Inhibition of ATP-Sensitive K⁺ Channels and L-Type Ca²⁺ Channels by Amiodarone Elicits Contradictory Effect on Insulin Secretion in MIN6 Cells

Atsushi Nishida¹,†, Taichi Takizawa¹,†, Akio Matsumoto¹, Takashi Miki², Susumu Seino³, and Haruaki Nakaya¹,*

¹Department of Pharmacology and ²Department of Medical Physiology, Chiba University Graduate School of Medicine, Chiba 260-8670, Japan
³Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan

Received November 18, 2010; Accepted March 10, 2011

Abstract. Some class I antiarrhythmic drugs induce a sporadic hypoglycemia by producing insulin secretion via inhibition of ATP-sensitive K⁺ (K_{ATP}) channels of pancreatic β-cells. It remains undetermined whether amiodarone produces insulin secretion by inhibiting K_{ATP} channels. In this study, effects of amiodarone on K_{ATP} channels, L-type Ca²⁺ channel, membrane potential, and insulin secretion were examined and compared with those of quinidine in a β-cell line (MIN6). Amiodarone as well as quinidine inhibited the openings of the K_{ATP} channel in a concentration-dependent manner without affecting its unitary amplitude in inside-out membrane patches of single MIN6 cells, and the IC₅₀ values were 0.24 and 4.9 μM, respectively. The L-type Ca²⁺ current was also inhibited by amiodarone as well as quinidine in a concentration-dependent manner. Although glibenclamide (0.1 μM) or quinidine (10 μM) significantly potentiated the insulin secretion from MIN6 cells, amiodarone (1 – 30 μM) failed to increase insulin secretion. Amiodarone (30 μM) and nifedipine (10 μM) significantly inhibited the increase in insulin secretion produced by 0.1 μM glibenclamide. Amiodarone (30 μM) produced a gradual decrease of the membrane potential, but did not produce repetitive electrical activity in MIN6 cells. Glibenclamide (1 μM) produced a slow depolarization, followed by spiking activity which was inhibited by 30 μM amiodarone. Thus, amiodarone is unlikely to produce hypoglycemia in spite of potent inhibitory action on K_{ATP} channels in insulin-secreting cells, possibly due to its Ca²⁺ channel–blocking action.

Keywords: ATP-sensitive K⁺ channel, amiodarone, quinidine, insulin, β-cell

Introduction

It has been reported that antiarrhythmic drugs induce a sporadic hypoglycemia by producing insulin secretion via inhibition of ATP-sensitive K⁺ (K_{ATP}) channels of pancreatic β-cells. It remains undetermined whether amiodarone produces insulin secretion by inhibiting K_{ATP} channels. In this study, effects of amiodarone on K_{ATP} channels, L-type Ca²⁺ channel, membrane potential, and insulin secretion were examined and compared with those of quinidine in a β-cell line (MIN6). Amiodarone as well as quinidine inhibited the openings of the K_{ATP} channel in a concentration-dependent manner without affecting its unitary amplitude in inside-out membrane patches of single MIN6 cells, and the IC₅₀ values were 0.24 and 4.9 μM, respectively. The L-type Ca²⁺ current was also inhibited by amiodarone as well as quinidine in a concentration-dependent manner. Although glibenclamide (0.1 μM) or quinidine (10 μM) significantly potentiated the insulin secretion from MIN6 cells, amiodarone (1 – 30 μM) failed to increase insulin secretion. Amiodarone (30 μM) and nifedipine (10 μM) significantly inhibited the increase in insulin secretion produced by 0.1 μM glibenclamide. Amiodarone (30 μM) produced a gradual decrease of the membrane potential, but did not produce repetitive electrical activity in MIN6 cells. Glibenclamide (1 μM) produced a slow depolarization, followed by spiking activity which was inhibited by 30 μM amiodarone. Thus, amiodarone is unlikely to produce hypoglycemia in spite of potent inhibitory action on K_{ATP} channels in insulin-secreting cells, possibly due to its Ca²⁺ channel–blocking action.

