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Bertosamil blocks HERG potassium channels in their open and inactivated states

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1 Bertosamil is chemically related to the class-III anti-arrhythmic drug tedisamil and has been developed as a bradycardic, anti-ischemic and anti-arrhythmic drug. Its anti-arrhythmic properties might in part be attributed to its block of voltage-dependent potassium channels $Kv_{1.2}$, $Kv_{1.4}$. and $Kv_{1.5}$. However, HERG-potassium channel block as an important target for class-III drugs has not yet been investigated.

2 We investigated the effect of bertosamil on the HERG potassium channel heterologously expressed in *Xenopus* oocytes with the two-electrode voltage-clamp technique.

3 Bertosamil (70 μM) inhibited HERG tail currrent after a test pulse to 30 mV by $49.3 \pm 8.4\%$ ($n = 5$) and the IC_{50} was 62.7 μM. Onset of block was fast, i.e. 90% of inhibition developed within 180 ± 8.22 s ($n = 5$), and block was totally reversible upon washout within 294 ± 38.7 s ($n = 5$).

4 Bertosamil-induced block of HERG potassium channels was state-dependent with block mainly to open- and inactivated channels. Half-maximal activation voltage was slightly shifted towards more negative potentials.

5 Steady-state inactivation of HERG was not influenced by bertosamil. Bertosamil block elicited voltage – but no frequency-dependent effects.

6 In summary, bertosamil blocked the HERG potassium channel. These blocking properties may contribute to the anti-arrhythmic effects of bertosamil in the treatment of atrial and particular ventricular arrhythmias.

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Keywords: Bertosamil; HERG; *Xenopus* oocytes; two-electrode voltage-clamp; anti-arrhythmic drug

Abbreviations: BASIS, Basel Anti-arrhythmic Study of Infarct Survival; GESICA, Grupo de Estudio de la Sobrevida en la Insuficiencia Cardiac en Argentina; IK_r, the rapid component of the delayed-rectifier potassium channel; HERG, 'human ether-a-go-go related gene'; LQT_2, the Long QT-syndrome type 2

Introduction

Bertosamil is chemically related to the class-III anti-arrhythmic drug tedisamil and has been developed as a bradycardic and atrial anti-arrhythmic drug inhibiting the atrial potassium channels $Kv_{1.2}$, $Kv_{1.4}$ and $Kv_{1.5}$ (Yonezawa *et al*., 1996; Godreau *et al*., 2002). However, a putative ventricular anti-arrhythmic potential of the compound has not been investigated before.

In atrial and ventricular cardiomyocytes, the rapid component of the delayed rectifier K^+ current, $I_{K(r)}$ (Sanguinetti & Jurkiewicz, 1990; Carmeliet, 1993), plays an important role in repolarization. During the plateau phase, $I_{K(r)}$ has only a small amplitude; this function of $I_{K(r)}$ supports the formation of the plateau potential and is a consequence of its inward-rectifying properties. As repolarization proceeds, a transient increase in $I_{K(r)}$ outward current occurs, due to fast recovery from inactivation and slow deactivation, which effectively repolarizes the cardiac cell (Bauer & Schwarz, 2001).

On the molecular level, $I_{K(r)}$ is encoded by the 'human ether-a-go-go-related gene' (HERG). This has been demonstrated in macroscopic current measurements (Sanguinetti *et al*., 1995; Trudeau *et al*., 1995) and single channel recordings (Kiehn *et al*., 1996; Zou *et al*., 1997).

One congenital form of the Long QT-syndrome (LQT2) has mutations in HERG, which leads to a reduction of $I_{K(r)}$. This prolongs the cardiac action potential. An extreme action potential lengthening can induce early after depolarizations, an increased tendency to heart arrhythmia and the occurrence of 'torsade de pointes' ventricular tachycardias, eventually leading to ventricular fibrillation and sudden death (Curran *et al*., 1995). Paradoxically, blocking the HERG channel by anti-arrhythmic drugs can prevent the occurrence of arrhythmias. It appears that reducing HERG-currents by anti-arrhythmic class-III agents does not simply produce an acquired Long QT-syndrome, although in both conditions the congenital and the acquired Long QT-syndrome (LQT_2) have reduced HERG currents.

One explanation for positive effects of some anti-arrhythmic drugs may be particularities in their pharmacological actions on the HERG currents. A positive frequency-dependence for amiodarone (Kiehn *et al*., 1999a) may be beneficial and account for the positive outcome of the clinical

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trials with amiodarone (BASIS, GESICA; Naccarelli *et al*., 2000). Therefore it is of particular interest to investigate if, and what type of action the anti-arrhythmic drug shows on the HERG potassium channel. Likewise, block of HERG channels by various drugs has been investigated before, i.e. azimilide (Busch *et al*., 1998), amiodarone (Kiehn *et al*., 1999a), RP 58866 or terikalant (Jurkiewicz *et al*., 1996), dofetilide (Kiehn *et al*., 1996), clofilium and its tertiary analogue LY97241 (Suessbrich *et al*., 1997a), haloperidol (Suessbrich *et al*., 1997b), terfenadine racemate and enantiomers (Yang *et al*., 1995) and carvedilol (Karle *et al*., 2001).

