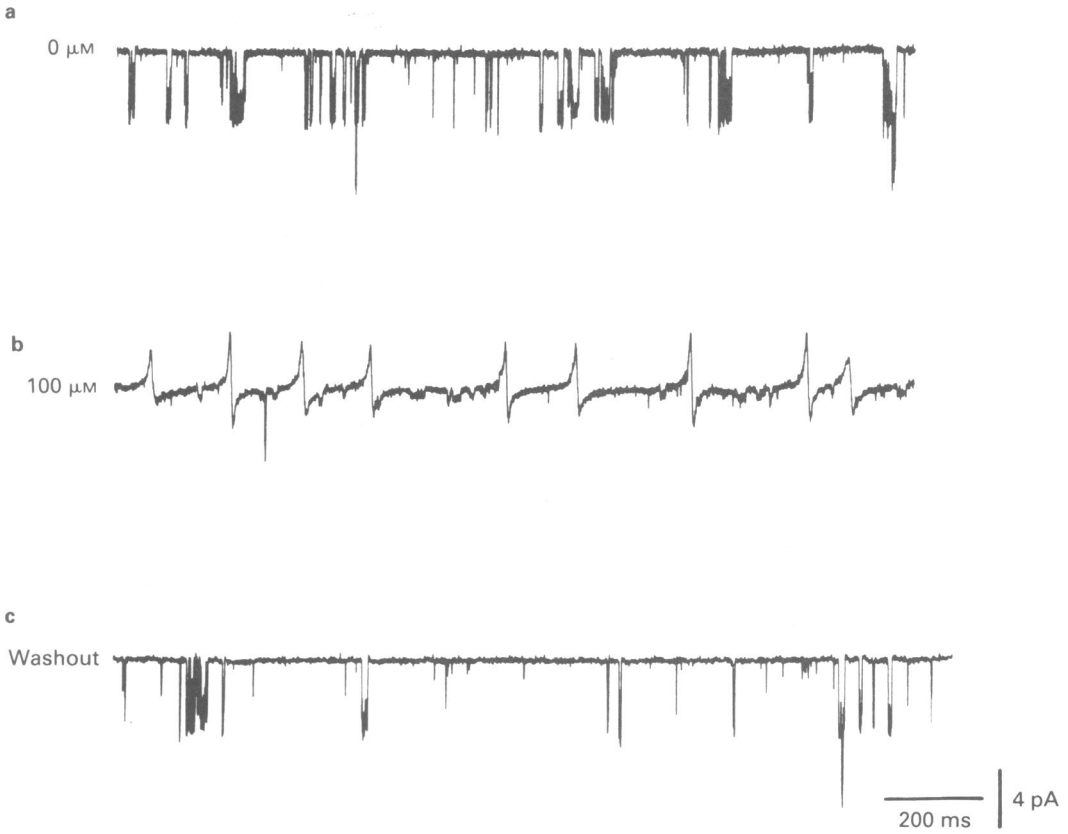

Figure 2 Effects on single K_{ATP} channel currents of 100 μM cibenzoline in the cell-attached membrane patch. The cell was superfused with 2.8 mM glucose containing KRB solution with 5 mM K^+ and the pipette solution contained 140 mM K^+. Since the pipette potential was 0 mV, the K_{ATP} channel current is driven by the resting membrane potential (− 70 mV), resulting in inward current as downward deflections of channel openings (a). Cibenzoline was contained in the bathing solution outside the patch pipette and as a consequence of the K_{ATP} channel inhibition, biphasic deflections of the baseline current level due to the action potentials of β-cells were seen (b). The lower trace (c) shows the K_{ATP} channel current recorded 15 min after washout. The current was low-pass filtered at 800 Hz and plotted on the chart recorder.

we conclude that cibenzoline induces action currents by depolarizing the membrane following inhibition of the K_{ATP} channel. During this action current, openings of a channel with a small amplitude were observed. We did not determine if this channel activity represents openings of the K_{ATP} channel with a decreased driving force due to membrane depolarization or is that of voltage-dependent potassium channels, as reported by Rorsman & Trube (1986). Mean patch current of the K_{ATP} channel was 0.12 pA in control and partially recovered to 0.06 pA after the inhibition by washing out cibenzoline in the cell-attached membrane patch (Figure 2, lower trace). We observed the reversible inhibition of channel activity in 4 out of 7 membrane patches. In other patches the channel activity did not reverse from the inhibition by cibenzoline in the concentration-range between 10 and 100 μM on washout.

