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Mechanism of block by tedisamil of transient outward current in human ventricular subepicardial myocytes

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1 Tedisamil is a new antiarrhythmic drug with predominant class III action. The aim of the present study was to investigate the blocking pattern of the compound on the transient outward current (I_{to}) in human subepicardial myocytes isolated from explanted left ventricles. Using the single electrode whole cell voltage clamp technique, I_{to} was analysed after appropriate voltage inactivation of sodium current and block of calcium current.

2 Tedisamil reduced the amplitude of peak I_{to}, but did not affect the amplitude of non-inactivating outward current. The drug accelerated the apparent rate of I_{to} inactivation. The reduction in time constant of I_{to} inactivation depended on drug concentration, the apparent IC_{50} value was 4.4 μM.

3 Tedisamil affected I_{to} amplitude in a use-dependent manner. After 2 min at -80 mV, maximum block of I_{to} was reached after 4–5 clamp steps either at the frequency of 0.2 or 2 Hz, indicating that the block was not frequency-dependent in an experimentally relevant range. Recovery from block was very slow and proceeded with a time constant of 12.1 ± 1.8 s. Also in the presence of drug, a fraction of channels recovered from inactivation with a similar time constant as in control myocytes (i.e. 81 ± 40 ms and 51 ± 8 ms, respectively, n.s.).

4 From the onset of fractional block of I_{to} by tedisamil during the initial 60 ms of a clamp step, we calculated $k_1 = 9 \times 10^6$ mol^{-1} s^{-1} for the association rate constant, and $k_2 = 23$ s^{-1} for the dissociation rate constant. The resulting apparent K_D was 2.6 μM and is similar to the IC_{50} value.

5 The effects of tedisamil on I_{to} could be simulated by assuming a four state channel model where the drug binds to the channel in an open (activated) conformation. It is concluded that in human subepicardial myocytes tedisamil is an open channel blocker of I_{to} and that this effect probably contributes to the antiarrhythmic potential of this drug.

Keywords: Human cardiac myocytes; voltage clamp; transient outward current; I_{to}, tedisamil; use-dependent block

Introduction

Patients with ischemic heart disease are particularly susceptible to episodes of ventricular tachycardia that may even culminate in sudden cardiac death. Attempts to prevent these life-threatening arrhythmias with drug treatment have not been rewarded with convincing success, because antiarrhythmic agents currently in clinical use may themselves dispose to proarrhythmic effects. Some sodium channel blockers like flecainide and encainide have actually *increased* the incidence of sudden cardiac deaths in patients after myocardial infarction (The Cardiac Arrhythmia Suppression Trial [CAST] Investigators, 1989). In search for new drugs with more favourable benefit-risk ratio, substances that prolong the cardiac action potential duration (APD) have received considerable attention as potential antiarrhythmic agents (for review see Woosley, 1991). Since the shape of the action potential is determined by the balance of several ionic currents, APD can, theoretically, be prolonged by increased or persisting inward current, or by reduced outward current, however, the most common mechanism of APD prolongation is blockade of K^+ currents.

Tedisamil (3,7-di(cyclopropylmethyl)-9,9-tetramethylene-3,7-diazabicyclo-[3.3.1]-nonane dihydrochloride) was originally developed as a bradycardic agent (Öxle *et al*., 1987) and subsequently found to be effective in patients with chronic stable angina, i.e. exhibiting antiischemic effects (Mitrovic *et al*., 1992; Raberger *et al*., 1992; Thormann *et al*., 1993). Tedisamil prolongs QT-interval of the electro-cardiogram in humans (Mitrovic *et al*., 1992) and primates (Adaikan *et al*., 1992) and this effect corresponds to prolongation of APD at the tissue level (Dukes & Morad, 1989; Ohler & Ravens, 1994). Tedisamil alters several ionic conductances involved in the shape of the action potential, i.e. transient outward current I_{to}, delayed rectifier potassium current I_K, and at higher concentrations, also sodium current I_{Na} (for recent review see Faivre *et al*., 1995). In addition, neuronal (Dukes *et al*., 1990) and vascular K^+ currents are also blocked (Pfründer & Kreye, 1991; 1992; Bray & Quast, 1992). The inward rectifier I_{K1} is not affected by tedisamil (Dukes & Morad, 1989; Dukes *et al*., 1990).