In this study, the effect of the new anti-arrhythmic drug bertosamil on the HERG potassium channels was investigated by the two-electrode voltage-clamp technique. A study like that should add to the knowledge about various HERG blockers and the consequences of inhibition characteristics for their future clinical use.

Methods

Solutions and drug administration

Two-microelectrode voltage clamp measurements of *Xenopus* oocytes were performed in a physiologically low K^+ solution containing (in mM: KCl 5, NaCl 100, $CaCl_2$ 1.5, $MgCl_2$ 2 and HEPES 10 (pH 7.3). Current and voltage electrodes were filled with 3 M KCl solution. Bertosamil (3-isobutyl-7-isopropyl-9,9-pentamethylene-3,7-diazabicyclo(3.3.1)nonane sesquihydrogenfumarate; kindly supplied by Solvay, Germany) was stored at $+4°C$. On the day of experiments, bertosamil was dissolved in the bath solution and pH was readjusted to 7.4, if necessary. All measurements were carried out at room temperature (20°C). The volume of the bath chamber was 150 μl; after solution switch, it took about 4 s for the new solution to reach the bath (tubing length of about 37 cm); at a flow rate of 1 ml min^{-1}, solution in the bath was totally exchanged within 9 s. In general, recording began 30 s after solution switch. All measurements were performed under steady-state conditions at least 2 min after total solution exchange.

Electrophysiology and data analysis

The two-microelectrode voltage-clamp configuration was used to record currents from *Xenopus laevis* oocytes. Microelectrodes had tip resistances ranging from 1 to 5 MΩ. Data were low-pass filtered at 1 to 2 kHz (−3 dB, four-pole Bessel filter) before digitalization at 5 to 10 kHz. Recordings were performed using a commercially available amplifier (Warner OC-725A, Warner Instruments, Hamden, U.S.A.) and pCLAMP software (Axon Instruments, Foster City, U.S.A.) for data acquisition and analysis. No leak subtraction was performed during the experiments. For activating currents, absolute values have been taken for analysis. For description of tails, currents were expressed relative to the baseline at the holding potential (−80 mV). Dose response curves were fitted with the Hill equation: $I/I_0 = I_0/(1 + X/IC_{50})^n$, where I/I_0 is the relative current, I_0 the unblocked current amplitude, X the bertosamil concentration, IC_{50} the dose for half maximal block and n the Hill coefficient.

Activation curves were fitted with a Boltzmann function: $Y = \{1 + \exp((V_{1/2} - V)/k)\}^{-1}$ where $V_{1/2}$ represents the half-maximal activation potential, Y the degree of activation and k is the slope factor. Statistical data are expressed as mean ± standard error where *n* represents the number of experiments performed. Statistical significance was evaluated using the paired Student's *t*-test. Differences were considered to be significant when the *P*-value was <0.05.

Expression of HERG channels in Xenopus *oocytes*

The HERG clone (GenBank accession no. hs04270) was a gift from M.T. Keating (Salt Lake City, Utah, U.S.A.). HERG complementary RNA was prepared from the HERG cDNA in the pSP64 plasmid with the mMESSAGE mMACHINE *in vitro* transcription kit (Ambion) by use of SP6 Polymerase after linearization with *Eco*RI (Boehringer Mannheim). Injection of RNA (50–500 ng μl^{-1}) into stage V and VI defolliculated oocytes was performed by using a Nanoject automatic injector (Drummond, Broomall, U.S.A.). The volume of injected cRNA solution was 50 nl per oocyte, and measurements were made 2–10 days after injection. The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Results

HERG potassium currents are blocked by bertosamil

To investigate the effect of bertosamil on cloned HERG potassium channels, two-microelectrode voltage clamp experiments were performed on *Xenopus* oocytes heterologously expressing the HERG channel. First the control measurements were obtained (Figure 1A), then bertosamil (70 μM) was washed into the bath for 10 min, before measuring with bertosamil (Figure 1B). From a holding potential of −80 mV test pulses from −80 to +80 mV in 10 mV increments (400 ms) were applied at a frequency of 0.2 Hz to measure activating currents. Each pulse was followed by a constant return pulse to −60 mV (400 ms) to evoke outward tail currents. HERG currents had an activation threshold of −40 mV, reached a current maximum at 10 mV before a considerable current reduction at higher test pulse amplitudes could be measured, as a result of inward rectification. Figure 1C shows the current-voltage relationship (I–V-curve) at the end of the test pulse. After application of 70 μM bertosamil (Figure 1B), the peak current amplitude (at 10 mV) was reduced by 52.5 ± 6.3% ($n = 4$; Figure 1C).

