Naunyn-Schmiedeberg's Arch Pharmacol (2000) 361 : 303–310
Digital Object Identifier (DOI) 10.1007/s002109900164

ORIGINAL ARTICLE

**János Magyar · Tamás Bányász · Péter Szigligeti
Ágnes Körtvély · Andrea Jednákovits · Péter P. Nánási**

Electrophysiological effects of bimoclomol in canine ventricular myocytes

Received: 30 June 1999 / Accepted: 1 October 1999 / Published online: 18 December 1999
© Springer-Verlag 1999

Abstract Concentration-dependent effects of bimoclomol, a novel heat shock protein (HSP) coinducer, were studied on the parameters of action potential and transmembrane ionic currents in enzymatically dispersed canine ventricular cardiomyocytes using conventional microelectrode and whole cell voltage clamp techniques. Bimoclomol (10–100 μM) decreased the maximum velocity of depolarization ($\dot{V}_{max}$) and amplitude of action potentials in a concentration-dependent manner. These effects were fully reversible after a 5-min period of washout in drug-free medium. Action potential duration measured at 50% or 90% level of repolarization (APD-50 and APD-90, respectively) was markedly shortened by bimoclomol. Both APD-50 and APD-90 were decreased, but the reduction in APD-50 was more pronounced. The APD-shortening effect of bimoclomol was significantly reduced in the presence of 20 nM charybdotoxin (inhibitor of the Ca-dependent K current) or 0.5 mM anthracene-9-carboxylic acid (inhibitor of the Ca-dependent Cl current) or 1 μM glibenclamide (inhibitor of the ATP-sensitive K current). In the presence of anthracene-9-carboxylic acid, APD-90 was lengthened by bimoclomol. The APD-shortening effect of bimoclomol was also partially antagonized by chelation of intracellular Ca^{2+} by application of the cell permeant form of BAPTA, or when using 10 mM EGTA-containing patch pipettes to record action potentials. The $\dot{V}_{max}$-depressant effect of bimoclomol was not affected by charybdotoxin, anthracene-9-carboxylic acid, glibenclamide, or BAPTA load.

In voltage clamped cardiomyocytes bimoclomol (100 μM) had no effect on the amplitude of I_{Ca}, but decreased significantly the inactivation time constant of I_{Ca} (from 19.8±1.6 ms to 16.8±1.2 ms at 0 mV). Bimoclomol also decreased significantly the amplitude of I_{K1} (from –20.5±1.1 pA/pF to –16.6±0.8 pA/pF at –135 mV), causing reduction in slope of the negative branch of the I-V curve. At positive potentials, however, bimoclomol increased outward current. The bimoclomol-induced current, therefore, was studied in the presence of $BaCl_2$, when I_{K1} current was blocked. The bimoclomol-induced current had a reversal potential close to –90 mV. Bimoclomol (100 μM) had no effect on the amplitude or kinetic properties of the transient outward K current (I_{to}) and the delayed rectifier K current (I_K).

It is concluded that bimoclomol exerts both Ca-independent (inhibition of I_{Na} and I_{K1}, activation of the ATP-sensitive K current) and Ca-dependent effects (mediated by Ca-activated Cl and probably K currents) in canine ventricular myocytes.

Key words Heat shock protein · Heart · Electrophysiology · Action potential duration · Ionic currents · Cytosolic calcium

Abbreviations *APD* action potential duration · *HSP* heat shock protein

Introduction

Bimoclomol, the recently developed cytoprotective hydroxylamine derivative [*N*-2-hydroxy-3-(1-piperidinyl) propoxy]-3-pyridine-carboximidoyl chloride maleate, BRLP-42; see Fig. 1), was shown to have a wide variety of effects in healthy and diseased animals. Chronic treatment with the compound was effective against several types of diabetic dysfunction, including neuropathy (Bíró et al. 1994), retinopathy (Hegedüs et al. 1994) and abnormal vascular reactivity (Jednákovits et al. 1994). Bimoclomol exerted beneficial effects in rat and canine hearts exposed to coronary artery occlusion and vasospasm (Jaszlits et al. 1994), effects associated probably with enhanced expression of the heat shock protein HSP70 (Vígh

J. Magyar · T. Bányász · P. Szigligeti · Å. Körtvély · P.P. Nánási (✉)
Department of Physiology, University Medical School of Debrecen, P.O. Box 22, 4012 Debrecen, Hungary
e-mail: nanasi@phys.dote.hu,
Tel.: +36-52-416634, Fax: +36-52-432289

Å. Körtvély · A. Jednákovits
Biorex Research and Development Co., P.O. Box 348, 8201 Veszprém-Szabadságpuszta, Hungary

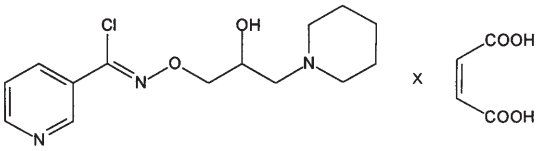

Fig. 1 Chemical structure of bimoclomol

et al. 1997). In addition, the drug showed strong dose-dependent acute antiarrhythmic action in isolated rat heart and also in anesthetized animals (Vígh et al. 1997). Currently the compound is in human phase 2 clinical trial. The present study was aimed to characterize the cellular electrophysiological actions of bimoclomol in isolated canine ventricular cardiomyocytes. Our further goal was to investigate the question whether the in vivo antiarrhythmic effect of the drug can or cannot be derived from its cardiac electrophysiological actions. Results indicate that bimoclomol may modify action potential configuration and transmembrane ionic currents in cardiac cells; however, the observed changes may hardly explain the antiarrhythmic action of the compound.