Potential antifibrillatory actions of tedisamil have been reported in ischaemia models of rat hearts (Bril *et al*., 1993; Rees *et al*., 1993), but it is not clear whether highly selective or less specific blockers should provide the better strategy against ischaemia-related arrhythmias. Since the APD-prolonging effect in rat myocardium is related to block of I_{to} which in this species is the most prominent outward current in subepicardial myocytes, the antifibrillatory protection could result from a selective inhibition of I_{to}. I_{to} is also the major outward current in human myocytes (Wettwer *et al*., 1994;

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Amos *et al*., 1996). Despite similar biophysical and pharmacological properties of I_{to} in rat and man (Wettwer *et al*., 1993), they appear to be encoded by different K^+ channel genes. While in rat heart both Kv/4.2 and Kv/4.3 contribute to I_{to}, Kv/4.3 seems to be the more abundant channel type in human heart (Dixon *et al*., 1996). In addition, the pharmacological profile of channels passing Ca^{2+} independent I_{to} may vary as shown for Kv1.4 and Kv4.2 (Yeola & Snyders, 1997) and this could also apply to different channels in the Kv4 group.

Therefore, the effects of tedisamil on I_{to} in human ventricular myocytes cannot be extrapolated from the rat or any expression system but require a separate study to characterize the nature of the putative block. Furthermore, a previous report of the effects of tedisamil on outward currents in human heart revealed differences in I_{to} block between atrial and ventricular myocytes (Ravens *et al*., 1997). However, this earlier study concentrated on atrial currents, hence I_{to} block by tedisamil in ventricular myocytes is still to be characterized. This detailed analysis is provided here. Since the amplitude of I_{to} varies in cells from different locations within the heart (Wettwer *et al*., 1994; Näbauer *et al*., 1996; Kääb *et al*., 1996), only myocytes of subepicardial origin were used. Some of the results have been published in preliminary form (Wettwer *et al*., 1995).

Methods

Ventricular myocardial tissue was obtained from seven transplant recipients suffering from severe heart failure (NYHA III–IV) because of ischaemic or dilative cardiomyopathy and from two donor hearts which were not suitable for transplantation. Details of diagnosis and drug treatment are listed in Table 1. Written informed consent was obtained from all transplant recipients prior to surgery and the investigation complies with the principles outlined in the Declaration of Helsinki.

The tissue specimens were transported to the laboratory in cold cardioplegic solution supplemented with 2,3-butanedione monoxime (30 mM; Wettwer *et al*., 1994). The subepicardial layers of the tissue, e.g. the first 3 mm below the epicardial surface were cut into small cubic chunks of 1 mm side length. The pieces were washed three times in nominally Ca^{2+}-free solution containing (in mM) NaCl, 100; KCl, 10; KH_2PO_4, 1.2; $MgSO_4$, 5; MOPS, 5; taurine, 50; glucose, 20; pH 7.0 at 37°C. They were then stirred for 40 min in enzyme solution containing type-I collagenase 1.5 mg ml^{-1}, type-III trypsin 1.0 mg ml^{-1}, bovine serum albumin 10 mg ml^{-1} (all from Sigma Chemicals). The chunks were then washed in Ca^{2+}-free solution supplemented with albumin (1.0 mg ml^{-1}) for 10 min and digestion was continued by incubation with a second enzyme solution (type-I collagenase 0.5 mg ml^{-1}). When elongated myocytes with clear cross striations appeared in the supernatant (within up to 150 min), the cells were harvested by washing twice in Ca^{2+}-free solution, in which the myocytes were also stored. The Ca^{2+} concentration ($[Ca^{2+}]$) was then slowly increased to a final concentration of 0.5 mM.

The myocytes were investigated as described in detail by Amos *et al*. (1996). In brief, a drop of cell suspension was placed in a small chamber perfused with modified Tyrode solution (1–2 ml min^{-1}, temperature 22–24°C). The composition of the solution was (in mM): NaCl, 150; KCl, 5.4; $MgCl_2$, 2.0; $CaCl_2$, 0.5; HEPES, 10; glucose, 10; pH 7.4. To block calcium current (I_{Ca}), the superfusion solution was supplemented with 0.1 mM $CdCl_2$.