The effect of cibenzoline on the membrane current was examined in the whole-cell clamp experiment. After disrupting the patch membrane at the pipette tip, a progressive increase in the time-independent outward current in response to 10 mV depolarization from the holding potential of − 70 mV was observed and the current level reached a steady state in 10 min (control in Figure 3a). This time-independent whole-cell current seemed to reflect activation of the K_{ATP} channel because of depletion of cytosolic ATP with the pipette solution containing less ATP (Trube *et al.*, 1986). This is supported by the fact that subsequent application of 0.1 mM tolbutamide reversibly decreased the current. We used a pipette solution with both 0.1 mM ATP and 0.1 mM ADP because the sensitivity of the K_{ATP} channel to ATP is decreased in the presence of ADP (Kakei *et al.*, 1986). Since 0.1 mM tolbutamide inhibits the K_{ATP} channel by approximately 90% of control (Trube *et al.*, 1986), the difference in current before and during the exposure to tolbutamide should be due to the current through the K_{ATP} channels.

In Figure 3a, the membrane current recorded at indicated voltage before, during and after an application of 100 μM tolbutamide (upper trace) and 30 μM cibenzoline (lower trace) are shown. Application of 100 μM tolbutamide reversibly depressed the membrane current as illustrated in the upper left panel in Figure 3a. Subsequent exposure of 30 μM cibenzoline inhibited the K_{ATP} channel current at both − 60 mV and + 30 mV. Cibenzoline inhibited the initial current level at the beginning of the depolarized pulse. The time-dependent outward current remained during the superfusion of cibenzoline (Figure 3a, right panel). In the present study, we did not examine the effects of cibenzoline on this time-dependent outward current.

Since the K_{ATP} channel current was poorly dependent on the membrane potential (Figure 3a, control), current-voltage relationships between initial current and each voltage applied were plotted in Figure 3b. The membrane conductance measured at − 70 mV was 2.7 nS at 100 μM tolbutamide and 1.7 nS at 30 μM cibenzoline in contrast to 28 nS in control. Both current-voltage relations obtained in the presence of tolbutamide or cibenzoline were superimposable at all the potentials tested and they showed linear relationship against the membrane potential. Thus we suggest that the channel block by cibenzoline or tolbutamide is not dependent on membrane voltage. In the whole-cell clamp experiments, the inhibition of the K_{ATP} channel current by cibenzoline was not reversible ($n = 12$) when tested at concentrations greater than 1 μM.

