Inhibition of G Protein-Coupled and ATP-Sensitive Potassium Currents by 2-Methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl)benzofuran (KB130015), an Amiodarone Derivative


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ABSTRACT

2-Methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl) benzofuran (KB130015; KB), a novel compound derived from amiodarone, has been proposed to have antiarrhythmic properties. Its effect on the G protein-coupled inward rectifying K⁺ current (IK(ACh) or IK(Ado)), ATP-sensitive K⁺ current (IK(ATP)), and background inward rectifying current (IK₁) were studied in guinea pig atrial and ventricular myocytes by the whole-cell voltage-clamp technique. Receptor-activated Ik(ACh/Ado) induced in atrial myocytes by the stimulation of either muscarinic or Ado receptors was concentration dependently (IC₅₀ value of 0.6–0.8 μM) inhibited by KB. Receptor-independent guanosine 5′-(3-thio)triphosphate-induced and background IK₁, which contributes to the resting conductance of atrial myocytes, were equally sensitive to KB (IC₅₀ value of ~0.9 μM). IK(ATP) induced in atrial myocytes during metabolic inhibition with 2,4-dinitrophenol (DNP) was also suppressed by KB, whereas IK₁ measured in ventricular myocytes was insensitive to the drug (KB ≤ 50 μM). Although being effective when applied from the outside, intracellular application of KB via the patch pipette affected neither IK(ACh) nor IK(ATP), 3,3′,5-triiodo-L-thyronin, which shares structural groups with KB, did not have an effect on the K⁺ currents. Consistent with the effects on single myocytes, KB did not depolarize the resting potential but antagonized the shortening of action potential duration by carbamylcholine-chloride or by DNP in multicellular preparations and antagonized the shortening of action potential duration by acetylcholine in single myocytes. It is concluded that KB inhibits IK(ACh) and IK(ATP) by direct drug-channel interaction at a site more easily accessible from extracellular side of the membrane.

Class III antiarrhythmic agents are effective drugs commonly used in ischemic heart disease. Suppression or prevention of arrhythmias is due to a prolongation of the action potential duration and of refractoriness. Amiodarone is considered as a class III drug and is known to be among the most effective drugs in the prevention of ventricular and supraventricular arrhythmias. Its action mechanisms at the cellular level include chronic effects, obtained after prolonged drug application, such as the suppression of the delayed repolarizing current IK of, and an interaction with the thyroid hormone signal transduction. The drug also has short-term or acute effects, including an inhibition of various ion currents (for review, see Kodama et al., 1999).

The clinical benefits of amiodarone are complicated by many undesirable side effects. To develop drugs with an improved pharmacological profile, new molecules based on its structural modification have been synthesized. 2-Methyl-3-(3,5-diido-4-carboxymethoxybenzyl)benzofuran; KB130015; KB) is one such novel compound that has been suggested to have antiarrhythmic properties and to inhibit the binding of T3 to nuclear receptors (Carlsson et al., 2002). Its relative effectiveness compared with amiodarone is not yet known. Given the structural similarity, some KB effects can be expected a priori. However, no prediction can be made about which of the amiodarone effects are conserved, or about their magnitude. As an example is the unexpected effect of KB on Na⁺ channel inactivation, which is

ABBREVIATIONS: T₃, 3,3′,5-triiodo-L-thyronine; Ado, adenosine; ACh, acetylcholine; CCh, carbamylcholine-chloride; APD, action potential duration; DNP, 2,4-dinitrophenol; I-V, current-voltage; GTPγS, guanosine 5′-O-(3-thio)triphosphate.

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nonexistent with amiodarone (Macianskiene et al., 2003b). Other structural analogs of amiodarone such as dronedarone (SR33589) have been shown to exert chronic and acute effects on ion channels similar to those of the parent agent amiodarone. The effects of KB on other K⁺ channels have not been investigated so far but could be important. Cardiac K⁺ currents that are acutely inhibited by amiodarone and its analog dronedarone include the background inward rectifying current (I_{K1}), the G protein-coupled inwardly rectifying currents [I_{KACH} or I_{KAdo}] and the ATP-sensitive current [I_{KATP}, see Kodama et al., 1997; Altomare et al., 2000; Guillemare et al., 2000]. In addition, although initial reports suggested that the fast component of the delayed rectifier, I_{K1}, is insensitive, a few recent studies indicate that it is inhibited by high concentrations of amiodarone (IC_{50} = 38 μM; Kamiya et al., 2001).

In the present study, we investigated the effects of KB on inward rectifying potassium currents in isolated guinea pig atrial and ventricular myocytes. I_{KACH} and I_{KATP} were measured in atrial cells. We found both currents to be markedly inhibited by KB, possibly via a direct drug-ion channel interaction. I_{K1} was studied in ventricular cells but was found to be insensitive to KB. In multicellular preparations, KB did not depolarize the resting membrane but antagonized action potential duration (APD) shortening by carbamylcholine-chloride (Cch) in atrial appendages, or by 2,4-dinitrophenol (DNP) in papillary muscles.