Membrane currents were measured with the single-electrode, whole-cell voltage clamp technique using a List EPC-7 amplifier (List-Medical, Darmstadt, Germany) and pClamp software (Axon Instruments, Foster City, CA, U.S.A.). Electrodes pulled from filamented borosilicate glass had tip resistances of 2–4 MΩ when filled with electrode solution (in mM): KCl, 130; $MgCl_2$, 4; $CaCl_2$, 5; HEPES, 10; EGTA, 10; Na_2-ATP, 4; pH 7.3; free $[Ca^{2+}]$ 50 nM, free $[Mg^{2+}]$ 300 μM. Access resistance was kept below 10 MΩ and series resistance was compensated by at least 70%. With a current amplitude of 1 nA, the potential error due to uncompensated series resistance amounted to less than 5 mV. Cell capacitance was measured before compensation using

Table 1 Origin of ventricular tissue for all cells investigated. Patient-related data from transplant recipients (1–7) and donors (8, 9)

Pat.	*Sex*	*Age* (yrs)	*Weight* (kg)	*Height* (cm)	*NYHA*	*Diagn.*	*Drugs*
1	m	52	60	180	IV	DCM	dobutamine, metoprolol β-acetyldigoxin, enoximone
2	f	59	61	168	III	RCM AVD	n.a.
3	m	58	57	165	III-IV	ICM	β-acetyldigoxin, captopril, furosemide, spironolactone, ISDN
4	m	50	72	170	IV	DCM	metildigoxin, captopril, spironolactone, furosemide, allopurinol
5	m	58	76	185	III	DCM	digitoxin, furosemide, ISMN, enalapril, hydrochlorothiazide
6	m	55	69	178	III	DCM	β-acetyldigoxin, furosemide, spironolactone, ramiprilate
7	f	57	75	n.a.	n.a.	n.a.	n.a.
8	m	37	n.a.	n.a.	–	SAB	norepinephrine, dopamine, desmopressin, lidocaine
9	m	37	n.a.	n.a.	–	n.a.	dopamine, desmopressin, lidocaine

AVD, aortic valve disease; DCM, dilated cardiomyopathy; ICM, ischaemic cardiomyopathy; ISDN, isosorbide dinitrate; ISMN, isosorbide mononitrate; RCM, restrictive cardiomyopathy; SAB, subarachnoidal bleeding; n.a., not available.

depolarizing ramps (1 V/s) from −40 to −35 mV (Amos *et al*., 1996). Up to 100 pF of cell capacitance was compensated.

Current amplitudes were either expressed in absolute values (pA) or corrected for cell size by dividing by cell capacitance (pA/pF). Values were given as means±s.e.mean, where the number of experiments *n* was given as number of cells/number of hearts. Fitting of equations to experimental data was performed using pClamp software or Prism (Graphpad Software, San Diego, CA, U.S.A.). Differences were tested for statistical significance using paired or unpaired Students *t*-tests as required. Significance was assumed if $P<0.05$.

Results

Concentration-dependence of I_{to} block by tedisamil

In order to separate the transient outward current I_{to} from overlapping currents, L-type I_{Ca} was blocked with Cd^{2+} (0.1 mM) and I_{Na} was inactivated by 20 ms long prepulses to −40 mV. Typical current traces recorded in human ventricular myocytes in response to depolarizing clamp steps to test potentials between −40 and +60 mV are shown in Figure 1A. Addition of tedisamil (10 μM) to the superfusion solution reduced the amplitude of the peak outward current and accelerated its inactivation time course, but left the non-inactivating current at the end of the 300 ms long clamp steps (I_{late}) essentially unaffected. Figure 1B shows the current-voltage relation for the peak and late outward current before and 5 min after addition of tedisamil. Between 0 and +60 mV, peak current amplitude decreased, whereas I_{late} was not changed. In 6 myocytes obtained from three hearts, the mean peak I_{to} current density at +60 mV was reduced from 10.0±2.6 pA/pF before to 6.7±1.5 pA/pF after 5 min of superfusion with 10μM tedisamil ($P=0.09$), the respective values for I_{late} were 2.4±0.5 pA/pF and 2.5±0.7 pA/pF (n.s.). However, the effect on peak current amplitude was too small for construction of a reliable concentration response curve.