Methods

Isolation of canine ventricular myocytes. Single canine ventricular myocytes were obtained from hearts of adult mongrel dogs using the segment perfusion technique. The animals (10–20 kg) were anesthetized with i.v. injection of 10 mg/kg ketamine hydrochloride (Calypsolvet) plus 1 mg/kg xylazine hydrochloride (Rometar). After opening the chest the heart was rapidly removed and the left anterior descending coronary artery was perfused using a Langendorff apparatus. Ca^{2+}-free JMM solution (Eagle's minimum essential medium, Joklik's modification; Sigma, product no. M-0518), supplemented with taurine (2.5 g/l), pyruvic acid (175 mg/l), ribose (750 mg/l), allopurinol (13.5 mg/l) and NaH_2PO_4 (200 mg/l), was used during the initial 5 min of perfusion to remove Ca^{2+} and blood from the tissue. After addition of $NaHCO_3$ (1.3 g/l), the pH of this perfusate was 7.0 when gassed with a mixture of 95% O_2 and 5% CO_2. Cell dispersion was performed for 30 min in the same solution containing also collagenase (660 mg/l, Worthington CLS-1), bovine albumin (2 g/l) and $CaCl_2$ (50 μM). During the isolation procedure the temperature was maintained at 37°C. Usually 40–60% of the cells were rod shaped and showed clear striation when the external calcium was restored. Before use, the cells were stored overnight at 14°C in modified JMM solution (pH=7.4).

Action potential recording. The viable cells were sedimented in a Plexiglass chamber allowing continuous superfusion (10 ml/min) with modified Krebs solution containing (in mM): NaCl 120, KCl 5.4, $CaCl_2$ 2.7, $MgCl_2$ 1.1, NaH_2PO_4 1.1, $NaHCO_3$ 24 and glucose 6. The solution was equilibrated with 95% O_2 plus 5% CO_2 at a temperature of 37°C and pH was adjusted to 7.4±0.05. Transmembrane potentials were recorded using glass microelectrodes filled with 3 M KCl and having tip resistance between 20 and 40 MΩ. These electrodes were connected to the input of an Axoclamp-2B amplifier (Axon Instruments). The cells were continuously paced through the recording electrode at steady cycle length of 1000 ms (unless otherwise indicated) using 1 ms wide rectangular current pulses with 120% threshold amplitude. Action potentials were digitized at 100 kHz using Digidata 1200 A/D card (Axon Instruments) and stored for later analysis. Some action potentials were recorded using patch clamp techniques in current clamp mode.

Voltage clamp. Ionic currents were recorded from Ca^{2+}-tolerant canine ventricular cells superfused with oxygenated Tyrode solution containing (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, Na_2HPO_4 0.35, HEPES 5, glucose 10 at a temperature of 37°C and pH 7.4. This external solution was supplemented with 0.5 mM $CdCl_2$ or 3 mM 4-aminopyridine when K currents or Ca currents were measured, respectively. Ionic currents were recorded using patch pipettes filled with internal solution, containing (in mM): K-aspartate 110, KCl 45, $MgCl_2$ 1, EGTA 10, K-ATP 3, GTP 0.25 and HEPES 5, when K currents were measured. For measurement of I_{Ca}, internal solution contained (in mM): KCl 110, KOH 40, EGTA 10, HEPES 10, TEACl 20, K-ATP 3 and GTP 0.25. In both cases internal pH was adjusted to 7.3 with KOH. Patch pipettes, fabricated from borosilicate glass, had tip resistance of 2 MΩ when filled with internal solution. This resistance increased to 2 GΩ after formation of gigaseal. Gentle suction combined with a series of electrical pulses was applied to achieve whole cell configuration (Hamill et al. 1981). Series resistance, pipette capacitance and junction potential were carefully compensated. Patch pipettes were connected to the input of an Axopatch-200B amplifier (Axon Instruments), controlled by a Digidata 1200 A/D converter card. Output signals of the amplifier were stored on magnetic tape for later analysis. Voltage protocols used to measure specific ionic currents are described below where appropriate.

Statistics. All values presented are arithmetic means±SEM. Statistical significance was determined using Student's *t* test for paired or unpaired data. When appropriate (results presented in Figs. 2, 3, 4) a single analysis of variance was first performed. Differences were considered significant when the *P* value was less than 0.05.

Drugs. Bimoclomol (obtained from Biorex Research and Development) was freshly dissolved in distilled water on the day of experiment. BAPTA-AM was purchased from Teflabs (Austin, Tex., USA), Charybdotoxin from Alomone (Jerusalem, Israel), Collagenase from Worthington (Lakewood, N.J., USA). Other chemicals were obtained from Sigma Chemicals (St. Louis, Mo., USA). The entire investigation conforms with the guidelines of the United States National Institutes of Health (1996) and with the principles outlined in the Declaration of Helsinki.

Results

Effect of bimoclomol on action potential characteristics

Bimoclomol (10–100 μM) decreased the maximum velocity of depolarization and amplitude of action potentials in a concentration-dependent manner. These effects were fully reversible after a 5 min period of washout in drug-free medium. Action potential durations measured at 50% or 90% level of repolarization (APD-50 and APD-90, respectively) were also significantly shortened by the drug. Both APD-50 and APD-90 decreased, however, reduction in APD-50 was more pronounced at concentrations higher than 10 μM. These effects of bimoclomol, associated with marked depression of action potential plateau, are presented in Fig. 2.