Materials and Methods

The experiments were done in guinea pig isolated atrial and ventricular myocytes or multicellular preparations. The study was performed in accordance with the Declaration of Helsinki and the institutional guidelines for the care and use of laboratory animals.

Isolation and Culture of Myocytes. Single atrial myocytes were isolated by a Langendorff perfusion method described previously (Banach et al., 1993). Atrial myocytes were kept in culture for up to 48 h at 37°C. The culture medium was the bicarbonate-buffered M199 (Invitrogen, Dreieich, Germany) containing gentamycin and kanamycin (each at 25 μg ml⁻¹; Sigma Chemie, Deisenhofen, Germany). The medium was not supplemented with fetal calf serum to minimize the loss of sensitivity to ACh or Ado (Bunnemann and Petz, 1995). Cells were plated at a density of several hundred per culture dish. About 50 to 70% of the cells attached within 16 to 24 h. No differences were found between freshly isolated and cultured myocytes in the effects studied here.

Multicellular Preparations. Left atrial appendages and left ventricular papillary muscles were dissected from guinea pig hearts. The preparations were pinned to the bottom of a tissue chamber and continuously superfused with the Tyrode’s solution equilibrated with 100% O₂. They were electrically stimulated with bipolar electrodes at 1 Hz with pulses of 2-ms duration and twice the diastolic threshold.

Solutions. For measurements in isolated myocytes the culture medium was replaced by a solution containing 120 mM NaCl, 20 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 10 mM HEPES-NaOH, pH 7.4. Pipettes were filled with a solution containing 110 mM K-aspartate, 20 mM KCl, 1 mM MgATP, 1 mM MgCl₂, 2 mM EGTA, 0.1 mM GTP, 0.1 mM NaOH, and 10 mM HEPES-NaOH, pH 7.4. For activation of I_{KATP}, MgCl₂ was substituted for MgATP in an equimolar concentration. The chosen K⁺ concentrations yield an equilibrium potential for K⁺ of ~48 mV. The Tyrode’s solution used for multicellular preparations contained 144 mM NaCl, 4 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES-NaOH, and 5 mM glucose, pH 7.4. Experiments on isolated cells were carried out at room temperature (21–24°C). For multicellular preparations, the bath temperature was kept at 36 ± 1°C, and pO₂ was 580 to 600 mm Hg.

Materials. Standard salts were from Merck (Darmstadt, Germany). HEPES, EGTA, MgATP, GTP, ACh-chloride, CCh, adenosine (Ado), and DNP were from Sigma Chemie. KB (free acid) was from Karo-Bio AB (Huddinge, Sweden) and was dissolved as a 100 to 200 mM stock solution in dimethyl sulfoxide. The highest dimethyl sulfoxide concentration used [0.1% (v/v)] had no direct effect on the membrane currents and potentials under study nor did it affect the action of ACh, Ado, or DNP.

Ion Current Measurements and Action Potential Measurements in Single Atrial Myocytes. Membrane currents were measured under whole-cell patch-clamp (Hamill et al., 1981). Pipettes were fabricated from borosilicate glass with filament (Clark Electro-medical, Pangbourne, UK) on a horizontal puller (DMZ, Munich, Germany) and were filled with the solution listed below. The d.c. resistance of the filled pipettes ranged from 3 to 6 MΩ. Currents were measured by means of a patch-clamp amplifier (List LM/EPF 7; List Electronics, Darmstadt, Germany). Signals were analog filtered (corner frequency 1–3 kHz) and were digitally stored on the hard disk of a computer equipped with a software package for voltage control, data acquisition, and data evaluation (ISO-2 by MFK, Frankfurt, Germany). Capacitance and series resistance were partially compensated. Unless stated otherwise the holding potential was ~90 mV, chosen to cause large inward K⁺ currents because of the strong inward rectifying properties of G protein-coupled currents. Fast voltage ramps (~120 to +60 mV, 500 ms) were generated to provide current-voltage relations and to monitor the electrical access to the cell. Drug-induced changes in outward current were measured during the voltage ramps, and no qualitative difference was detected in the observed drug effects on inward and outward currents. Rapid exchange of superfusion solution was performed by means of solenoid-operated valves, which permitted switching between up to six different solutions. The half-time of solution exchange was about 0.5 s, as measured from the time course of I_{KACH}, block upon switching from the standard (high-K⁺) to a K⁺–free, 5 mM Cs⁺-containing solution.

Current-clamp experiments were also performed in the whole-cell recording mode at 37 ± 1°C. External and pipette solutions were the same as those used in the whole-cell voltage-clamp experiments. However, a physiological external K⁺ concentration was chosen (5 mM). After the establishment of the whole-cell clamp mode, rectangular 2-ms currents were applied at a rate of 0.2 Hz. After a stabilization of action potential configuration, experiments were performed.