Keywords: ATP-sensitive K⁺ channel, amiodarone, quinidine, insulin, β-cell
(13) and rat (14) by using patch clamp techniques. If amiodarone blocks $K_{ATP}$ channels in pancreatic $\beta$ cells as well as in cardiac cells, the drug may cause hypoglycemia in patients. As far as we know, however, an amiodarone-induced hypoglycemia has not been reported. Therefore, this study was undertaken to examine the effect of amiodarone on the $K_{ATP}$-channel activity in insulin-secreting cells (MIN6 cells) using patch clamp techniques in comparison with quinidine. In addition, we also evaluated the effects of amiodarone on the insulin secretion and membrane potentials in the cell line.

Materials and Methods

Cell culture

MIN6 cells, a pancreatic $\beta$-cell line showing glucose-inducible insulin secretion (15, 16), were cultured in a Dulbecco’s modified Eagle’s medium (Sigma Chemical Co., St. Louis, MO, USA) containing 25 mM glucose, 10% fetal bovine serum (Hyclone Laboratories, Logan, UT, USA), penicillin G at 100 U/mL, and streptomycin at 100 $\mu$g/mL in a humidified atmosphere of 5% CO$_2$ – 95% air at 37°C.

Patch clamp study

Single channel current recordings were performed by the inside-out mode of the patch clamp method at room temperature (20°C – 25°C). A cover glass with cultured MIN6 cells was placed in a recording chamber (1-mL volume) attached to an inverted microscope (Olympus IMT-2; Olympus, Tokyo) and superfused with HEPES-Tyrode solution at a rate of 3 mL/min. The composition of the normal HEPES-Tyrode solution was as follows: 143 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl$_2$, 0.5 mM MgCl$_2$, 0.33 mM NaH$_2$PO$_4$, 5.5 mM glucose, and 5.0 mM HEPES, and was adjusted to pH 7.4 with KOH. The pipette solution used for the measurement of the L-type Ca$^{2+}$ current ($I_{Ca.L}$). The external solution was composed of 143 mM NaCl, 5.4 mM CsCl, 1.8 mM CaCl$_2$, 0.5 mM MgCl$_2$, 0.33 mM NaH$_2$PO$_4$, 15 mM glucose, and 5 mM HEPES and was adjusted to pH 7.4 with KOH. The pipette solution used for the measurement of $I_{Ca.L}$ contained 110 mM CsOH, 110 mM l-aspartate, 1 mM MgCl$_2$, 20 mM CsCl, 5 mM ATP-K$_2$, 10 mM EGTA, and 5 mM HEPES, and was adjusted to pH 7.4. The patch pipettes, made from glass capillaries with a diameter of 1.5 mm using a vertical microelectrode puller, were filled with the internal solution, and their resistance was 5 – 10 M$\Omega$. After the G$\Omega$ seal between the tip of the electrode and the cell membrane was established, the membrane patch was disrupted by more negative pressure for the internal perfusion with the cesium-containing pipette solution. The electrode was connected to the CEZ-2300 patch clamp amplifier. Recordings were filtered at 1-kHz bandwidth and series resistance was compensated. Command pulse signals were generated by a digital to analog converter controlled by pCLAMP software (Axon instruments, Inc.). A liquid junction potential between the internal solution and the bath solution was corrected. The capacitance of the membrane was calculated from the steady-state current in response to a ramp pulse (–5 mV/2.5 ms) from 0 mV.

Current-clamp experiments using MIN6 cells were performed in the whole-cell recording mode at room temperature. The nystatin-perforated whole-cell current clamp mode was used to record the membrane potential. The composition of the external solution was 143 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl$_2$, 0.5 mM MgCl$_2$, 0.33 mM NaH$_2$PO$_4$, 15 mM glucose, and 5.0 mM HEPES-NaOH buffer (pH 7.4). The composition of the pipette solution was 110 mM KOH, 110 mM l-aspartate, 20 mM KCl, 1 mM MgCl$_2$, 0.5 mM EGTA, and 10 mM HEPES-KOH (pH 7.2). Nystatin was dissolved in methanol at a...
concentration of 10 mg/ml and added to the pipette solution in a concentration of 300 – 500 μg/ml just prior to the experiments. The resistance of the patch pipette filled with these pipette solutions was 2 – 3 MΩ. After a stabilization of the membrane potentials, effects of various drugs on the membrane potential of MIN6 cells were evaluated.