The amplitude of the outward tail currents in the return pulse partially exceeded the amplitude of the activating current due to slow activation, fast inactivation, rapid recovery from inactivation and slow deactivation. (This has been described elsewhere (Kiehn *et al*., 1996; 1999b; Sanguinetti *et al*., 1995; Spector *et al*., 1996). Tail currents saturated after a test pulse amplitude of +30 mV or above (Figure 1D). Bertosamil in a concentration of 70 μM reduced the peak tail current amplitude (after a test pulse to 30 mV) by 49.3 ± 8.4% ($n = 5$; Figure 1D). Normalized activating and normalized tail currents for control and 70 μM bertosamil were scaled in Figure 1E,F. Bertosamil had no effect on the

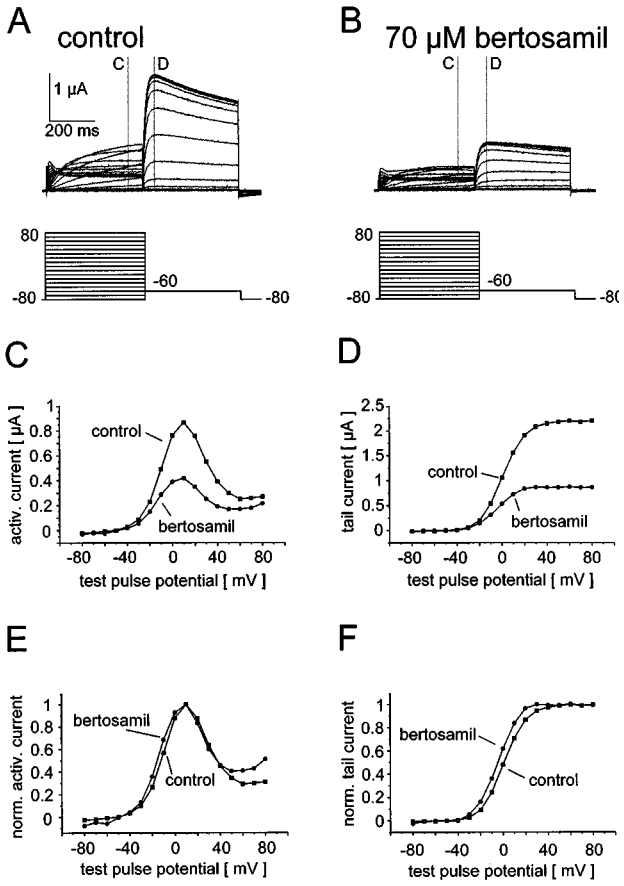

Figure 1 Inhibition of HERG channels by bertosamil. Control currents (A) and inhibition by 70 μM bertosamil of HERG currents heterologously expressed in *Xenopus* oocytes (B). Corresponding activating current amplitude at the end of the first test pulse as a function of the test pulse potential (C). The maximal current amplitude at +10 mV was reduced by 49.4%. (D) Tail current amplitudes as a function of the preceding test pulse potential. The peak tail current after the test pulse to +30 mV was reduced by 55.1%. Normalized activating (E) and tail currents (F). Normalized tail currents show a small shift of −4.02 mV of the half-maximal activation voltage $V_{1/2}$. Protocol: Holding potential −80 mV, test pulse −80 mV to +80 mV (400 ms) in 10 mV-increments, return pulse constant −60 mV (400 ms).

I–V-relationship of the activating current and it caused only a small shift of the activation curve of HERG tail currents which is described in detail later in this text.

Concentration dependence of block

The concentration-dependence of bertosamil-induced inhibition was measured on HERG K^+ tail current amplitudes. From a holding potential of −80 mV, test pulses to +20 mV (2000 ms) and return pulses to −40 mV (1600 ms) were applied to elicit large, slowly decaying outward tail currents (Figure 2A). The effect of bertosamil on the tail current at −40 mV was concentration dependent, i.e. bertosamil at a concentration of 1, 10, 70 , 100, 300 and 1000 μM reduced the tail currents by 2.2±2.8%, 4.8±4.4%, 51.6±3.1%, 64.4±3.0%, 90.4±0.6% and 96.8±1.1%, respectively ($n=5$). The normalized dose response curve was fitted with the Hill equation and gave an IC_{50} of 62.7 μM; the Hill coefficient was 1.53 (Figure 2B).

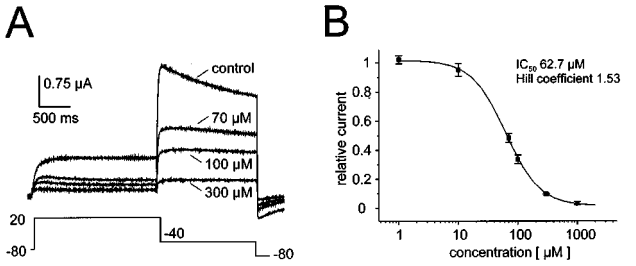

Figure 2 Concentration-dependence of block. (A) HERG activating and outward tail currents under control conditions and after superfusion with different concentrations of bertosamil (70, 100 and 300 μM). (B) Concentration response curve fitted with the Hill equation: The IC_{50} was 62.7 μM. Protocol: repetitive pulsing at a frequency of 0.1 Hz, holding potential −80 mV, test pulses to +20 mV (2000 ms) and return pulses to −40 mV (1600 ms) Mean±s.e.m. ($n=5$).