The bimoclomol-induced shortening of action potential duration was frequency-dependent: it was stronger at higher driving rates. APD-50 was decreased by 100 μM bimoclomol from 162±6 to 45±13 ms (reduction of 71±9%) at 1 Hz, whereas at 0.2 Hz the respective values were 198±8 and 101±21 ms (49±11% reduction). Under these conditions APD-90 decreased by 36±5% and 16±9% at 1 Hz and 0.2 Hz, respectively. These differences were sta-

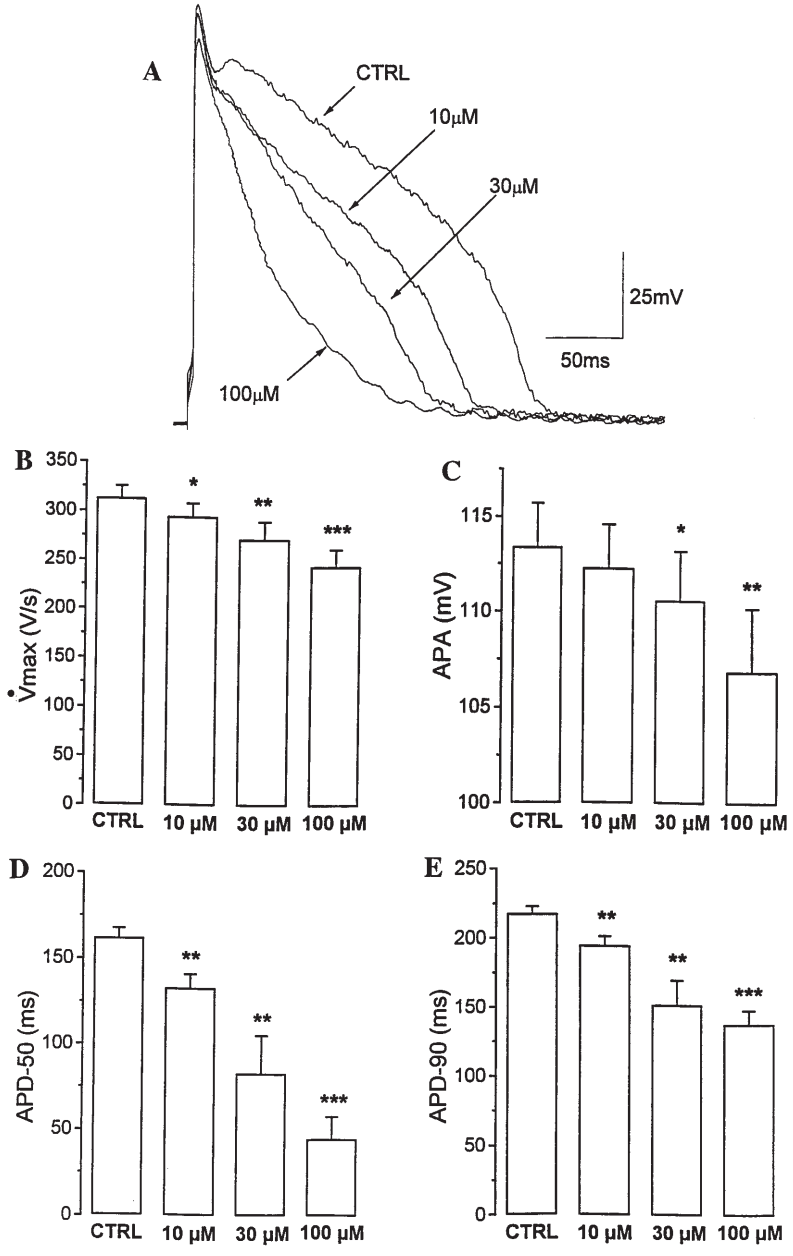

Fig. 2 Concentration-dependent effects of bimoclomol on action potential configuration (**A**), maximum velocity of depolarization ($\dot{V}_{max}$; **B**), action potential amplitude (*APA*; **C**), action potential duration measured at 50% (*APD-50*; **D**) and 90% (*APD-90*; **E**) of repolarization in isolated canine ventricular myocytes (n=6). *Columns*, *bars* mean data± SEM obtained at a constant pacing frequency of 1 Hz; *asterisks* significant changes from predrug control (*CTRL*) values determined using Student's t test for paired data (*P<0.05, **P<0.01, ***P<0.001)

tistically significant (P<0.05, n=6). Results given above were obtained using conventional sharp microelectrodes. When patch pipettes, containing 10 mM EGTA, were used to record action potentials at 1 Hz, the APD-shortening effect of bimoclomol was partially attenuated: only 28±6% reduction in APD-50 and 21±6% reduction in APD-90 were observed on the effect of 100 μM bimoclomol (P<0.05, n=6). These changes were significantly (P<0.05) less than those obtained with conventional microelectrodes. On the basis of these results the possibility arises that changes in intracellular Ca^{2+} may probably be involved in the effects of bimoclomol on action potential configuration. Indeed, when the effect of bimoclomol (30 and 100 μM) was studied in BAPTA-loaded myocytes, the APD-shortening effect of the drug was significantly less than in unloaded cells. In these experiments the cells were first superfused with 10 μM BAPTA-AM (the cell permeant acetoxymethylester form of BAPTA) for 10 min and then equilibrated in Krebs solution for further 10 min before exposure to bimoclomol. As shown in Fig. 3, the bimoclomol-induced reduction in APD-50 decreased, and shortening of APD-90 was fully abolished in BAPTA-loaded cardiomyocytes.

If the effect of bimoclomol is associated with elevation in cytosolic Ca^{2+}, it is likely to be mediated – at least in part – by Ca-dependent outward currents. In this case pharmacological inhibition of these currents is expected to prevent or diminish the APD-shortening effect of bimoclomol. Application of 0.5 mM anthracene-9-carboxylic acid (inhibitor of Ca-dependent Cl current; Hwang and Gadsby 1994) or 20 nM charybdotoxin (inhibitor of Ca-dependent K current; Krause et al. 1993; Miller et al.