Measurement of insulin secretion
MIN6 cells were seeded in 48-well plates at an initial density of 5 × 10⁴ cells/well with 0.5 mL medium/well and were cultured at 37°C. Five days later, the cells were washed three times with Krebs-Ringer bicarbonate (KRB) buffer medium containing 0.2% bovine serum albumin (BSA). The composition of KRB medium was as follows: 154 mM NaCl, 6.2 mM KCl, 3.3 mM CaCl₂, 1.6 mM MgSO₄, 1.5 mM KH₂PO₄, 12.4 mM NaHCO₃, and 20 mM HEPES-NaOH (pH 7.4). The cells were then preincubated for 30 min in KRB-BSA containing 3.3 mM glucose and incubated for 30 min in KRB-BSA containing various concentrations of glucose or drugs at 37°C. After each incubation, 0.4 mL of the medium was withdrawn, centrifuged at 15000 rpm for 2 min at 4°C, and 0.3 mL of the medium was withdrawn and stored at −80°C.

Immunoreactive insulin (IRI) was measured by a double-antibody radioimmunoassay using human insulin as standard.

Drugs
The following drugs were used: amiodarone (Sigma Chemical), quinidine (Wako, Osaka), and glibenclamide (Sigma Chemical). Amiodarone was dissolved in absolute ethanol at a concentration of 10 mM and then added to the bath solution containing bovine serum albumin (0.03% – 1.0%), as described by Honjo et al. (18). It was confirmed that the solvent of amiodarone did not affect the Kₐ₅₆ₐ₆- and L-type Ca²⁺-channel current. Glibenclamide was dissolved in dimethylsulfoxide at a concentration of 100 mM. Quinidine was dissolved in distilled water.

Statistics
All values are presented in terms of the mean ± S.E.M. Intergroup comparisons were performed using one-way analysis of variance followed by the Bonferroni method if analysis of variance was significant. P < 0.05 was considered statistically significant.

Results
Effects of amiodarone on the Kₐ₅₆ₐ₆-channel current in MIN6 cells
As shown in Fig. 1, a single channel current was recorded from an inside-out membrane patch of a MIN6 cell at various clamp potentials. The slope conductance of the unitary current from the current–voltage relationship was 85 ± 2 pS (n = 3) and the current was inhibited by the application of 1 mM ATP to the internal solution. These findings implied that the unitary current was the single Kₐ₅₆ₐ₆-channel current.

Figure 2A illustrates effects of quinidine and amiodarone on the single Kₐ₅₆ₐ₆-channel current recorded from an inside-out membrane patch. A single channel current was recorded at a holding potential of −40 mV with an internal solution containing a trace amount of ATP. UDP

![Fig. 1. Single channel current recordings in the inside-out mode from a MIN6 cell. Unitary K⁺ currents recorded in a membrane patch held at various voltage levels are shown in the left upper panel. Inward currents are shown as downward deflection. Current–voltage relationship for the single channel current is shown in the right panel. Linear slope conductance was 84.6 pS. Effects of ATP (1 mM) on the unitary K⁺-channel current recorded from an inside-out patch held at −40 mV with an internal solution of 1 μM ATP are shown in the left lower panel. The K⁺ current was completely inhibited by the application of 1 mM ATP to the internal solution.](image-url)
(1 mM) was added to the solution to stabilize the channel activity. Quinidine and amiodarone inhibited the openings of the K<sub>ATP</sub> channel without affecting the amplitude of unitary current. Figure 2B shows relative channels activities in the presence of various concentrations of the drugs. Quinidine and amiodarone inhibited the K<sub>ATP</sub>-channel current in a concentration-dependent manner, and the IC<sub>50</sub> values were 4.9 and 0.24 μM, respectively.