Inactivation of I_{to} during an activating clamp step followed an exponential time course (Figure 2A). Computer-assisted curve fitting to the experimental data resulted in the best fit of a monoexponential function. The time constant of inactivation τ_i depended on test potential resulting in a shallow U-shaped curve (Figure 2B). Tedisamil (10 μM) significantly reduced τ_i in the whole voltage range studied with significantly larger residual values at 0 mV than at test potentials positive to +20 mV. This apparent inactivation-accelerating effect of tedisamil was concentration dependent, the IC_{50} value was 4.4 μM (Figure 2C).

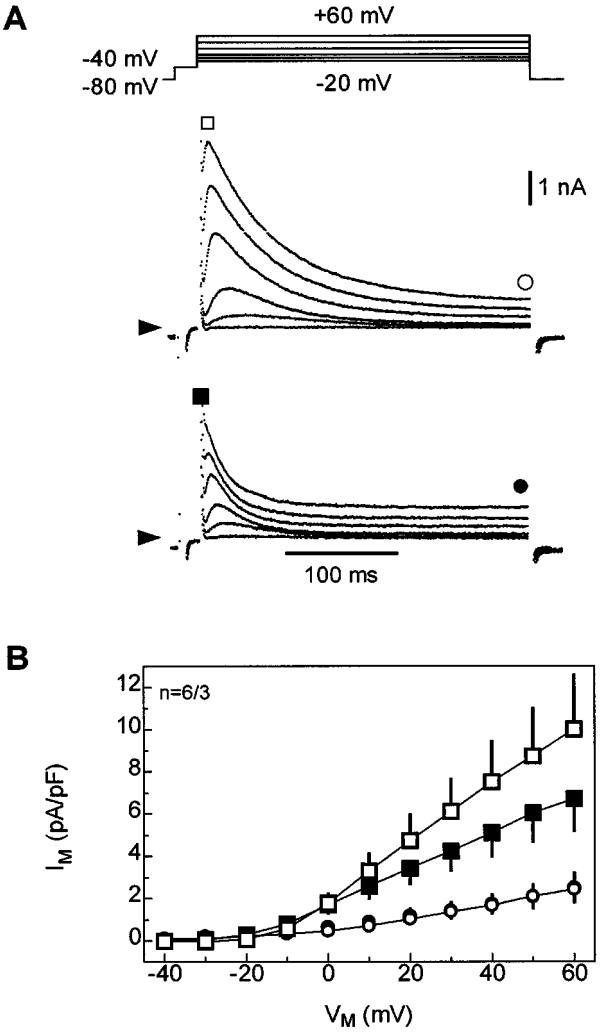

Figure 1 Effects of tedisamil on I_{to} of human ventricular myocytes. (A) Current traces obtained with 300 ms long clamp steps in the range of −20 to +60 mV from a holding potential of −80 mV. A short prepulse to −40 mV was used to inactivate I_{Na}. The top family of traces were obtained before, the bottom traces after 5 min of exposure to 10 μM tedisamil. At all potentials currents inactivated with a single exponential time course (see Figure 2). The arrow heads point at zero current. Calibrations as indicated. (B) Current voltage relations of I_{to} before (open symbols) and after exposure to tedisamil (closed symbols). Squares indicate peak outward current, circles indicate late current. Means±s.e.mean of six myocytes from three failing hearts.