Onset of block and wash-out of the bertosamil-effect

The onset of block by bertosamil was analysed in further detail. From a holding potential of −80 mV and at a frequency of 0.07 Hz, test pulses to +30 mV (400 ms) were applied before a second pulse to −60 mV (400 ms) followed, to elicit outward tail currents (Figure 3A). Peak tail currents were measured to determine the amount of block. After a control period of 3 min, which demonstrated the stability of the experimental conditions, 100 μM bertosamil was applied by perfusing the bath. The onset of block was fast, i.e. 50% of the maximal inhibition occurred within 54±3.67 s, 75% within 93±3.0 s, and 90% within 180±8.22 s ($n=5$). Steady-state block was obtained after 3 min of drug application, with a further increase of inhibition (less than 10%) within the following 9 min (Figure 3C).

Wash-out of bertosamil followed the same protocol (Figure 3B). After observation of steady-state block (for 3 min), the drug was washed out with the bath solution. The inhibitory effect of bertosamil was completely reversible within 6 min (Figure 3C) with 50% recovery from block after 135±21.2 s, and a complete recovery after 294±38.7 s ($n=5$; Figure 3D).

Bertosamil blocks HERG channels mainly in the open state and in the inactivated state

For HERG channels, a five-state-model has been suggested (open, inactivated and three closed states (Kiehn *et al.*, 1999b)). In this study, two different approaches were chosen to investigate the state-dependent block of HERG channels by bertosamil.

A first voltage protocol served to open the channels by a single long test pulse from −80 mV to 0 mV (7500 ms). After the control measurement, HERG channels were kept in the closed state at a potential of −80 mV, and 70 μM bertosamil was perfused into the bath for 10 min. Then the step protocol was repeated under bertosamil ($n=5$; Figure 4A). By division of currents and presentation in a normalized form, the time-course of relative block was obtained (Figure 4B). The block was phasic from the beginning of the test pulse, i.e. the relative block was 0 at 0 s and developed steadily within 2 s, an argument for open- and against closed-channel block. The development of block was fast, as approximately 80% of block were achieved within the first second. In contrast, the

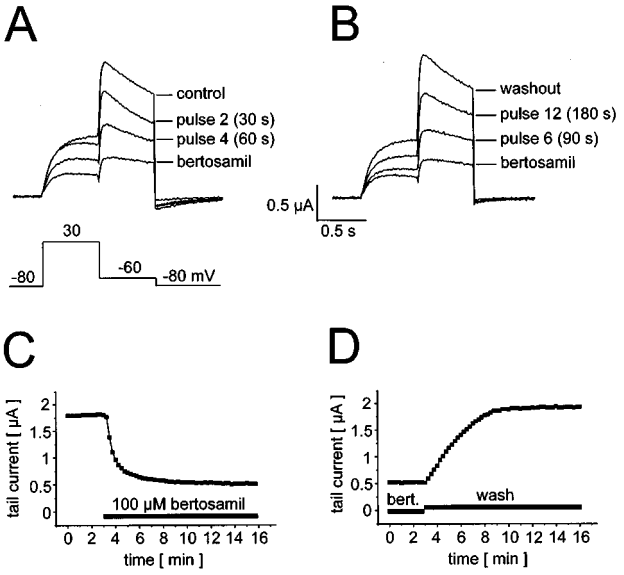

Figure 3 Time course of onset and wash-out of block. After 3 min of control, demonstrating the stability of experimental conditions, 100 μM bertosamil was perfused into the bath. Superfusion with bertosamil for 3 min resulted in a tail current reduction of 65.2%. After 12 min the inhibition reached steady-state with 71.1% inhibition (A and C). Block was completely reversible after a 6-min wash out period (B and D). Protocol: repetitive pulsing at a frequency of 0.07 Hz, holding potential -80 mV, first pulse to $+30$ mV (400 ms), followed by a second pulse to -60 mV (400 ms), representative experiment.