**Influence of amiodarone on insulin secretion in MIN6 cells**

Application of 25 mM glucose and 0.1 μM glibenclamide increased the insulin secretion by 132 ± 21% (n = 12, *P* < 0.05) and 151 ± 25% of control (n = 8, *P* < 0.05, Fig. 3), respectively. In concentrations higher than 10 μM, quinidine significantly increased the insulin secretion, as shown in Fig. 3. After the application of 10, 30, and 100 μM quinidine, insulin secretion was increased by 75 ± 22%, 105 ± 23%, and 159 ± 15% (n = 8, *P* < 0.05) of the control, respectively. However, amiodarone at concentrations of 1 – 30 μM, which inhibited the K<sub>ATP</sub>-channel current in the inside-out patch, did not increase insulin secretion. Furthermore, we evaluated the effect of amiodarone on the increase in insulin secretion by glibenclamide. As shown in Fig. 4, amiodarone (30 μM) and nifedipine (10 μM), a Ca<sup>2+</sup>-channel blocker, significantly inhibited the increase in insulin secretion by 0.1 μM glibenclamide. Glibenclamide per se increased the insulin secretion to 240 ± 29% of the control, and addition of amiodarone and nifedipine decreased it to 145 ± 28% (n = 8, *P* < 0.05) and 86 ± 10% (n = 8, *P* < 0.05) of the control. Values are expressed as the mean ± S.E.M. of 8 experiments.

---

**Fig. 2.** Effects of amiodarone and quinidine on K<sub>ATP</sub> channels in MIN6 cells. A: Representative tracing demonstrating effects of quinidine (100 μM, upper panel) and amiodarone (AMI, 3 μM, lower panel) on the unitary current of the K<sub>ATP</sub> channels in MIN6 cells. UDP (1 mM) was added to stabilize the channel activity. The current was recorded from an inside-out patch held at −40 mV with an internal solution of 1 μM ATP. Note that both quinidine and amiodarone inhibited the opening of the K<sub>ATP</sub> channels. B: Effects of quinidine (closed circle) and amiodarone (open circle) on relative open probability (NP/NPc) of the inward K<sub>ATP</sub>-channel current in the inside-out patch. Values are expressed as the mean ± S.E.M. of 2 – 8 experiments.

**Fig. 3.** Effects of amiodarone, quinidine, high glucose, and glibenclamide on insulin release from MIN6 cells. Left panel: Effects of quinidine (closed circle) and amiodarone (open circle) on insulin release from MIN6 cells. Right panel: Effects of high glucose (25 mM) and glibenclamide (GLIB, 0.1 μM) on insulin release from MIN6 cells. Data are expressed as a percentage of the insulin release in KRB containing a non-stimulating (3.3 mM) concentration of glucose. Values are expressed as the mean ± S.E.M. of 8 experiments. *P* < 0.05 vs. control.

**Fig. 4.** Influence of amiodarone (AMI, 30 μM) and nifedipine (NIF, 10 μM) on the glibenclamide (GLIB, 0.1 μM)-induced insulin release from MIN6 cells. Data are expressed as a percentage of the insulin release in KRB containing a non-stimulating (3.3 mM) concentration of glucose. Values are expressed as the mean ± S.E.M. of 8 experiments. *P* < 0.05 vs. glibenclamide (0.1 μM) alone.
Effects of amiodarone on the L-type Ca\(^{2+}\) current in MIN6 cells

Effects of amiodarone on the L-type Ca\(^{2+}\) current \((I_{Ca,L})\) were examined and compared with those of quinidine in MIN6 cells. The \(I_{Ca,L}\) was isolated from other membrane currents by using Cs-rich solutions and membrane currents were elicited by 300-ms step pulses to various potentials from a holding potential of \(-40\) mV at 0.1 Hz. Representative current traces after amiodarone and nifedipine and the summarized data of current–voltage relations in control conditions are shown in Fig. 5. Amiodarone at a concentration of 1 \(\mu\)M decreased \(I_{Ca,L}\) and application of 5 \(\mu\)M nifedipine further decreased the current elicited by depolarizing test pulses to 0 mV from a holding potential of \(-40\) mV. We designated the nifedipine-sensitive current as \(I_{Ca,L}\) and the effects of various concentrations of amiodarone and quinidine on \(I_{Ca,L}\) were evaluated. Amiodarone at a concentration of 1, 3, 10, and 30 \(\mu\)M decreased the amplitude of the \(I_{Ca,L}\) at 0 mV by 48.6 ± 6.3\%, 66.9 ± 5.9\%, 83.1 ± 5.9\%, and 97.3 ± 0.5\% (n = 5), respectively. Quinidine at a concentration of 10, 30, and 100 \(\mu\)M decreased the amplitude at the \(I_{Ca,L}\) at 0 mV by 12.2 ± 5.5\%, 46.3 ± 7.6\%, and 84.2 ± 3.7\% (n = 5), respectively (Fig. 5).