Use-dependent block of I_{to}

Acceleration of I_{to} inactivation can be explained by preferential drug binding to channels in their open state with channel block as a consequence of this binding. If the channels do not fully recover during the interval between successive activation, 'use-dependency' of block is expected. We have tested this hypothesis by the following protocol. Myocytes were clamped to −80 mV for 2 min before I_{to} was activated at a frequency of 2 or 0.2 Hz. Under control conditions, the mean amplitude of peak I_{to} was 9.0±1.5 pA/pF with the first and 9.1±1.5 pA/pF with the eighth clamp step in a train of pulses at 2 Hz ($n=7$), the respective values were 7.6±0.8 pA/pF and 7.7±0.9 pA/pF at 0.2 Hz ($n=6$). Lack of decline in I_{to} amplitude with subsequent pulses under control conditions indicates that the intervals of 180 and 4680 ms between clamp steps (at 2 and 0.2 Hz, respectively) were sufficient for complete I_{to} recovery from inactivation without drug. In the presence of tedisamil (5 μM), peak I_{to} amplitude decreased from 8.0±1.0 pA/pF (first clamp step) to 5.7±0.7 pA/pF (eighth clamp step at 2 Hz, $n=5$), the respective values at 0.2 Hz were 8.7±1.8 and 5.4±1.1 pA/pF ($n=3$), a representative experiment is depicted in Figure 3. Figure 4A shows the time course of the development of tedisamil block after 2 min of 'rest', when currents are normalized to the first amplitude in the train. The block developed faster at 2 Hz than at 0.2 Hz, the respective time constants for block development were 0.7±0.2 s and 3.8±0.1 s. Plotting the development of block as a function of pulse number (Figure 4B) reveals that the block reached a steady state after five to eight clamp steps at both pulse frequencies. Thus the blocking effect of tedisamil on peak I_{to} was clearly use-dependent. However, since a similar number of

clamp steps was required for steady-state block at 0.2 and 2.0 Hz, it is expected that only minor recovery from block occurs during the intervals between steps at these two frequencies. Therefore, complete recovery should require much more time.

Recovery from block

In the following experiments, the time course of I_{to} recovery from block was measured. In a series of conditioning clamp pulses (i.e. eight 200 ms long steps to +60 mV at 2.5 Hz), the full block of I_{to} by tedisamil was allowed to develop. This was followed by a single test interval lasting between 10 ms and 120 s at −80 mV. The same pulse protocol was used to measure recovery from inactivation in controls. The amplitude of I_{to} in response to the test pulse was normalized to the amplitude measured after 2 min at −80 mV and plotted against the test interval on a logarithmic scale for even spacing of the data points (Figure 5). In control myocytes, I_{to} recovery from *inactivation* was about 90% complete after 200 ms. Curve fitting to the data points of six individual experiments yielded a single exponential function with a fraction of total current $A = 0.94 \pm 0.01$ (0.94) recovering with a time constant $\tau_r = 51 \pm 8$ ms (51 ms), where the numbers in brackets are the values used for curve fitting to the mean data points. In the presence of tedisamil, I_{to} recovery from *block* occurred in two distinct phases and required more than 100 s to be complete. The two exponential functions used for optimal curve fitting had the following characteristics: fraction $A = 0.32 \pm 0.07$ (0.39) recovered with $\tau_{ra} = 81 \pm 40$ ms (47 ms) and fraction $B = 0.66 \pm 0.06$ (0.60) recovered with $\tau_{rb} = 12.1 \pm 1.8$ s (14.6 s). The similarity in recovery time constants of control myocytes and of the initial phase in tedisamil-exposed cells suggests that the same recovery process, i.e. recovery from inactivation, is involved. If this holds true, it can be concluded that approximately one third of I_{to} channels remain unblocked in presence of 5 μM tedisamil.