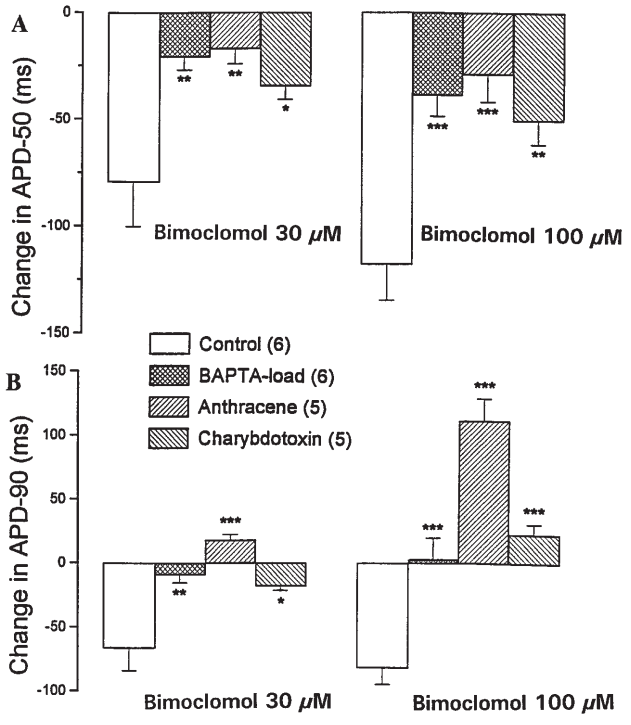

Fig. 3 Effect of 10 μM BAPTA-acetoxymethylester, 0.5 mM anthracene-9-carboxylic acid and 20 nM charybdotoxin pretreatment on the effect of bimoclomol (30 μM *left*, 100 μM *right*) on action potential duration measured at 50% APD-50 (**A**) and 90% APD-90 (**B**) of repolarization. *Numbers in parenthesis* number of cells studied; *asterisks* statistical differences between control (exposed to bimoclomol alone) and pretreated groups (drug indicated followed by bimoclomol exposure) determined using Student's *t* test for unpaired data (**P*<0.05, ***P*<0.01, ****P*<0.001)

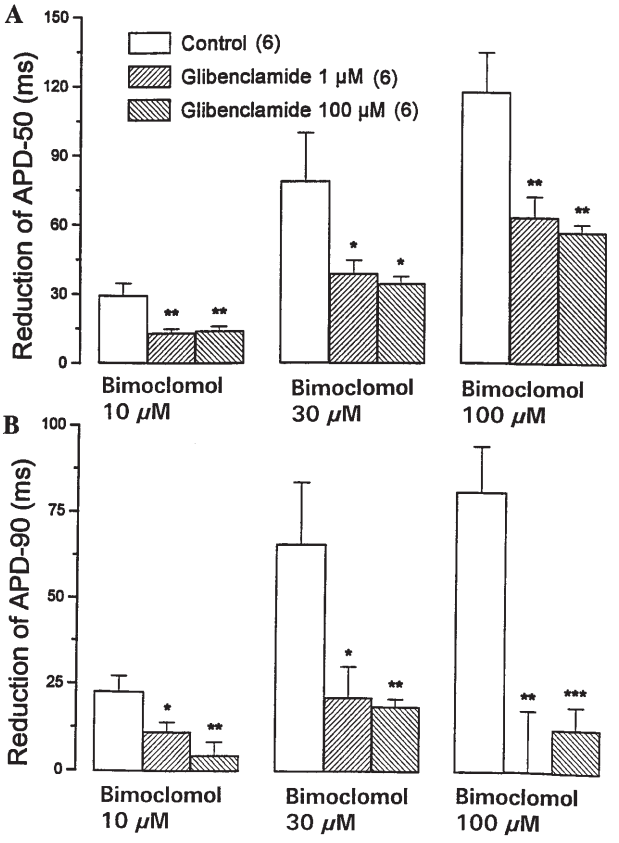

Fig. 4 Effect of bimoclomol (10, 30, and 100 μM) on action potential duration measured at 50% APD-50 (**A**) and 90% APD-90 (**B**) of repolarization in absence and presence of 1 μM or 100 μM glibenclamide. *Numbers in parenthesis* number of cells studied; *asterisks* statistical differences between control (bimoclomol alone) and pretreated groups (glibenclamide + bimoclomol) determined using Student's *t* test for unpaired data (**P*<0.05, ***P*<0.01, ****P*<0.001)

1985) strongly attenuated the shortening effect of bimoclomol on APD-50 (Fig. 3A). Furthermore, APD-90 was significantly lengthened by 100 μM bimoclomol in the presence of 0.5 mM anthracene-9-carboxylic acid or 20 nM charybdotoxin (Fig. 3B). Although these results indeed support the contribution of Ca-dependent outward currents, their involvement may not be exclusive. Glibenclamide, the inhibitor of ATP-sensitive K channels (Escande et al. 1989; Noma and Shibasaki 1985) also significantly decreased the APD-shortening effect of bimoclomol (Fig. 4). Pretreatment with glibenclamide (1 μM) alone failed to alter action potential duration (not shown, n=6). It is important to note that the $\dot{V}_{max}$-depressant effect of bimoclomol was not affected by charybdotoxin, anthracene-9-carboxylic acid, glibenclamide or BAPTA-load.