Action Potentials in Multicellular Preparations. For measurements in the multicellular preparation transmembrane potentials were recorded with glass microelectrodes filled with 3 M KCl (resistance 7–10 MΩ). The microelectrodes were connected to the input stage of a high-impedance amplifier (MEZ-7101; NIHoden America, Inc., Foothill Ranch, CA). The amplified signals were displayed on a dual-beam oscilloscope (C1–69) and sampled at 10 kHz using a 16-bit analog-to-digital converter (PCL816; Advantech France, Levallois Perret, France). After an equilibration period of 50 to 60 min with stable impedance, control recordings were first made. Then the atrial preparations were exposed to CCh (10 μM, for 10–15 min) followed by a washout of the agonist (15–25 min). Afterward, the same preparations were exposed to KB (50 or 200 μM; for 15–20 min), and CCh was reapplied and washed out in the presence of KB. In ventricular preparations, control measurements were followed by an application of 2,4-dinitrophenol (DNP; 50–100 μM) for 10 min, and of KB (200 μM; 25 min) added on top of DNP. Action potential durations were measured at 50% (APD_{50}) and 90% (APD_{90}) repolarization.

Results

KB Inhibits Agonist-Induced and Background Muscarinic Potassium Current. In guinea pig atrial myocytes freshly isolated or kept in serum-free culture for several
Inhibition by KB of $I_{K(ACh)}$, activated via muscarinic receptors. A, current continuously recorded at the holding potential (−90 mV) and during regular short voltage ramps (showing as vertical current deflections) under basal conditions, during exposure to ACH (10 μM) and during exposure to KB (50 μM) added on top of ACh. Periods of exposure to ACh and KB are indicated by horizontal bars. Top dotted line, zero current level. Bottom dotted line, current level at holding potential under basal conditions. Notice 1) the increase of inward current by ACh, 2) the suppression of the ACh effect in the presence of KB, and 3) a decrease of the inward current to a lower than basal level in the presence of KB. B, I-V relationships of the ACh-induced current (unfilled squares) and of the KB-sensitive current (filled squares). The ACh-induced current was obtained by subtracting the trace labeled as a from the trace labeled as b in A. The KB-sensitive current was obtained by subtracting the trace labeled as c from the trace labeled as b in A.

Figure 1.

Reversibility and concentration dependence of the KB effect on $I_{K(ACh)}$. A, current continuously recorded at the holding potential (−90 mV) and during regular short voltage ramps (showing as vertical current deflections) under basal conditions, during exposure to ACh (10 μM) and during exposure to KB (1 μM) added on top of ACh. Periods of exposure to ACh and KB are indicated by horizontal bars. Dashed line, zero current level. Notice the rapid removal of 1 μM KB effect upon washout of this concentration. B, relationship between relative inhibition of $I_{K(ACh)}$ and drug concentration. $I_{K(ACh)}$ induced by either ACh ($n = 7$), Ado ($n = 6$), or GTPγS ($n = 5$). The amplitude of inhibition was measured as the difference between currents just before and at the end of the KB application. Data were fitted to the Hill equation (relative inhibition = $1/(1 + (IC_{50}/[KB])^p)$, where $IC_{50}$ is the drug concentration, IC50 is the half-maximum effective concentration, and $p$ is the Hill coefficient).

Fig. 1. Inhibition by KB of $I_{K(ACh)}$, activated via muscarinic receptors. A, current continuously recorded at the holding potential (−90 mV) and during regular short voltage ramps (showing as vertical current deflections) under basal conditions, during exposure to ACh (10 μM) and during exposure to KB (50 μM) added on top of ACh. Periods of exposure to ACh and KB are indicated by horizontal bars. Top dotted line, zero current level. Bottom dotted line, current level at holding potential under basal conditions. Notice 1) the increase of inward current by ACh; 2) the suppression of the ACh effect in the presence of KB; and 3) a decrease of the inward current to a lower than basal level in the presence of KB. B, I-V relationships of the ACh-induced current (unfilled squares) and of the KB-sensitive current (filled squares). The ACh-induced current was obtained by subtracting the trace labeled as a from the trace labeled as b in A. The KB-sensitive current was obtained by subtracting the trace labeled as c from the trace labeled as b in A.

Hours, saturating concentrations of ACh (10 μM) induced the G protein-coupled inward rectifying current $I_{K(ACh)}$, with a mean current density of −74 ± 9 pA/pF at −90 mV ($n = 7$). Figure 1A shows a representative current trace. Adding KB (50 μM) to the superfusion solution in the continued presence of ACh caused a complete inhibition of $I_{K(ACh)}$. The current-voltage (I-V) relationship of the ACh-induced current, obtained by subtracting currents in basal conditions (Fig. 1A, a) from the current in the presence of ACh (Fig. 1A, b), is displayed in Fig. 1B (filled squares) and shows the strong inward rectification typical for $I_{K(ACh)}$. The KB-inhibited current (Fig. 1B, unfilled squares), obtained by subtracting the current in the presence of KB (Fig. 1A, c) from that in the presence of ACh (Fig. 1A, b), exhibited characteristics identical to those of $I_{K(ACh)}$, suggesting that the drug inhibited this current.