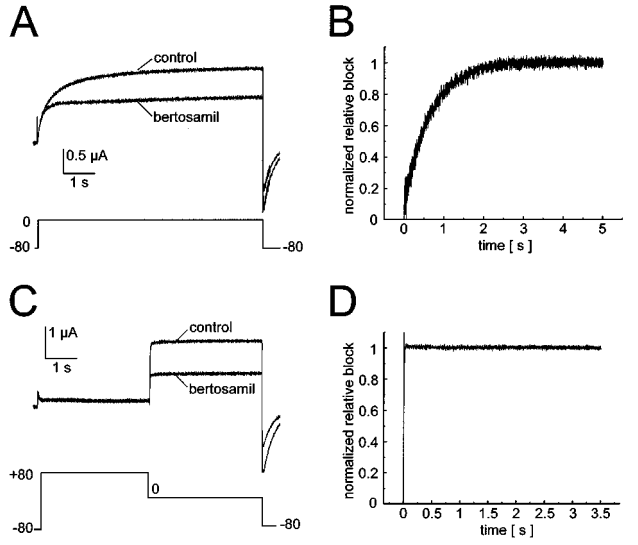

Figure 4 Bertosamil blocks HERG channels mainly in the open and inactivated states. Channels were held at -80 mV for 5 min, i.e. in their closed state, before a test pulse to 0 mV (for 7500 ms) was applied in the presence of the drug. Shown are overlay experiments (A) for control and after incubation with 70 μM bertosamil (for 10 min, without any intermittent test pulse). (B) Normalized relative block is plotted versus time after the voltage step to 0 mV. This gave the bertosamil-sensitive current which showed phasic development of complete inhibition, a sign for open channel block. (C) In a second approach, HERG channels were inactivated by a first voltage step to $+80$ mV (3500 ms), before opening by a second step to 0 mV (3500 ms) followed. Corresponding normalized relative block is shown in (D). During the first voltage step in the inactivated state, maximal block was already achieved in the inactivated state with no further development of open channel block.

step protocol investigating the onset of bertosamil block showed 50% of the maximal inhibition after 54 s only. In this protocol, the test pulses (400 ms) were shorter and intervening pulses back to -80 mV closed the channels. Although, the closed state is not a target for bertosamil-dependent block of HERG channels. Therefore, a whole train of short pulses is necessary to obtain comparable effects as one long pulse.

To address the question whether HERG channels are blocked in the inactivated state, a long test pulse was applied from -80 mV to $+80$ mV (3500 ms) to inactivate the channels. In a second test pulse to 0 mV (3500 ms), the channels were opened ($n=4$). Typical current traces under control conditions and after 10 min incubation with 70 μM bertosamil while holding the cell at -80 mV are displayed in Figure 4C and relative block is displayed in Figure 4D. Block by bertosamil is obtained during the inactivated state and block had already reached its maximum when the channels were opened by the second step. From these experiments, we conclude that bertosamil blocks HERG channels mainly in the open state and in the inactivated state, but not in closed states.

HERG current activation is not markedly affected by bertosamil

Activation curves were obtained with a protocol, where variable test pulses were applied ranging from -120 mV to $+100$ mV (400 ms) in 10 mV-increments, measuring the corresponding inward tail current amplitude at -120 mV (400 ms) for control (Figure 5A) and 70 μM bertosamil (Figure 5B). Inverted peak tail amplitudes of $n=8$ cells were normalized and statistically analysed. Plotted as a function of the preceding test pulse potential (Figure 5C), these curves were a measure of steady-state activation. As an effect of bertosamil, a slight shift of the mean half-maximal activation voltage $V_{1/2}$ by 3.07 ± 0.36 mV towards more negative potentials was observed, which was significant ($P<0.05$ for $n=8$). After a wash-out period of 10 min, the effect was completely reversible. However, this small effect is not expected to contribute considerably to the effects of bertosamil *in vivo*.

Effects of bertosamil on inactivation of HERG currents

Two different protocols were used to analyse inactivation of HERG currents. Firstly, the effect of bertosamil on time course of inactivation was investigated. From a holding potential of -80 mV, a long test pulse to $+40$ mV (900 ms) was applied to inactivate the channel. Short pulses to -100 mV (16 ms), and voltage steps to potentials ranging from -60 mV to $+40$ mV (150 ms, in 20 mV-increments) followed to elicit large outward inactivating currents. Control inactivating currents (Figure 6A) and currents under 70 μM bertosamil (Figure 6B) were fitted with single exponential functions to obtain time constants (Figure 6C, $n=5$). The two graphs describing the time constants under control conditions and with bertosamil were significantly different ($P<0.05$).