Effect of bimoclomol on transmembrane ionic currents

I_{Ca} was measured using depolarizing voltage pulses of 400 ms duration clamped from a holding potential of –40 mV to test potentials increasing up to +50 mV in 10 mV steps. Families of I_{Ca} recorded before and after bimoclomol treatment (100 μM) and current-voltage relations obtained for I_{Ca} are presented in Fig. 5A and B. Amplitudes of I_{Ca}, defined as a difference between peak inward current and the steady-state current measured at the end of the test pulse, were plotted against their corresponding test potentials. The average I-V curve, obtained in six cells, indicates that bimoclomol has no effect on the amplitude of I_{Ca} at test potentials ranging from –40 to +50 mV. In these experiments test depolarizations were repeated at a frequency of 0.2 Hz. In additional three experiments I_{Ca} was first measured at 0.2 Hz, and then a train of test pulses to 0 mV were delivered at 3 Hz. I_{Ca} slightly decreased in response to increasing stimulation frequency, however, this reduction, characterized by the ratio of the last and first I_{Ca} amplitudes of the 3 Hz train, was the same in control and in the presence of 100 μM bimoclomol (0.88±0.08 and 0.89±0.06, respectively). This finding indicates that peak I_{Ca} is not reduced by bimoclomol even at high stimulation frequencies. Bimoclomol accelerated inactivation kinetics of I_{Ca} (Fig. 5C). The inactivation time constant obtained for I_{Ca} at 0 mV was decreased significantly by 100 μM bimoclomol from 19.8±1.6 to 16.8±1.2 ms (P<0.05, n=6). Accordingly, the integral of I_{Ca}, deter-

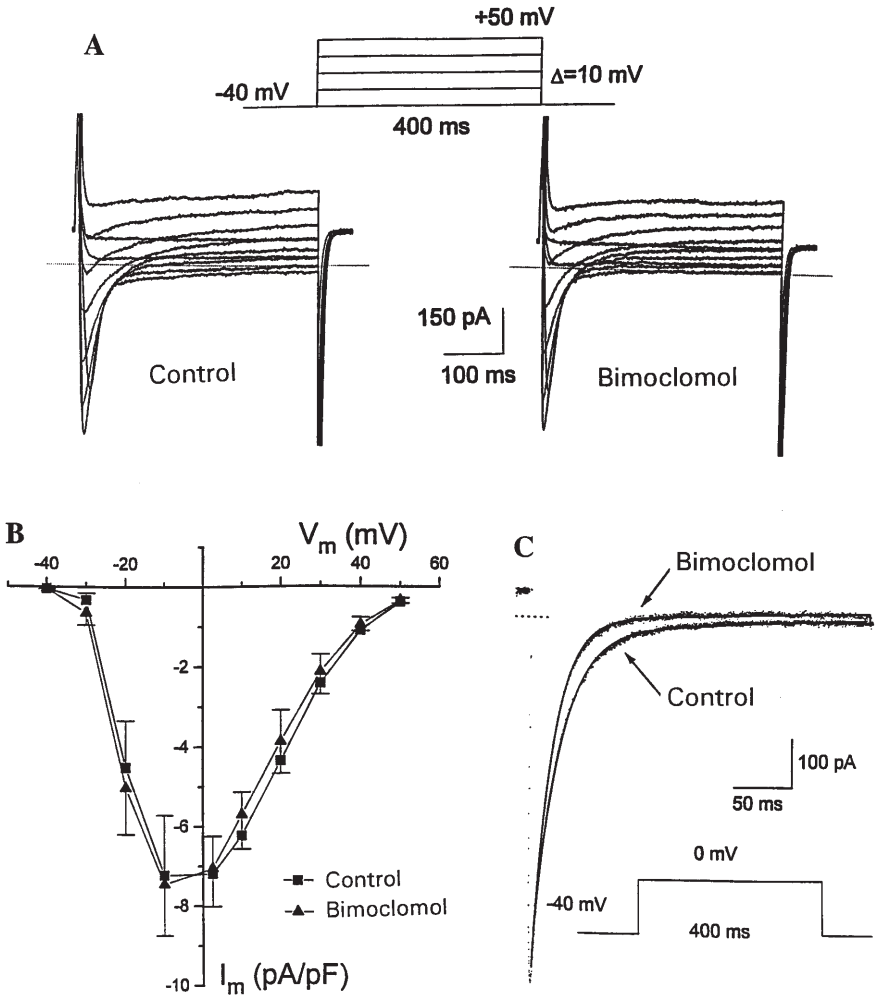

Fig. 5A–C Effect of bimoclomol (100 μM) on I_{Ca}. **A** Families of membrane currents evoked by depolarizations to potentials ranging between –50 and +50 mV in the absence and presence of bimoclomol. Holding potential: –40 mV, pulse duration: 400 ms. **B** Current-voltage relationship for peak $I_{Ca,}$ obtained by plotting peak currents against corresponding test potentials. *Symbols, bars* mean±SEM obtained in six cells. **C** Representative records showing inactivation kinetics of I_{Ca} at 0 mV in absence and presence of 100 μM bimoclomol. *Solid curves* monoexponential fit of current decay

mined during the initial 150 ms of depolarization to 0 mV, also decreased from –9.3±0.54 to –8.1±0.63 pC (P<0.05, n=6).

I_{to} was studied in seven cells using depolarizing voltage pulses of 200 ms duration, arising from a holding potential of –80 mV to test potentials ranging from –20 to +50 mV. Na current was eliminated by application of 5 ms prepulses to –40 mV before each test depolarization. Bimoclomol (100 μM) had no effect on peak current amplitude (7.7±1 vs. 6.9±1 pA/pF, absolute values), on the inactivation time constants of I_{to} (7.6±0.9 vs. 7.7±0.7 ms), and on the time constant for recovery from inactivation (28.2±0.9 vs. 27.9±1.3 ms), measured with a paired-pulse protocol by applying interpulse intervals ranging from 10 to 120 ms. All parameter was determined at +50 mV. The voltage dependence of steady-state inactivation of I_{to} was measured during test depolarizations to +50 mV, preceded by a set of prepulses increasing in 5 mV steps from the –80 mV holding potential up to +10 mV. Both pulses lasted for 500 ms. The outward current amplitude measured after each prepulse during the test pulse was normalized to that measured without prepulse. These current ratios were plotted against corresponding prepulse potentials and fitted to a Boltzmann function. Bimoclomol (100 μM) had no effect on the voltage-dependence of inactivation of I_{to}, the estimated midpoint potentials were –34.0±4.1 and –34.9±3.5 mV, respectively (not shown).