Influence of amiodarone on the membrane potential of MIN6 cells

At a glucose concentration of 15 mM, the MIN6 cell was electrically silent with a resting membrane potential of \(-40\) to \(-70\) mV. As shown in Fig. 6, amiodarone (30 \(\mu\)M) produced a gradual depolarization of the resting membrane, but did not produce electrical repetitive firing as bursts. Similar results were obtained from other 3 cells. Glibenclamide (1 \(\mu\)M) produced a slow depolarization, followed by burst firing, which was inhibited by 30 \(\mu\)M amiodarone (n = 5). It was confirmed that nifedipine (5 \(\mu\)M) also inhibited the oscillatory membrane depolarizations elicited by glibenclamide (1 \(\mu\)M) in MIN6 cells (n = 4). In contrast with amiodarone, quinidine at a concentration of 100 \(\mu\)M produced a gradual depolarization of the resting membrane, which was followed by repetitive firings (Fig. 6). Similar results were obtained in 4 MIN6 cells. Quinidine (100 \(\mu\)M) failed to block the electrical repetitive firing as bursts that were induced by glibenclamide (1 \(\mu\)M) in 4 MIN6 cells.

Discussion

Amiodarone has several electropharmacological actions such as class I, II, III, and IV action (7, 19 – 23). In terms of K\(^+\) channel–blocking action, amiodarone is reported to inhibit the delayed rectifier K\(^+\) channel, inward rectifier K\(^+\) channel, Na\(^+\)-activated K\(^+\) channel, and acetylcholine-sensitive muscarinic K\(^+\) channel (8 – 11). In addition, previous reports from our laboratory and others have indicated that amiodarone inhibits sarcolemmal K\(_{ATP}\) channels in cardiomyocytes (13, 14). In the present study, we have demonstrated for the first time that amiodarone can also inhibit the K\(_{ATP}\)-channel current in insulin-secreting cells.

Some of class I antiarrhythmic drugs were reported to block the K\(_{ATP}\) channels (3, 4, 24 – 26). It is well-known that class Ia antiarrhythmic drugs such as quinidine, disopyramide, and cibenzoline induce a hypoglycemia (1, 2, 27, 28). Several studies have indicated that these drugs increase insulin secretion from pancreatic \(\beta\)-cells by inhibiting K\(_{ATP}\) channels in a manner similar to sulfonylurea antidiabetic drugs (3, 4, 25). Consistent with previous reports, quinidine inhibited K\(_{ATP}\) channels and increased insulin secretion in MIN6 cells in this study.

Amiodarone also inhibited the K\(_{ATP}\)-channel current in a concentration-dependent manner. However, amio-

![Fig. 5. Effects of amiodarone and quinidine on the L-type Ca\(^{2+}\) current \((I_{Ca,L})\) in MIN6 cells. A: Actual current traces elicited by 300-ms depolarizing pulses from a holding potential of \(-40\) mV to 0 mV in the control condition and after addition of amiodarone (1 \(\mu\)M) and nifedipine (5 \(\mu\)M). B: The I–V relationship of the L-type Ca\(^{2+}\) current recorded from MIN6 cells under control conditions. Data represent the mean ± S.E.M. of 16 cells. C: Concentration–response curves for the inhibitory effects of amiodarone and quinidine on the nifedipine-sensitive currents elicited by 300-ms depolarizing pulses to 0 mV from a holding potential of \(-40\) mV. Each point represents the mean ± S.E.M. of 5 cells.](image-url)
darone at concentrations of 1 – 30 μM did not increase insulin secretion. The effective concentration to inhibit the K<sub>ATP</sub>-channel current was similar to or less than those needed to inhibit the other cardiac ion channels (7 – 11, 19 – 23). A therapeutic plasma concentration of amiodarone is considered to be a range between 0.8 and 3.1 μM (29). However, it has not been reported that amiodarone induces a hypoglycemia, as far as we are aware. Why does amiodarone not increase insulin secretion or induce a hypoglycemia even though it inhibits the K<sub>ATP</sub> channels? The reason is not immediately apparent but several possibilities may be considered.