KB effectively inhibited both inward and outward components of $I_{K(ACh)}$. The KB-sensitive current was quantitatively larger than the ACh-induced current (see less inward current at holding potential in the presence of KB compared with predrug level in Fig. 1A). Because an agonist-independent opening of $I_{K(ACh)}$ channels has been shown to contribute to the background current in atrial cells (Sakmann et al., 1983; Okabe et al., 1990; Kaibara et al., 1991), our results thus suggest that KB inhibited not only the ACh-induced $I_{K(ACh)}$ but also the basal G protein-activated current. The I-V relationship of the KB-inhibited background current (not illustrated) also displayed the same strong inward rectification as $I_{K(ACh)}$.

The effect of KB was concentration-dependent and reversible. Reversibility of the inhibition to the expected current level, taking into account the concurrent desensitization, was observed using low KB concentrations (Fig. 2A), but the effects of high concentrations (≥10 μM) persisted for several minutes during drug washout. The concentration-effect curve obtained from experiments in 7 cells could be fitted by a Hill equation with IC50 of 0.82 μM and Hill coefficient of 1.12 (Fig. 2B, continuous line).

To examine whether KB inhibition of $I_{K(ACh)}$ was due to an interaction at the muscarinic receptor level, or involved mechanisms downstream of the receptor, we tested its effect on G protein-coupled K+ current induced by other receptors. We therefore activated $I_{K(ACh)}$ by adenosine. Figure 3A illustrates an experiment that is representative for six different atrial myocytes, in which KB inhibited the adenosine-induced $I_{K(Ado)}$ (which is identical to $I_{K(ACh)}$). Again the effect was concentration-dependent (IC50 = 0.57 μM; Fig. 2B, dashed line) and reversible within minutes. I-V relationships

Fig. 2.
The KB-sensitive current displayed the same strong inward rectifying properties as $I_{K_{\text{Ado}}}$.

T$_3$, which shares structural groups with KB and whose nuclear receptors can bind KB (Carlsson et al., 2002), failed to mimic the inhibitory action of this drug on the adenosine-induced potassium current (concentrations tested). These experiments indicate that KB inhibits $I_{K_{\text{Ado}}}$ either by interfering with the G protein (βγ) subunit-channel interaction or by directly acting on $K_{\text{Ado}}$ ion channels.

KB Inhibits the ATP-Sensitive K$^+$ Channel. To investigate the sensitivity of $I_{K_{\text{ATP}}}$ to KB, we performed experiments in atrial myocytes and superfused the cells with DNP (100 μM). Figure 5 shows a representative experiment for six cells superfused with DNP. After application of the metabolic uncoupler, $I_{K_{\text{ATP}}}$ was slowly activated and resulted in an increase of inward current at the holding potential of $-90$ mV. Superfusion with KB in the continued presence of DNP resulted in a suppression of the inward current increase. I-V relationship of the DNP-induced current showed weak inward rectification.

Fig. 3. Inhibition by KB of $I_{K_{\text{Ado}}}$ activated via $A_1$ adenosine receptors. A, current continuously recorded at the holding potential ($-90$ mV) and during regular short voltage ramps (showing as vertical current deflections) under basal conditions, during exposure to adenosine (Ado; 100 μM) and during exposure to KB (50 μM) or T$_3$ (30 nM) added on top of Ado. Periods of exposure to Ado, KB, or T$_3$ are indicated by horizontal bars. Dotted line, zero current level. Notice the suppression of the Ado effect in the presence of KB but not in the presence of T$_3$. B, I-V relationships of the Ado-induced current (filled squares and of the KB-sensitive current (filled squares). The Ado-induced current was obtained by subtracting the trace labeled as a from the trace labeled as b in A. The KB-sensitive current was obtained by subtracting the trace labeled as c from the trace labeled as b in A.

KB Inhibits GTP$\gamma$S-Induced $I_{K_{\text{Ado}}}$r. The above-mentioned experiments, in which KB inhibits $I_{K_{\text{Ado}}}$ coupled to different receptors, make it unlikely that the drug acts at the receptor level. To further determine the level of KB action along the G protein-channel signal transduction cascade, we tested the effect of KB on $I_{K_{\text{Ado}}}$, activated by GTP$\gamma$S (500 μM). The nonhydrolysable nucleotide was added to the patch pipette solution and yielded an irreversible activation of $I_{K_{\text{Ado}}}$ upon repeated exposure to ACh. Figure 4 illustrates representative results of similar experiments in five atrial myocytes. While dialyzing with GTP$\gamma$S, the application of ACh resulted in an activation of $I_{K_{\text{Ado}}}$ to its maximal level (Fig. 4A). The effect of ACh was only partially reversible after the first application and became irreversible after the third agonist application. Sustained $I_{K_{\text{Ado}}}$ obtained after washout of ACh under these conditions was inhibited by KB (IC$_{50}$ = 0.89 μM; Fig. 2B, dotted line). The KB-sensitive current displayed strong inward rectifying properties (Fig. 4B) and was larger than the current induced by GTP$\gamma$S and ACh (see less inward current at holding potential in the presence of KB compared with predrug level in Fig. 4A), suggesting that KB inhibited both GTP$\gamma$S-induced and background $I_{K_{\text{Ado}}}$.