I_K was activated during long lasting (3000 ms) depolarizations to positive membrane potentials (not shown). Transient currents (I_{Na}, I_{Ca}, and I_{to}) were inactivated by clamping to –30 mV before applying test depolarizations to +50 mV. Tail currents were recorded upon repolarization to –30 mV. No significant change was observed in tail current amplitudes (0.56±0.09 vs. 0.53±0.13 pA/pF, n=5) in absence or presence of 100 μM bimoclomol. The amplitude of the outward current, activating slowly during the 3000 ms long depolarization to +50 mV, was increased slightly by bimoclomol (from 3.6±0.3 to 3.9±0.2 pA/pF, n=5), however, this increase was not significant statistically.

Steady-state current-voltage relationship was determined using 400 ms long test potentials ranging from –135 to +55 mV and increasing in 10 mV steps. Each test pulse was preceded by a 5 ms long prepulse to –40 mV in order to inactivate Na current. Membrane current measured at the end of each test pulse was plotted against the corresponding test potential. Bimoclomol (100 μM) decreased significantly the amplitude of I_{K1} between –135

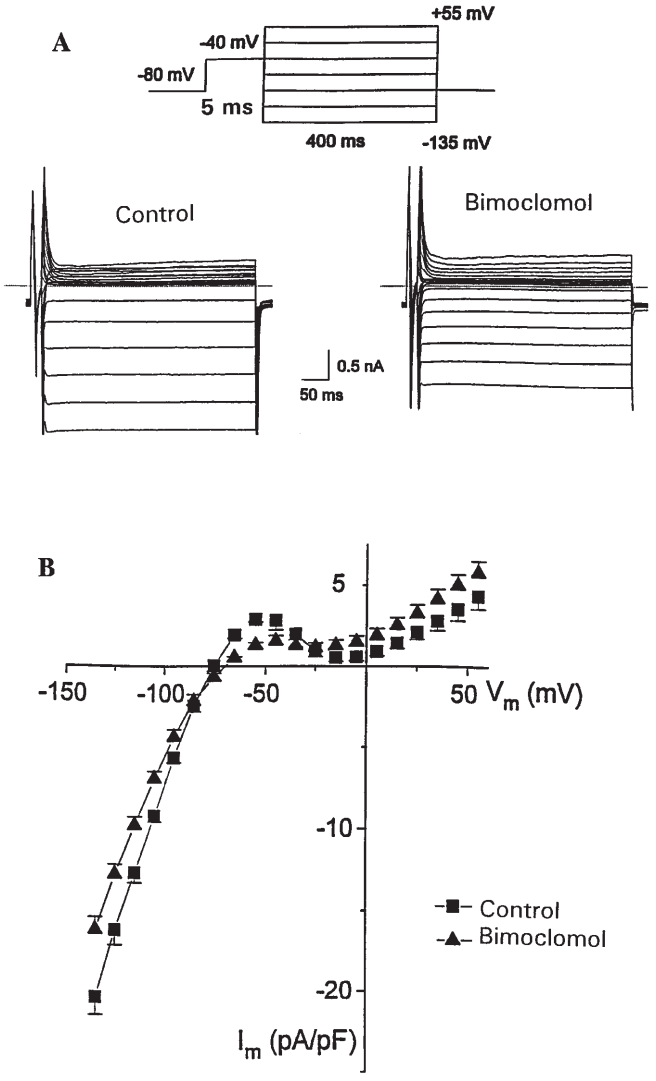

Fig. 6 Effect of bimoclomol (100 μM) on A I_{K1} and B steady-state current-voltage relationship in eight myocytes. Test potentials ranging between –135 and +55 mV were applied for 400 ms. The I-V curve was obtained by plotting steady-state currents as a function of test potentials. *Symbols, bars* means±SEM