In Figure 4, the relative whole-cell current decreased by

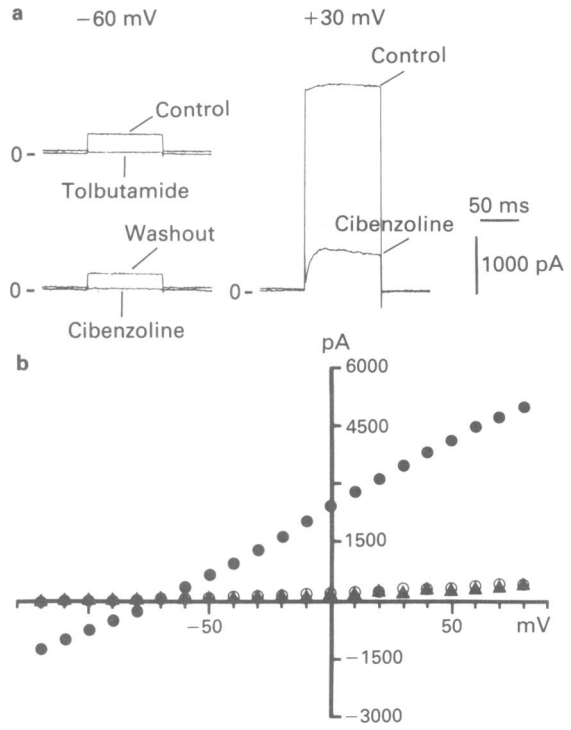

Figure 3 Effect of 30 μM cibenzoline on the membrane current and current-voltage relationship in whole-cell clamp experiments. The membrane was voltage-clamped at the holding potential of − 70 mV and depolarized to various potentials with a pulse duration of 100 ms every 5 s. The pipette solution contained 0.1 mM ATP and 0.1 mM ADP. (a) The membrane currents produced by a depolarized pulse to − 60 mV (left panel) and + 30 mV (right panel) before (Control), during (Tolbutamide) and after (Washout) exposure to 100 μM tolbutamide are illustrated. Cibenzoline 30 μM was then superfused (Cibenzoline). (b) Current-voltage relationships between initial current and membrane potential in the absence (control; ●) and in the presence of either 100 μM tolbutamide (○) or 30 μM cibenzoline (▲) are plotted.

cibenzoline is plotted as a function of its concentration between 1 and 100 μM. The mean membrane capacitance of single β-cells was 6.7 ± 0.6 pF ($n = 9$) and the input conductance, when measured from the current amplitude produced by the 10 mV depolarization pulses in the presence of 0.1 mM ATP and 0.1 mM ADP, was 2400 ± 430 pS/pF ($n = 9$). Input membrane conductance was measured after reaching a steady state level during the superfusion of various concentrations of cibenzoline (usually 1, 3, 10, 30 and 100 μM as a sequence) and the normalized conductance was plotted in Figure 4.

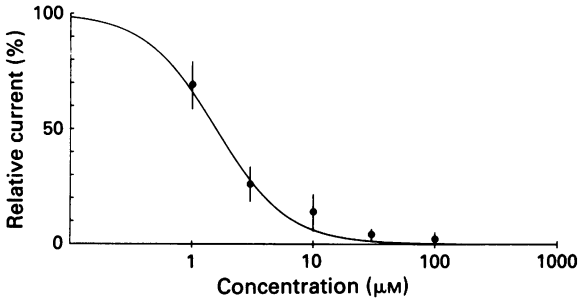

Figure 4 Dose-response relations of cibenzoline on the whole-cell K_{ATP} channel current produced by a reduction of cytosolic ATP concentration. The whole-cell membrane current was monitored during application of 10 mV depolarized pulses with 100 ms duration from the holding potential of − 70 mV every 5 s. The pipette solution containing 0.1 mM ATP and 0.1 mM ADP was used to evoke the K_{ATP} channel current. The membrane conductance between − 70 mV and − 60 mV was measured in control and test solutions containing various concentrations of cibenzoline. The conductance measured during exposure of cibenzoline was normalized with respect to the fraction of tolbutamide-sensitive conductance determined by difference between the conductance in control and that on superfusing 100 μM tolbutamide at the beginning of experiments. The data at required concentration of cibenzoline were collected from the separate membrane patches ($n = 12$) and then plotted as a function of the concentration of cibenzoline. Vertical bars represent the mean ± s.e.mean. The curve was drawn according to the Hill equation with IC_{50} of 1.5 μM and the Hill coefficient of 1.5.