Fig. 4. Inhibition by KB of $I_{K_{\text{Ado}}}$ activated via the nonhydrolysable GTP analog GTP$\gamma$S. A, current continuously recorded at the holding potential ($-90$ mV) and during regular short voltage ramps (showing as vertical current deflections) under basal conditions, during repeated exposures to ACh (10 μM) and during exposure to KB (50 μM) or T$_3$ (30 nM) added on top of Ado. Periods of exposure to Ado, KB, or T$_3$ are indicated by horizontal bars. Dotted line, zero current level. Notice the suppression of the Ado effect in the presence of KB but not in the presence of T$_3$. B, I-V relationships of the Ado-induced current (unfilled squares) and of the KB-sensitive current (filled squares). The Ado-induced current was obtained by subtracting the trace labeled as a from the trace labeled as b in A. The KB-sensitive current was obtained by subtracting the trace labeled as c from the trace labeled as b in A.
ward rectification, which is typical for $I_{KATP}$, and was superimposable to the KB-inhibited current, indicating that $I_{KATP}$ was the target of both agents.

**No Effect on the Background Inward Rectifier ($I_{K1}$) Channel.** If KB is an inhibitor of G protein-coupled and ATP-sensitive channels, other ion channels sharing structural similarities with these channels might be sensitive to the drug as well. Therefore, we studied the effect of KB on inward rectifying potassium current $I_{K1}$. No effect of KB (10–50 μM) applied from the external side of the membrane completely inhibited $I_{K1}$. In line with the experiments described above, the background current was not affected by internal KB but was decreased by external KB. These results exclude that KB inhibits $I_{K1}$ by accessing its action site from the internal side of the membrane.

**Influence of KB on Action Potentials in Multicellular Preparations and in Single Atrial Myocytes.** Given the above-mentioned KB effects on membrane currents, the drug may be expected to antagonize the shortening of action potential induced by muscarinic receptor activation in atrial cells. Figure 7A illustrates the effect of CCh before or after pretreatment with KB in multicellular preparations. Under control conditions (in the absence of KB), action potentials were markedly shortened in the presence of 10 μM CCh (Fig. 7A). After KB pretreatment, the action potential duration was significantly prolonged, indicating that KB inhibited $I_{K1}$ and $I_{K1}$.

**Fig. 5.** Inhibition by KB of $I_{KATP}$ activated during metabolic inhibition with DNP. A, current continuously recorded at the holding potential (~90 mV) and during regular short voltage ramps (showing as vertical current deflections) under basal conditions, during exposure to DNP (100 μM) and during exposure to KB (50 μM) applied in the presence of DNP. Cell dialyzed with ATP-free pipette solution. Periods of exposure to DNP or KB are indicated by horizontal bars. Dotted line, zero current level. Notice the slow activation of $I_{KATP}$ during the exposure to DNP and its suppression after exposure to KB. ACh induced $I_{K1}$ is inhibited as well. B, I-V relationships of the DNP-induced (unfilled squares) and the KB-sensitive (filled squares) currents. The DNP-induced current was obtained by subtracting the trace labeled as a from the trace labeled as b in A. The KB-sensitive current was obtained by subtracting the trace labeled as c from the trace labeled as b in A. Weak inward rectification indicates $I_{KATP}$ to be the activated and inhibited current, respectively.

**Fig. 6.** Lack of effect of intracellularly applied KB of $I_{K1}$. Current recorded at the holding potential (~90 mV) and during regular short voltage ramps (showing as vertical current deflections) under basal conditions and during two exposures to ACh (10 μM). Dashed line, zero current level. Cell dialyzed with pipette solution containing 50 μM KB. Periods of exposure to ACh or to extracellular KB are indicated by horizontal bars. First exposure to ACh, 10 s after patch rupture. Second exposure to ACh, 30 min after patch rupture. Dotted line, zero current level. Notice similar effects of ACh during both applications.
comparing APD90 in the presence of CCh before exposure to KB versus potential induced upon K ATP channel opening with DNP.

Both CCh and 200 M significantly (P < 0.05) increased the resting potential (Vrest) and reduced the amplitude of action potential (APA) compared with control conditions. Dashed lines, zero potential level. B, pooled data of action potential duration measured at APD90, in control and in the presence of KB. Dashed lines, zero potential level. B, pooled data of action potential duration measured at APD90, in control and in the presence of KB. Dashed lines, zero potential level. B, pooled data of action potential duration measured at APD90, in control and in the presence of KB. Dashed lines, zero potential level.