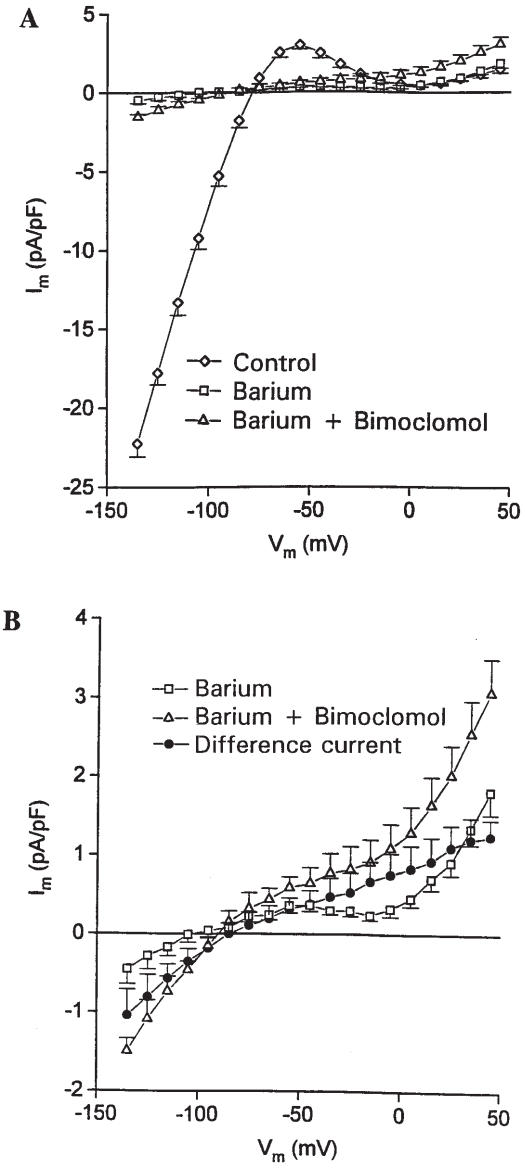

Fig. 7A, B Effect of bimoclomol (100 μM) on steady-state current-voltage relationship in the presence of 0.3 mM $BaCl_2$. Similar pulse protocol as used in Fig. 6; *n*=6. **A** The whole experiment is shown. **B** Results obtained in the presence of $BaCl_2$ are presented only using an enlarged ordinate. *Filled circles* the bimoclomol-induced current (as a difference recorded in the presence and absence of bimoclomol in the continuous presence of $BaCl_2$); *symbols, bars* means±SEM

and –55 mV, causing a reduction in the slope of the negative branch of the I-V curve (Fig. 6). At –135 mV bimoclomol decreased I_{K1} current density from –20.5±1.1 to –16.6±0.8 pA/pF (P<0.05, n=8). In contrast to voltages negative to –50 mV, where bimoclomol decreased both inward and outward currents, an increase in outward current was observed at potentials positive to –25 mV in the presence of bimoclomol.

Since the inhibition of I_{K1} might mask the bimoclomol-induced current at negative potentials, the effect of 100 μM bimoclomol on the I-V curve was studied also in the presence of 0.3 mM $BaCl_2$ (n=6). As shown in Fig. 7A, $BaCl_2$ effectively inhibited I_{K1} (inward current density, measured at –135 mV, was reduced from –22.2±0.9 to –0.45±0.2 pA/pF) and abolished N-shaped I-V characteristics. In the presence of $BaCl_2$, bimoclomol increased the current density at both negative and positive membrane potentials. The amplitude of the bimoclomol-induced current was –1.04±0.33 pA/pF at –135 mV and +1.25±0.21 pA/pF at +45 mV. The current had a reversal potential close to –90 mV (Fig. 7B).

Discussion

The most prominent effect of bimoclomol in canine ventricular myocytes was the acceleration of repolarization (shortening of APD) which appears to involve complex

mechanisms. Some of these are likely associated with elevation in cytosolic Ca^{2+}, because chelation of Ca^{2+}_i by EGTA or BAPTA-AM effectively diminished the bimoclomol-induced reduction in APD. Since bimoclomol decreased rather than increased Ca entry through Ca channels, the elevation in cytosolic Ca^{2+} may be due to changes in the function of SR. Indeed, preliminary results indicate that bimoclomol increases the opening probability of the Ca-release channel (ryanodine receptor) in SR vesicles prepared from canine heart (Jóna, personal communication). Furthermore, both systolic and diastolic Ca concentrations, monitored using the fluorescent dye Fura-2, were increased by bimoclomol in working guinea pig heart (Szigeti, unpublished). According to pharmacological studies with anthracene-9-carboxylic acid and charybdotoxin, the APD-shortening effect of bimoclomol is likely to be mediated by activation of the Ca-dependent Cl current (Sipido et al. 1993; Zygmunt and Gibbons 1992) and probably the Ca-dependent K current (Hermann and Hartung 1983). The acceleration of inactivation of I_{Ca}, observed in voltage clamp experiments, may also likely be explained by elevation in cytosolic Ca^{2+} (Lee et al. 1985).

Beyond these Ca-dependent actions of bimoclomol, there are three additional, probably Ca-independent, effects documented. The drug decreased the amplitude of the inward rectifier K current I_{K1}, an effect expected to lengthen action potential duration, especially at the terminal phase of repolarization (Harvey and Ten Eick 1988). Accordingly, APD-90 was markedly increased by bimoclomol after suppression of the Ca-dependent Cl current by anthracene-9-carboxylic acid. In addition, the shortening effect of bimoclomol was always more prominent on APD-50 than on APD-90, independently of chelation of Ca^{2+}_i. This can be explained by considering that moderate inhibition of I_{K1} has little effect on APD-50 but causes marked prolongation of APD-90. Elevation in cytosolic Ca^{2+} was shown to increase APD in many mammalian cardiac preparations due to activation of the Na^+/Ca^{2+} exchange current (Egan et al. 1989; Kirby et al. 1993; Mitchell et al. 1984; Noble et al. 1991). Prolongation of APD-50, however, was never been observed after bimoclomol treatment. This contradiction can be explained by assuming that activation of the ATP-sensitive K-current by bimoclomol (together with the shortening effect of both Ca-dependent outward currents) may exceed the lengthening effect of $I_{Na}+_{/Ca}2+$ in canine ventricular cells. Contribution of the ATP-sensitive K current to the effect of bimoclomol is supported by the partial loss of the shortening effect in the presence of 1 μM glibenclamide, a drug which is known to selectively block ATP-sensitive K current at this low concentration (Faivre and Findlay 1989; Ogbaghebriel and Shrier 1995). On the basis of the results given above, bimoclomol may activate three types of current (a Cl and probably two different K currents). This is congruent with the reversal potential of approx. -90 mV, obtained for the bimoclomol-induced current in the presence of $BaCl_2$. Finally, bimoclomol caused a moderate but significant suppression of $\dot{V}_{max}$, an effect generally attributed to inhibition of fast Na current (Hondeghem 1978). The $\dot{V}_{max}$-depressant effect of bimoclomol was not affected by chelation of Ca^{2+}_i by EGTA or BAPTA-AM.