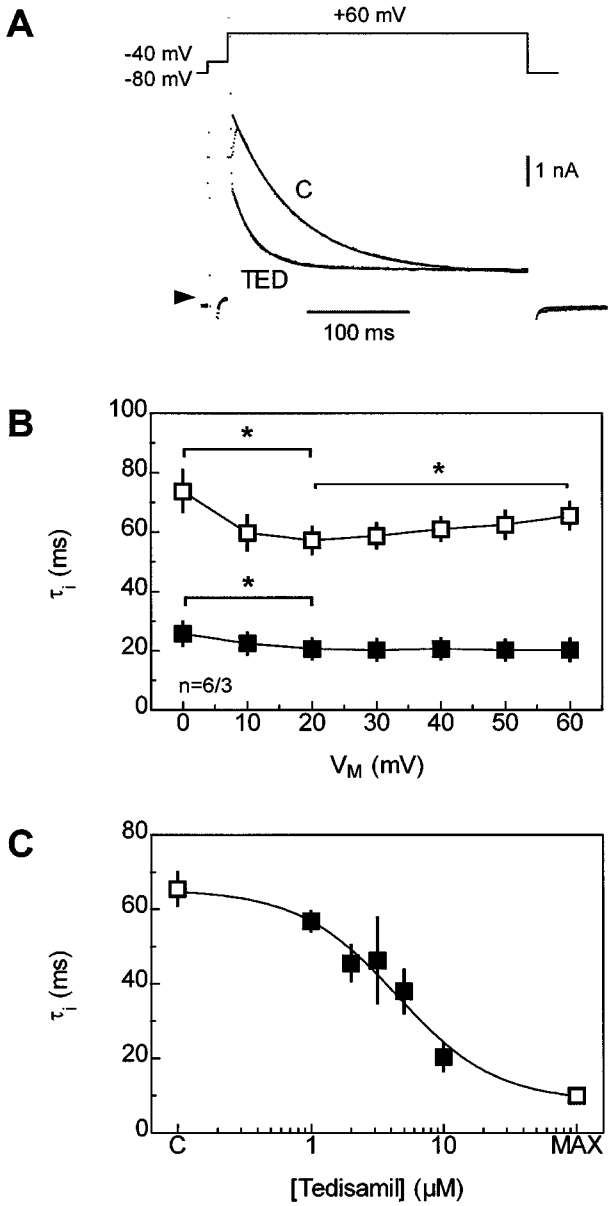

Figure 2 (A) Current tracings before (C) and 5 min after addition of 10 μM tedisamil (TED). Clamp step to +60 mV. A monoexponential fit was adapted to the raw data to describe the inactivation time course (continuous line extrapolated to the origin of the clamps step). (B) Potential dependence of inactivation time constant τ_i before and after exposure to 10 μM tedisamil. τ_i was obtained by computer-assisted fitting of a monoexponential function to the inactivation time course of I_{to}. Student's paired *t*-test, $^*P < 0.05$. (C) Concentration-dependence of the tedisamil-induced decrease of τ_i (test pulse to +60 mV). Please note, that the apparent acceleration of inactivation was considered to be maximum at τ_i of 10 ms, which corresponds to twice the value of the decay time constant for the capacitive current. A Langmuir adsorption isotherm with a IC_{50} value of 4.4 μM and a Hill coefficient of 1.2 was fitted to the data points. $n = 4-6/1-3$.

Fractional block of I_{to}

The K_D value for drug-channel interaction can be calculated from analysis of the onset of block during a clamp step (compare Snyders *et al*., 1992). In the presence of tedisamil, I_{to} activated initially as in the control but then decreased more rapidly (compare original tracings in Figure 1A). The fractional block (FB) was calculated by subtracting from unity the ratio of the current amplitudes after (I_T) and before (I_C) addition of tedisamil ($1 - I_T/I_C$) and FB was plotted against time of the depolarizing voltage step (Figure 6A). The onset of block as it developed during the first 60 ms of a clamp step to +60 mV could be fitted by a monoexponential function that yielded concentration-dependent time constants τ_{on}. Assuming

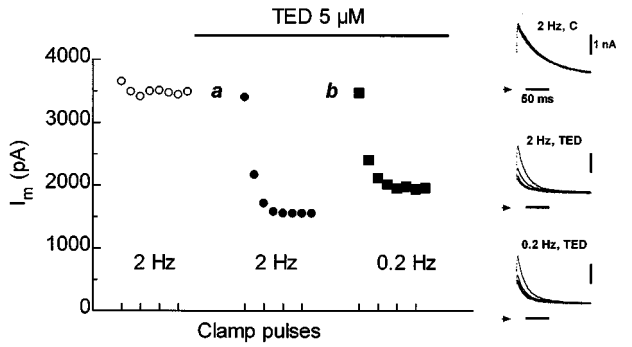

Figure 3 Use-dependence of tedisamil block of I_{to}. The development of block in presence of 5 μM tedisamil was measured at clamp pulse frequencies of 0.2 Hz (squares) and 2 Hz (circles) following a 2-min rest period at −80 mV. Representative experiment. (○) control stimulation of I_{to} at 2 Hz. a, 2 min of rest after application of tedisamil (5 μM) followed by I_{to} activation at 2 Hz (●) in presence of tedisamil, b, 2 min of rest, I_{to} activation at 0.2 Hz (■). Corresponding 1st, 2nd, 4th and 8th current tracings at +60 mV demonstrating development of block (right). Top, middle and bottom tracings: Control (C) at 2 Hz, in presence of 10 μM tedisamil (TED) at 2.0 Hz and 0.2 Hz, respectively.