Fig. 7A, left). After washout of the CCh effect (not illustrated; but see Table 1), treating the same preparation with 50 M KB for 20 min caused no major change of the action potential. Reapplication of CCh after stabilization with KB caused less marked APD shortening compared with control conditions (Fig. 7A, right). Similar results were obtained in five preparations, as summarized in Fig. 7B. APD90 in the presence of both CCh and 200 M KB was not significantly different from the value in the presence of 200 M KB alone, but was significantly (P < 0.05) longer than the value in the presence of CCh before treatment with KB (Table 1). The inhibition of CCh effect by KB was concentration-dependent and was less than 50 M KB. In single atrial myocytes KB 50 M almost completely reversed an ACh (10 M)-induced action potential shortening. An experiment being representative for six different cells is shown in Fig. 8A. The summary of the data of six different experiments (APD90) is shown in Fig. 8B.

We also expected KB to antagonize the shortening of action potential induced upon KATP channel opening with DNP.

Fig. 7. Reversal by KB of the effect of CCh in multicellular atrial preparations. A, typical action potentials measured in one single preparation in various conditions. Left, under basal control conditions, and after application of 10 M CCh. Right, during application of 200 M KB (after washout from the first CCh exposure) and after re-exposure to CCh in the presence of KB. Dashed lines, zero potential level. B, pooled data of action potential duration measured at APD90, in control and in the presence of CCh (10 M) applied in 10 preparations before exposure or after 20-min pre-exposure to 50 M (n = 5) or 200 M KB (n = 5). * P < 0.05 comparing APD90 in the presence of CCh before exposure to KB versus after pre-exposure to 50 M KB.

This antagonism was tested in papillary muscles. It was rather difficult to stably record action potentials in the presence of DNP (100 M), because of a frequent occurrence of contracture or of a complete loss of excitability in several experiments. Successful recordings in four preparations using 50 M DNP (n = 2) or 100 M DNP (n = 2) showed that KB could partly reverse the APD shortening caused by the metabolic poisoning. Figure 9A illustrates a typical result, and Fig. 9B and Table 2 summarize the data from all preparations. The APD prolongation caused by KB was significant (P < 0.01) but remained modest and did not restore the duration to its initial value.

We also expected KB to antagonize the shortening of action potential induced upon KATP channel opening with DNP.

TABLE 1

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<th>Control (n = 10)</th>
<th>CCh (10 M)</th>
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V_rest, resting potential; APA, amplitude of action potential; APD measured at 50% (APD50) or at 90% (APD90) repolarization.

* P < 0.05; ** P < 0.01; *** P < 0.001 compared with control (ANOVA and Tukey-Kramer multiple comparison test).

Fig. 8. Effect of KB 50 M on action potential of atrial myocytes in the presence of ACh. A, representative experiment where ACh 10 M induces a marked shortening of the action potential. Superfusion of the myocyte with KB completely reverses the ACh effect. B, summarized data of changes of action potential duration at APD90. * P < 0.05 comparing APD90 in the presence of ACh before exposure to KB versus after pre-exposure to 50 M KB.

Influence of KB on the effect of CCh on membrane potentials of guinea pig atrial multicellular preparations

Values are given as mean S.E.M. Same preparations were used for control and either 50 M KB or 200 M KB. Recordings were made at 37°C.

This antagonism was tested in papillary muscles. It was rather difficult to stably record action potentials in the presence of DNP (100 M), because of a frequent occurrence of contracture or of a complete loss of excitability in several experiments. Successful recordings in four preparations using 50 M DNP (n = 2) or 100 M DNP (n = 2) showed that KB could partly reverse the APD shortening caused by the metabolic poisoning. Figure 9A illustrates a typical result, and Fig. 9B and Table 2 summarize the data from all preparations. The APD prolongation caused by KB was significant (P < 0.01) but remained modest and did not restore the duration to its initial value.
TABLE 2

Influence of KB on the effect of DNP on membrane potentials of guinea pig papillary muscle

Values are given as mean S.E.M. (n = 4). Recordings were made at 37°C.

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<tbody>
<tr>
<td><strong>V_{rest}</strong> (mV)</td>
<td>-83 ± 0.4</td>
<td>-81 ± 0.3</td>
<td>-81 ± 0.2</td>
</tr>
<tr>
<td>APA (mV)</td>
<td>112 ± 1.4</td>
<td>101 ± 7.0</td>
<td>100 ± 4.7</td>
</tr>
<tr>
<td>APD_{90} (ms)</td>
<td>205 ± 12.3</td>
<td>33 ± 12.1***</td>
<td>76 ± 18.2*** **</td>
</tr>
<tr>
<td>APD_{99} (ms)</td>
<td>255 ± 7.6</td>
<td>85 ± 13.3***</td>
<td>129 ± 24.3*** *</td>
</tr>
</tbody>
</table>

**V_{rest},** resting potential; APA, amplitude of action potential; APD measured at 50% (APD_{50}) or at 90% (APD_{90}) repolarization.

***P < 0.001 compared with control (ANOVA and Tukey-Kramer multiple comparison test).

*P < 0.05, **P < 0.01, compared with DNP alone.