In a second approach, steady-state inactivation currents for control and bertosamil were measured with the following protocol: Channels were inactivated at a holding potential of

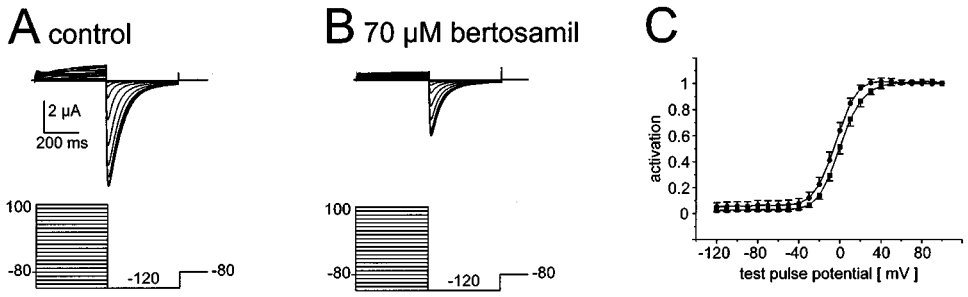

Figure 5 Bertosamil does not markedly affect HERG current activation. Activation curves of HERG K^+ channels were measured in a protocol with inward tail currents in a representative experiment for control (A) and with 70 μM bertosamil (B). In (C) the inverted tail current amplitude is displayed as a function of the previous test pulse potential resulting in an activation curve. Bertosamil caused a small negative shift of the half-maximal activation voltage $V_{1/2}$ by 3.0 ± 0.36 mV ($n=8$). Protocol: Holding potential -80 mV, test pulses from -120 mV to $+100$ mV in 10 mV-increments (400 ms), followed by a constant pulse to -120 mV (400 ms) to elicit inward tail currents.

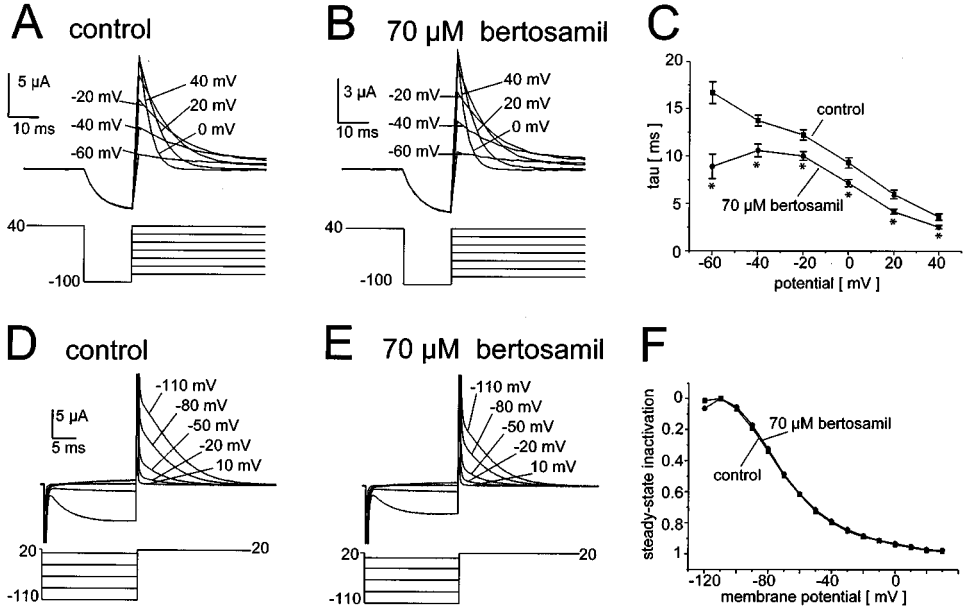

Figure 6 Effects of bertosamil on the inactivation of HERG currents. Direct inactivating currents were measured for control (A) and with bertosamil (B) and time constants for direct inactivation are displayed as a function of voltage in (C). Bertosamil caused significant changes in direct inactivation time constants ($P<0.05$). Protocols for A, B, C: Test pulse 1 $+40$ mV (900 ms), test pulse 2 to -100 mV (16 ms), inactivating currents were elicited by a test pulse 3 to potentials ranging from -60 mV to $+40$ mV (150 ms) in 20 mV-increments. The holding potential was -80 mV. *Significant difference between bertosamil and control ($P<0.05$). Steady-state inactivation was measured for control (D) and after incubation with 70 μM bertosamil (E). Outward current amplitudes were normalized and plotted as a function of the preceding test pulse potential in (F). Only a small shift of the inactivation curve was observed in $n=7$ experiments. Protocol: Holding potential $+20$ mV, test pulse potentials ranging from -120 mV to $+10$ mV in 10 mV-increments (15 ms). Return pulses to $+20$ mV evoked outward inactivating currents.

$+20$ mV before short test pulses to potentials ranging from -120 mV to $+10$ mV (15 ms, 10 mV-increments) were applied to recover the channels from inactivation. Returning to the holding potential of $+20$ mV after these test pulses evoked large outward inactivating currents. After having obtained a measurement under control conditions, the oocyte was clamped at a holding potential of -80 mV during a wash-in period of 10 min (70 μM bertosamil), necessary to avoid destruction of the cell which occurs if it is clamped at $+20$ mV for several minutes. Typical recordings before (Figure 6D) and after exposure to bertosamil (Figure 6E) are displayed. Outward current amplitudes measured 2 ms after the return to the holding potential were normalized and fitted to a Boltzmann function, which did not elicit any significant shift in the steady-state inactivation curve ($n=7$, Figure 6F).