In this study, high glucose (25 mM) and glibenclamide (0.1 μM) markedly increased insulin secretion from MIN6 cells. Since the EC<sub>50</sub> value for the insulinotropic effect and the K<sub>a</sub> value of the high affinity binding site of glibenclamide are reported to be between 1 and 10 nM (30, 31), glibenclamide was considered to produce almost maximal insulin secretion at the concentration used in the MIN6 cells. It is well established that glucose- or sulfonylurea-stimulated insulin release is dependent on electrical activity (32 – 34). Glucose, which stimulates the rate of metabolism and increases the ATP:ADP ratio, or sulfonylurea drugs lead to the closure of K<sub>ATP</sub> channels and membrane depolarization. The membrane depolarization results in the activation of voltage-dependent Ca<sup>2+</sup> channels and the initiation of spiking electrical activity. The increased Ca<sup>2+</sup> influx leads to a rise in cytoplasmic Ca<sup>2+</sup> concentration, activation of calmodulin, and thereby insulin secretion (35). The rhythmic membrane oscillations are elicited by 2-step depolarization (36). First, the resting membrane gradually depolarizes and second, the spiking electrical activity occurs. The slow membrane depolarization was produced by the K<sub>ATP</sub>-channel blocker glibenclamide, whereas a burst of spiking electrical activity was inhibited by the Ca<sup>2+</sup> channel–blocker nifedipine in this study. Therefore, K<sub>ATP</sub>-channel and Ca<sup>2+</sup>-channel functions would be important for changes of membrane potential and insulin secretion.

In the present study, amiodarone and nifedipine inhibited the increase in the insulin secretion by glibenclamide. Furthermore, amiodarone and nifedipine inhibited the spiking electrical activity elicited by glibenclamide. One possible explanation for the failure of amiodarone to increase the insulin secretion might be ascribed to the effective blockade of the L-type Ca<sup>2+</sup> channel by the antiarrhythmic drug. The inhibitory effect of amiodarone on L-type Ca<sup>2+</sup> current was more potent than that of quinidine. In order to stop the spiking electrical activity in MIN6 cells, strong inhibition of the L-type Ca<sup>2+</sup> channel might be needed. The therapeutic plasma concentrations of amiodarone and quinidine are assumed to be less than 3.1 and 15.4 μM, respectively (29). Amiodarone at this concentration would inhibit the L-type Ca<sup>2+</sup> current and...
the $K_{\text{ATP}}$ current by about 70% and 100%, respectively. On the other hand, quinidine at this concentration would be expected to inhibit the L-type $Ca^{2+}$ current and the $K_{\text{ATP}}$ current by about 25% and 70%, respectively. Both amiodarone and quinidine showed a potency sufficient to block the $K_{\text{ATP}}$ current in the clinical concentrations. However, the potency of quinidine at its clinical concentrations might be insufficient to block the L-type $Ca^{2+}$ current. Indeed, quinidine even at a toxic concentration of 100 $\mu$M failed to stop the spiking electrical activity, although it reduced the frequency of the repetitive electrical activity in this study.

It is acknowledged that $K_{\text{ATP}}$ channels in insulin-secreting cells comprise inwardly rectifying $K^+$-channel member Kir6.2 subunits and sulfonylurea receptor (SUR1) subunits (37). Several studies were conducted to determine a molecular target for antiarrhythmic drugs of insulinotrophic action, such as cibenzoline and disopyramide (4, 38, 39). Radioligand binding studies have indicated that binding sites of these antiarrhythmic drugs are distinct from the glibenclamide-binding site in insulin-secreting cells (data not shown). Therefore, amiodarone might act on a site different from that of glibenclamide in $K_{\text{ATP}}$-current. Indeed, quinidine even at a toxic concentration of 100 $\mu$M failed to stop the spiking electrical activity, although it reduced the frequency of the repetitive electrical activity in this study.

Acknowledgments

The authors thank Mr. H. Maruyama and Ms. Y. Reien for their excellent technical assistance and Ms. I. Sakashita for her secretarial assistance. This work was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

1145.