Discussion

The objective of the present study was to examine the effects of the new amiodarone-like compound KB130015 (KB) on cardiac K⁺ currents. The results show that KB inhibits the G-coupled inward-rectifying K⁺ current I_{K(ACh)} and the ATP-sensitive current I_{K(ATP)} in atrial cells. KB suppressed I_{K(ACh)} activated indirectly via various receptors or directly by GTPγS. Consistent with the suppression of I_{K(ACh)} measured in single cells, KB decreased the APD shortening caused by the muscarinic agonist CCh in multicellular preparations. Similarly, the drug suppressed I_{K(ATP)} activated by metabolic inhibition with DNP in myocytes, and partially reversed the APD shortening by DNP in papillary muscles. In contrast, KB had no effect on I_{K1} recorded in myocytes and on the resting potential in multicellular preparations.

KB shares structural similarity with amiodarone, from which it was derived by chemical substitution (Carlsson et al., 2002), with the aim to produce a drug with less side effects and improved bioavailability. Preliminary animal toxicity studies of KB suggest that side effects after its chronic administration occur at doses similar to or higher than those of amiodarone, used as reference compound (Carlsson et al., 2002; supplementary materials at http://pubs.acs.org). Amiodarone, which is one of the most effective antiarrhythmic drugs, exerts many effects at the cellular level after either acute or chronic administration (Kodama et al., 1997). Although it is still unclear which of the complex effects of amiodarone are critical for its antiarrhythmic action, its effects on the function and expression of various ion channels are likely to play a predominant role. Amiodarone or its analogs, when acutely applied, decrease the availability or the open probability of many ion channels, including voltage-dependent Na⁺ channels, L-type Ca²⁺ channels, and various K⁺ channels (Kodama et al., 1997). Hence, KB could be expected to exert similar effects. However, besides the recently described and unexpected effects of KB on voltage-dependent Na⁺ and Ca²⁺ channels (Macianskiene et al., 2003b), its effects on other ion channels are largely unknown. I_{K(ACh)} represents the prototypical G protein-gated K⁺ channel and can be activated via the M₂ muscarinic receptor (for reviews, see Kurachi, 1995; Dascal, 1997; Dascal, 2001) or via the A₁ adenosine receptor (Kurachi et al., 1986). Our data show that I_{K(ACh)} activated by agonists to either of these receptors (ACh and Ado, respectively) was suppressed by KB. The KB-sensitive current had the same reversal potential (~48 mV) and a similar (inward-rectifying) shape of the I-V relationship as the current induced by the agonists. Under our experimental conditions, I_{K(ACh)} reached a peak shortly after the start of the agonist application and then decayed to a steady level due to desensitization (Carmeliet and Mubagwa, 1986). KB applied on top of the agonists suppressed I_{K(ACh)} with a time course markedly faster than that of desensitization. In addition, upon washout of low KB concentrations, I_{K(ACh)} rapidly returned to its steady-state level. The fast onset of and recovery from KB effect indicates that I_{K(ACh)} suppression by KB involved a mechanism different from desensitization. The KB effect is likely to involve a mechanism downstream from the agonist-receptor interaction, at the G protein or K_{ACh} channel level. This was suggested by the equal potency to suppress ACh and Ado effects (Fig. 2B) and was confirmed by the ability of KB to suppress I_{K(ACh)} induced by GTPγS. These results are similar to those obtained with amiodarone and dronerodone, which also inhibit I_{K(ACh)} via various receptors or by internal GTPγS (Watanabe et al., 1996; Altomare et al., 2000; Guillemare et al., 2000). The potency of KB to suppress I_{K(ACh)} (IC_{50} = 0.8 μM) is similar to that of amiodarone (IC_{50} = 1–2.4 μM; Watanabe et al., 1996; Guillemare et al., 2000), but much lower compared with that reported for dnonerodone (10–63 nM; Altomare et al., 2000; Guillemare et al., 2000). Our finding that KB suppresses activation of G protein-gated K⁺ channels by muscarinic and adenosine agonists does not exclude the possibility that it also acts at the receptor level. The parent compound amiodarone interferes with binding to
the muscarinic receptor (Cohen-Armon et al., 1984; Colvin et al., 1989). As in the case of amiodarone, KB actions may be more potent at, but not restricted to, the channel.