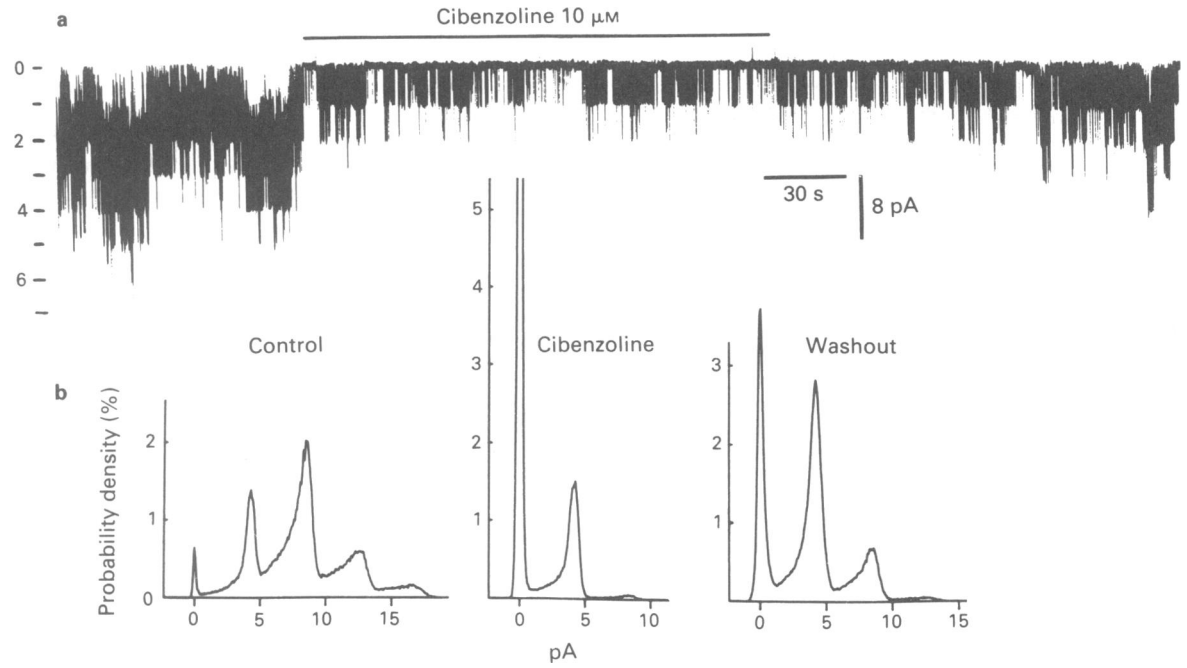

Figure 5 The effect of cibenzoline on the K_{ATP} channel in the inside-out patch. The K^+ concentration of both internal and pipette solutions was 140 mM and the patch membrane potential was held at − 60 mV. The bathing solution contained 0.3 mM ATP and 0.1 mM ADP. (a) Single K_{ATP} channel current was recorded in an inside-out membrane patch in the presence and absence of 10 μM cibenzoline. The number beside the current trace indicates the open channel level. (b) The amplitude histograms corresponding to that in the control (left), during cibenzoline exposure (middle) and after washout of the agent (right). The ordinate scale expresses the percentage of total sampled points and the abscissa scale the bin size in units of 0.04 pA. The data were low-pass filtered at 1 kHz and sampled at 5 kHz.

Cibenzoline inhibited the K_{ATP} channel in a dose-dependent manner. The curve was drawn by fitting the data to the equation (see Methods) with an IC_{50} of 1.5 μM and a Hill coefficient of 1.5 by using a least squares method.

Cibenzoline was also an effective inhibitor of K_{ATP} channel activity when exposed to the channels via the inside-out membrane configuration. In Figure 5a, the chart recording of the opening of K_{ATP} channels is illustrated, where at least 7 channels were present in this membrane patch and 10 μM cibenzoline inhibited these channels. The inhibitory effect of cibenzoline was partially reversible as described later at a concentration of 10 μM but irreversible at 100 μM ($n = 5$) for at least 30 min superfusion with washout solution. Amplitude histograms constructed by continuous sampling for more than 30 s before, during and after applying 10 μM cibenzoline are shown (Figure 5b). The single channel amplitude was unaffected and was 4.3 pA in the presence and absence of cibenzoline. The conductance of the single K_{ATP} channel was also unchanged by cibenzoline (not shown). The mean patch current was 8.1 pA, 1.0 pA and 3.6 pA before, during and after exposure of cibenzoline, respectively. Consequently the open probability of the K_{ATP} channel was 0.27 in control, assuming the total number of the channels to be 7. Cibenzoline decreased the mean patch current to 12% of control and this was in good agreement with that found in the whole-cell clamp experiment with respect to the channel inhibition by the agent (Figure 4). It can be suggested that cibenzoline decreased the probability of the channel being open or that this agent decreased the number of the channels in the membrane patch. In the presence of cibenzoline the number of openings of overlapped K_{ATP} channels was 2 (the middle histogram in Figure 5b). When we attempted to fit the probability that a given number of channels were open by using binominal analysis, we found that channel inhibition by cibenzoline could be due to both a reduction of the number of channels and open probability or due to an equal decrease in open probability without a change in the number of channels.