Bertosamil block of HERG currents shows voltage-dependence

To find out whether the effect of bertosamil on HERG current varies with voltage, the following voltage protocol was used: From a holding potential of -80 mV, where HERG currents are in the closed state, a long depolarizing pulse to potentials ranging from -40 mV to $+80$ mV was applied (35 s) to reach steady-state conditions. A subsequent

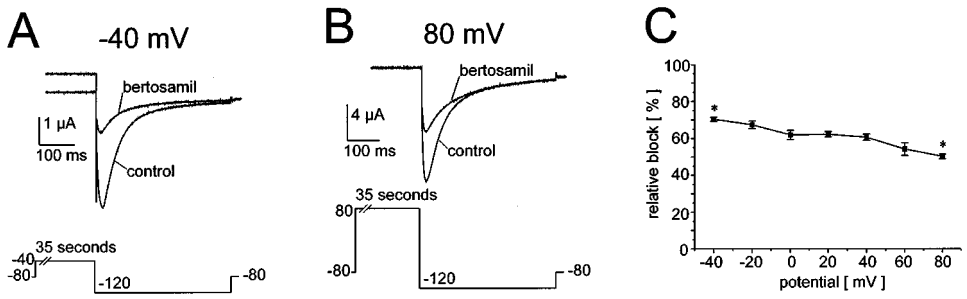

Figure 7 Bertosamil block of HERG currents shows significant voltage dependence. Representative current traces after long depolarizing pulses to −40 mV (A) and +80 mV (B), before and after superfusion with 100 μM bertosamil for 10 min. Relative block of peak tail currents is plotted as a function of various test pulse potentials in (C). Only a small decrease of block could be observed at more positive potentials ($n=4-5$) with a significant difference between −40 and +80 mV. Protocol: Holding potential −80 mV, after a long depolarizing test pulse to potentials ranging from −40 to +80 mV (35 s), peak tail currents were measured during a repolarizing step to −120 mV (400 ms). *These values are significantly different.

repolarizing step to −120 mV (400 ms) was used to elicit inward tail currents. Only one experiment at each potential could be carried out with a single oocyte, as mainly open channels are blocked by bertosamil and unblocking is slow. After control (Figure 7A), the cell was exposed to 100 μM bertosamil for 10 min at −80 mV without pulsing and then the protocol was repeated (Figure 7B). The peak inward tail currents, which were elicited by the short second pulse, were measured to determine the amount of block ($n=4-5$, Figure 7C). Bertosamil caused strong inhibition at all potentials, with a slightly weaker block at more positive potentials. As HERG channels are mainly in the inactivated state at +60 mV and +80 mV, this is possibly due to a lower affinity of bertosamil to inactivated channels. However, significant voltage dependence of bertosamil block was observed, as values at −40 mV ($70.4\pm1.1\%$, $n=4$) and +80 mV ($50.2\pm1.3\%$, $n=5$) were significantly different ($P<0.05$).

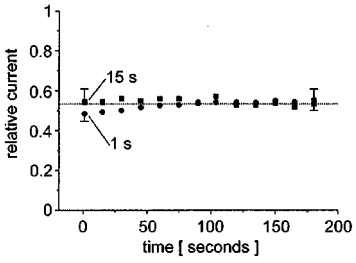

Figure 8 Bertosamil block of HERG channels is not frequency dependent. Pulses were applied at time intervals of 1 s and 15 s under control conditions and after exposure to 70 μM bertosamil to investigate frequency-dependence. In four experiments frequency dependence of block was observed. For 1-s intervals, only every fifteenth measurement is displayed and only four error bars are displayed to achieve a clearer presentation. Protocol: From a holding potential of −80 mV, test pulses to +20 mV (300 ms) were applied to activate HERG currents, and tail currents were elicited by a second step to −40 mV (300 ms).

Bertosamil block is not frequency-dependent

To investigate frequency-dependence, a short voltage protocol was repeated at intervals of 1 s and 15 s (for 180 s). From a holding potential of −80 mV, a depolarizing test pulse to 20 mV (300 ms) was used to activate HERG currents, and a second repolarizing step to −40 mV (300 ms) elicited tail currents. Peak tail current amplitudes were measured to determine the amount of block.

After control, the oocyte was exposed to 70 μM bertosamil for 10 min without pulsing. Only one oocyte was used at each frequency. Seventy μM Bertosamil reduced peak tail currents by $44.5\pm5.3\%$ (1 s) and $46.7\pm7.5\%$ (15 s), respectively ($n=4$, Figure 8). Therefore, neither the development of block nor the amount of steady-state block was frequency-dependent. The lack of frequency-dependence can be interpreted as the result of fast onset of open channel block within the first one or two pulses, and a slow unblocking.