During KB application the current at holding potential usually became smaller than the basal level. I-V relationships also showed quantitatively larger KB-sensitive currents compared with agonist- or GTPγS-induced currents. This indicates that KB had another effect in addition to suppressing the effect of agonists and GTPγS. The extra current suppressed by KB showed inward rectification and reversal potential similar to those of $I_{\text{K(ACh)}}$. Agonist-independent opening of $K_{\text{ATP}}$ channels has been shown to contribute to the background current in atrial cells (Sakmann et al., 1983; Okabe et al., 1990) and reflects basal activation of $G_{\text{K1}}$ molecules by nonliganded receptors (Kaibara et al., 1991). Our results thus suggest that in the presence of KB not only the ACh-induced $I_{\text{K(ACh)}}$ but also basal G protein-activated current was inhibited. Because agonist-independent opening of $I_{\text{K(ACh)}}$ may contribute to basal current in atrial cells, it has been proposed that APD prolongation by amiodarone and its analogs could contribute to antiarrhythmic action. Our experiments on atrial preparations, KB was applied alone after washout of CCh. At 50 μM there was no effect, but at 200 μM the drug caused a slight prolongation of the APD (Table 1). It is not possible to interpret this effect solely in terms of an effect on $I_{\text{K1}}$, because effects on other ion channels could contribute to the net change. KB has effects on Na$^+$ and Ca$^{2+}$ channels (Machainskiena et al., 2003a). Other ion currents (e.g., the Na$^+$-K$^+$ pump, the Na$^+$-Ca$^{2+}$ exchange, or the Na$^+$-activated K$^+$ currents that are sensitive to intracellular Na$^+$ concentrations) might also be indirectly affected by the expected increase in Na$^+$ due to prolonged Na$^+$ channel opening (Machainskiena et al., 2003b).

In addition, the contribution of $I_{\text{K(ACh)}}$ might be overwhelmed by a larger contribution of $I_{\text{K1}}$, especially at the resting potential.

In the present study, $I_{\text{KATP}}$ in the whole-cell configuration was activated by uncoupling mitochondrial respiration from oxidative phosphorylation with DNP. Both the DNP-induced current and the current suppressed by KB in the presence of DNP showed similar inward-going rectification. Although outward rectifying currents have been induced by metabolic inhibition in other studies (Isenberg et al., 1983), inward rectification may be induced by intracellular Mg$^{2+}$ and Na$^+$ (Horie et al., 1987; Ciani and Ribaiet, 1988). Inward rectifying K(ATP) currents have been obtained under experimental conditions similar to those used in the present study (Brandts et al., 2000). Hence, we can conclude that $I_{\text{KATP}}$ induced by DNP was reversibly suppressed by KB. A similar effect ($IC_{50} = 2.3 \mu M$) has been previously noted using amiodarone on $I_{\text{KATP}}$ induced by ATP depletion or by bimakalim in inside-out membrane patches of rat ventricular cells (Holmes et al., 2000). However, other studies showed a lower potency ($IC_{50} = 19 \mu M$) of amiodarone on the K$^+$ conductance induced by ATP depletion in rat cardiocytes or a lack of effect on $I_{\text{KATP}}$ induced by the K$^+$ channel opener KN2391 in Xenopus oocytes (Sakuta et al., 1992). The reason for this discrepancy remains unclear, but the difference in results might have been due to the cell type or the experimental conditions.

Of the K$^+$ currents tested, $I_{\text{K1}}$, which represents the strong inward rectifying background current responsible for setting the resting potential in atrial and ventricular myocytes, was the least sensitive to KB effect. Our results with guinea pig ventricular cells are similar to those obtained in pig cells, where KB was found to have no effect on $I_{\text{K1}}$ or on the resting potential (Macianskiene et al., 2003a).

Our data also show that KB could antagonize the APD shortening induced by $M_3$ muscarinic receptor activation in intact atrial muscle and isolated atrial myocytes. Similarly, the drug partially reversed the APD shortening induced by DNP in the multicellular preparation of ventricular muscle. In the multicellular preparation, KB concentrations of 50 μM were little or not effective and a concentration as high as 200 μM had to be given to obtain an effect. The reasons for the low potency of KB in multicellular preparations are unknown but could involve diffusion limitations imposed by superfusing instead of perfusing the preparations. This may result in reduced drug availability in the cell from which recordings are made. The same reason can be invoked to explain only partly the low efficacy of KB to oppose DNP-induced APD shortening. Additional factors (e.g., effects on other ion channels) implicated in the DNP effects or a change of sensitivity of $I_{\text{KATP}}$ channels by DNP are likely to be involved.

Although KB is highly lipophilic and its effect at high concentrations could not be easily washed out, the onset and washout of the effect of low concentrations was fast (Fig. 2). The latter finding makes it likely that KB was acting from a site easily accessible from the extracellular medium. This is supported by experiments in which intracellularly diazyl KB failed to inhibit $I_{\text{K(ACh)}}$ (Fig. 6). A similar failure of intracellular KB (in contrast to extracellular KB) to modify Na$^+$ channels has been observed (Machainskiena et al., 2003b). For amiodarone, based on 1) the high lipophilicity, 2) the slow time course of its action, and 3) the ability of bath-applied molecules to block $I_{\text{K(Ca)}}$ in cell-attached patches, it has been proposed that its effect on $I_{\text{K(ACh)}}$ is caused by drug diffused into the intracellular medium (Altomare et al., 2000). However, the same criteria would apply if the drug can diffuse into the membrane to reach its action site. Given the above-mentioned failure of intracellular KB to cause any effect, we propose that KB inhibits $I_{\text{K(ACh)}}$ and $I_{\text{KATP}}$ by binding to the channels or their subunits from within the cell membrane, at a site more easily accessible from the external side of the bilayer.

References

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