Discussion

In the present paper we have demonstrated that the ATP-sensitive K^+ channels in pancreatic β-cells are inhibited by the antiarrhythmic agent, cibenzoline. It is likely that such inhibition of the K_{ATP} channel results in depolarization of the β-cell membrane and evokes an increase in the cytosolic Ca^{2+} concentration followed by stimulation of insulin secretion even at subthreshold glucose levels. We suggest that this effect is, in part, a cause of hypoglycaemia with hyperinsulinemia induced by this agent (Houdent *et al.*, 1991; Bertrand *et al.*, 1992). Bertrand *et al.* (1992) demonstrated that cibenzoline directly stimulates insulin secretion from an isolated pancreas. They showed that cibenzoline at 2 μM did not increase insulin release when the glucose concentration was 4.2 mM but 6 μM cibenzoline did. Since the concentrations they used were located on the steepest part of the dose-inhibition curve of the K_{ATP} channel for cibenzoline (Figure 4), these concentrations seem to be critical for the stimulation of insulin secretion.

Since cibenzoline is used clinically as an antiarrhythmic drug, it certainly affects ionic channels other than the K_{ATP} channel. In cardiac myocytes, the voltage-dependent Ca^{2+} channel has been reported to be inhibited by cibenzoline (Holck & Osterrieder, 1986; Matsuoka *et al.*, 1991). The 50% inhibition for the peak Ca^{2+} currents was 14 μM (Holck & Osterrieder, 1986) or 30 μM (Matsuoka *et al.*, 1991). These values are greater than that found (1.5 μM) in the present study.

Cibenzoline blocked the K_{ATP} channel from both sides of the membrane and can reach its site of action in the channel even when applied via the bathing solution in the cell-attached mode without direct exposure to the patch membrane. These observations suggest that cibenzoline can diffuse within the lipid membrane. These actions of the agent resemble those of tolbutamide which inhibits the K_{ATP} channel from both the outside and inside of the β-cell membrane (Trube *et al.*, 1986). We observed poor reversibility of the channel inhibition by cibenzoline over 1 μM in whole-cell voltage clamp experiments. Bertrand *et al.* (1992) demonstrated a sustained increase in insulin release by 6 μM cibenzoline during washout of the agent. We speculate that cibenzoline may be retained in the lipid membrane and may not diffuse out of the membrane. The ability of cibenzoline to block the channel was also similar to that of tolbutamide in that 50% inhibition occurred at 6–10 μM (Trube *et al.*, 1986).

It has been reported that cibenzoline-induced hypoglycaemia is associated with hyperinsulinemia (Lefort *et al.*, 1988; Houdent *et al.*, 1991). In such cases, cibenzoline may potentiate insulin secretion in response to the plasma glucose level and induce inappropriate secretion of insulin even at non-stimulatory concentrations of plasma glucose. In clinical use, it is known that the plasma cibenzoline concentration in normal man can reach a maximum of approximately 1.0 μM (Massarella *et al.*, 1986). This level may be exceeded when renal function is reduced as the kidney is the major organ by which this agent is excreted (Aronoff *et al.*, 1991). However, we do not exclude the possibility that the extra-islet actions of cibenzoline, such as enhanced glucose utilization, may cause cibenzoline-induced hypoglycaemia with normal insulin levels as has been reported elsewhere (Hilleman *et al.*, 1987; Gachot *et al.*, 1988; Jeandel *et al.*, 1988; Houdent *et al.*, 1991).

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