Discussion

This study demonstrates that the class-III anti-arrhythmic drug bertosamil blocks the HERG potassium channel with an IC_{50} of 62.7 μM. The onset of block was fast and inhibition was completely reversible upon washout. Bertosamil affected HERG potassium channels mainly in their open and inactivated states. The effect was slightly voltage- but not use-dependent. There was no relevant effect of the drug on HERG channel activation. The time-course of inactivation was influenced significantly.

Bertosamil has been developed as a bradycardic, anti-arrhythmic and anti-ischemic drug. However, little information is available on the pharmacological properties of this compound, e.g. the drug distribution to predict effective plasma concentrations in animals or humans is not available. Only application doses are given in the literature. Bertosamil at concentrations of 0.5–5.0 μmol kg^{-1} caused elevation of the blood pressure, reduction of heart rate and possessed ventricular and atrial anti-arrhythmic activity in anaesthetized cats (Papp *et al.*, 1992). Beside the block of HERG-potassium channels, demonstrated in this study, bertosamil blocks other ion channels in the heart as well. It can be speculated about individual pharmacological targets and properties of bertosamil supported by the distribution of the targets. Atrial anti-arrhythmic effects of bertosamil might be explained by inhibition of the potassium channels $Kv_{1.2}$, $Kv_{1.4}$ and $Kv_{1.5}$ (Yamagishi *et al.*, 1995; Godreau *et al.*, 2002) as these channels resemble native correlates in atrium (Boyle

Table 1 Comparison of different HERG blockers

Characteristics of block	*BRL-32872*	*Dofetilide*	*Amiodarone*	*dl-Sotalol*	*d-Sotalol*	*Carvedilol*	*Bertosamil*
IC_{50}	241.5 nM	595 nM (30 nM)	9.8 μM	ca. 900 μM	ca. 900 μM (ca. 200 μM)	9.8 μM	62.7 μM
State	Open, (inactivated)	Open	Open, closed, inactivated	(Open)	Open, block stabilized by inactivation	Open	Open and inactivated
Voltage-dependence	–	(+)	+	?	?	–	(+)
Sidedness of block	Membraneous, pore-trapping	Cytoplasmatic	Cytoplasmatic membraneous	?	Membraneous, pore-trapping	(Cytoplasmatic)	?
Kinetics	Fast	Slow (τ ca. 12 s)	Medium (τ ca. 1.3 s)	?	Fast	Fast (τ ca. 0.1 s)	Fast
Use-dependence	Positive	(Reverse)	Positive	(Reverse)	(Reverse)	–	–
Additional characteristics	Ca^{2+}-channel blockade	–	β-blocker, Na^+, Ca^{2+}	β	–	β	Ca^{2+}
Clinical outcome	Unknown	Neutral	Positive	Neutral	Negative	Positive	Unknown

& Nerbonne, 1991; Escande *et al*., 1987; Godreau *et al*., 2002; Shibata *et al*., 1989). For $Kv_{1.5}$, the IC_{50} of bertosamil-dependent block was 39 μM for the transient component of current and 11 μM for the sustained component, i.e. values in the same range as the IC_{50} of HERG-block in this study. These effects were use-dependent; however, they were explained by both an open-channel block and an acceleration of the rate of channel inactivation. This mechanism resembles the mode of bertosamil-action on HERG.

For $Kv_{1.2}$, the IC_{50} of bertosamil-dependent block was 354.8 μM, for $Kv_{1.4}$, it was 323.6 μM, i.e. values much higher than for HERG block in this study. Part of the atrial effects might derive from accelerated inactivation of sustained outward current which leads to an apparent increase of its transient component and to a decrease of its sustained component in human atrial myocytes (Tessier *et al*., 1997). This effect could represent a new anti-arrhythmic mechanism in the management of atrial dysrhythmias, i.e. atrial fibrillation or atrial flutter.

In electrophysiological studies, bertosamil decreased the maximum rate of depolarization, lengthened the action potential duration, and prolonged the effective refractory period markedly in atrial and ventricular myocardial cells of the rabbit (Krassoi & Papp, 1992).

Bertosamil caused transient positive, followed by continuous negative, chronotropic responses and positive inotropic responses in atria, and increased the left ventricular contractile force in isolated dog heart preparations (Yonezawa *et al*., 1996). Effects were explained by inhibition of negative cardiac responses mediated by an ATP-sensitive K^+ channel but not by the acetylcholine muscarinic receptor. At high dose, bertosamil attenuates the L-type Ca^{2+} channel-mediated positive cardiac responses in isolated dog hearts (Yonezawa *et al*., 1996). However, clinical data about effects of bertosamil in human has not been provided so far.

In summary bertosamil shows HERG channel block, demonstrated in this study. In contrast to many pure class-III anti-arrhythmic drugs (dofetilide, d-sotalol, see Table 1) with strong pro-arrhythmic side effects such as 'torsade de pointes' tachycardias, bertosamil shows additional pharmacological actions, such as Ca-channel blockade at higher concentrations which might prevent Ca-overload and proarrhythmias making this compound saver than pure HERG channels blockers.

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