Summarizing our results it can be concluded that bimoclomol exerts both Ca-independent (inhibition of I_{Na} and I_{K1}, activation of the ATP-sensitive K current) and Ca-dependent effects (mediated by Ca-sensitive Cl and probably K currents) in canine ventricular myocytes. These changes (except suppression of I_{Na}) cannot explain the antiarrhythmic action of the compound observed in vivo. It seems likely therefore that other mechanisms (e.g., bimoclomol-induced vasodilaton) may rather be involved. The question, how cellular electrophysiological effects of bimoclomol are related to the well documented HSP coinducer property of the drug (Vígh et al. 1997), also remains in the field of speculation. One possible link between the observed electrophysiological changes and enhancement of HSP expression might be the elevation in cytosolic Ca^{2+}. Further studies, including measurement of intracellular Ca^{2+} concentrations, are required to clarify this point.

Acknowledgements This study was supported by grants to P.P.N. (OTKA-T025577 and ETT-098/1996) from the Hungarian Research Found and Hungarian Ministry of Health. Further support was obtained from Biorex Research and Development Co.

References

Bíró K, Jednákovits A, Jaszlits L, Hegedüs E, Kukorelli T (1994) Improvement of streptozotocin-diabetic neuropathy by BRLP-42. Diabetologia 37 [Suppl 1]:282

Egan TM, Noble D, Noble SJ, Powell T, Spindler AJ, Twist VW (1989) Sodium-calcium exchange during the action potential in guinea pig ventricular cells. J Physiol (Lond) 411:639–661

Escande D, Thuringer D, LeGuern S, Courteix J, Laville M, Cavero I (1989) Potassium channel openers act through an activation of ATP-sensitive K^+ channels in guinea-pig cardiac myocytes. Pflugers Arch 414:669–675

Faivre J-F, Findlay I (1989) Effects of tolbutamide, glibenclamide and diazoxide upon action potentials recorded from rat ventricular muscle. Biochim Biophys Acta 984:1–5

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

Harvey RD, Ten Eick RE (1988) Characterization of the inward-rectifying potassium current in cat ventricular myocytes. J Gen Physiol 91:593–615

Hegedüs E, Bíró K, Jaszlits L, Jednákovits A, Kürthy M, Bácsy E (1994) Protective effect of BRLP-42 on experimental retinopathy. Diabetologia 37 [Suppl 1]:137

Hermann A, Hartung K (1983) Ca^{2+} activated K^+ conductance in molluscan neurons. Cell Calcium 4:387–405

Hondeghem LM (1978) Validity of V_{max} as a measure of the sodium current in cardiac and nervous tissues. Biophys J 23: 147–152

Hwang TC, Gadsby DC (1994) Chloride ion channels in mammalian heart cells. Curr Top Membr 42:317–346

Jaszlits L, Jednákovits A, Koltai M. Zs, Bíró K, Hegedüs E, Udvardy É, Vereczkey L, Literáti-Nagy P, Pogátsa G (1993) Antiischaemic efficacy of BRLP-42. J Mol Cell Cardiol 25 [Suppl 1]:20

Jednákovits A, Jaszlits L, Bíró K, Pogátsa G, Hegedüs E, Kürthy M (1994) Effect of BRLP-42 on vascular reactivity of diabetic arterial beds. Diabetologia 37 [Suppl 1]:19

Kirby MS, McCall E, Orchard CH, Boyett MR (1993) The role of Na^+-Ca^{2+} exchange in paired pulse potentiation of ferret ventricular muscle. J Physiol (Lond) 472:415–442

Krause PC, Rardon DP, Miles WM, Klein LS, Mahomed Y, King RD, Zipes DP (1993) Characteristics of Ca^{2+}-activated K^+ channels isolated from the left ventricle of a patient with idiopathic long QT syndrome. Am Heart J 126:1134–1141

Lee KS, Marban E, Tsien RW (1985) Inactivation of Ca channels in mammalian heart cells: joint dependence on membrane potential and intracellular calcium. J Physiol (Lond) 364:395–411

Miller C, Moczydlowski E, Latorre R, Philips M (1985) Charybdotoxin, a protein inhibitor of single Ca^{2+} activated K^+ channels from mammalian skeletal muscle. Nature 313:316–318

Mitchell MR, Powell T, Terrar DA, Twist VW (1984) Strontium, nifedipine and 4-aminopyridine modify the time course of the action potential in cells from rat ventricular muscle. Br J Pharmacol 81:551–556

National Institutes of Health (1996) Guide for the care and use of laboratory animals, revised. Publ no. 85-23. NIH, Bethesda

Noble D, Noble SJ, Bett GCL, Earm YE, Ho W-K, So I-S (1991) The role of sodium-calcium exchange during the cardiac action potential. Ann NY Acad Sci 639:334–353

Noma A, Shibasaki T (1985) Membrane current through adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. J Physiol (Lond) 363:463–480

Ogbaghebriel A, Shrier A (1995) Differental responsiveness of atrial and ventricular myocytes to potassium channel openers. J Cardiovasc Pharmacol 25:65–74

Sipido KR, Callewaert G, Carmeliet E (1993) $[Ca^{2+}]_i$ transients and $[Ca^{2+}]_i$-dependent chloride current in single Purkinje cells from rabbit heart. J Physiol (Lond) 468:641–667

Vígh L, Literáti-Nagy P, Horváth I, Török Zs, Balogh G, Glatz A, Kovács E, Boros I, Ferdinándy P, Farkas B, Jaszlits L, Jednákovits A, Korányi L, Maresca B (1997) Bimoclomol: a nontoxic hydroxylamine derivative with stress protein-inducing activity and cytoprotective effects. Nat Med 3:1150–1154

Zygmunt AC, Gibbons WR (1992) Properties of the calcium-activated chloride current in heart. J Gen Physiol 99:391–414