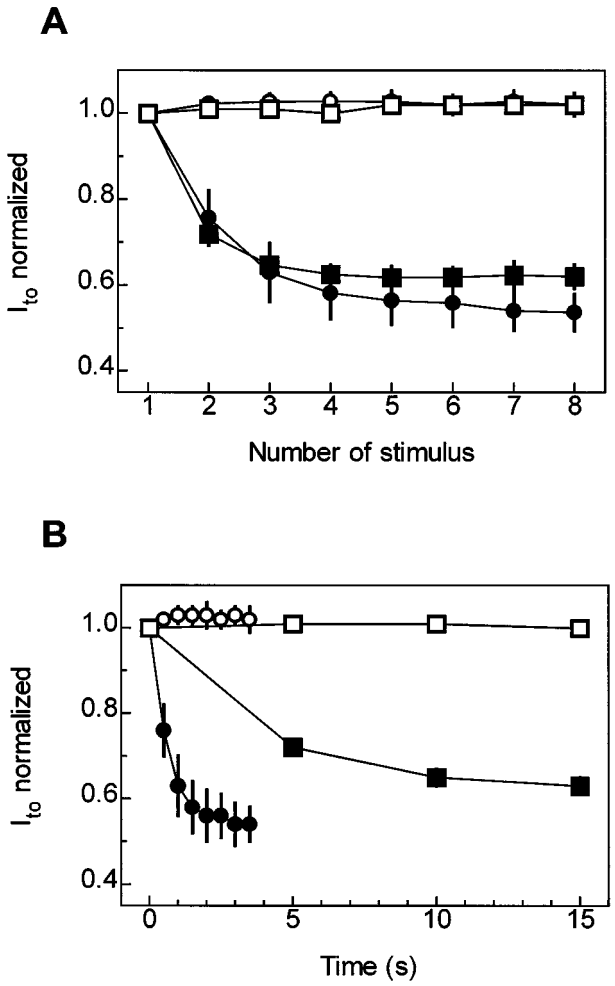

Figure 4 (A, B) Time course of the blocking effect of tedisamil on I_{to} after 2 min of rest. I_{to} is normalized to the first I_{to} amplitude after the 2-min rest period and is plotted *versus* the number of stimuli following rest (A) or against time within a train of stimuli (B). 2 Hz: ○, controls, n = 8/2; ●, tedisamil, $n = 4/2$; 0.2 Hz: □, controls, $n = 6/1$; ■, tedisamil, $n = 3/1$.

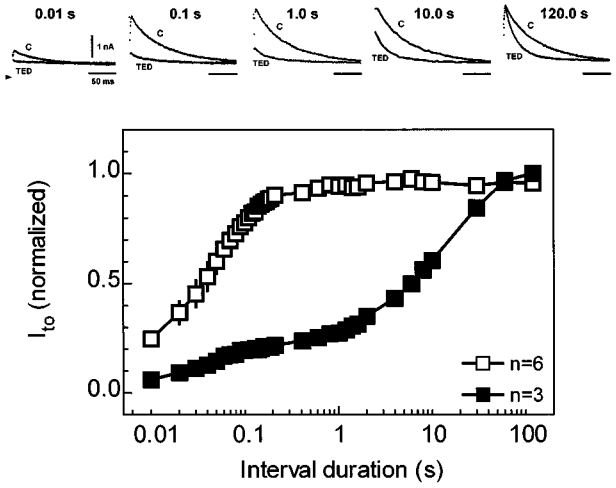

Figure 5 Recovery of I_{to} from inactivation (control, open symbols) and from block (tedisamil, 5 μM; filled symbols). I_{to} is normalized to fully recovered current and plotted *versus* the test interval at −80 mV on a logarithmic scale. Full block of I_{to} was obtained during eight clamp steps at 2.5 Hz to +60 mV. Mean values from six and three human ventricular myocytes. Top: Original tracings of control currents (C) and in presence of 5 μM tedisamil (TED) after recovery intervals of 0.01 s, 0.1 s, 1.0 s, 10.0 s and 120 s.

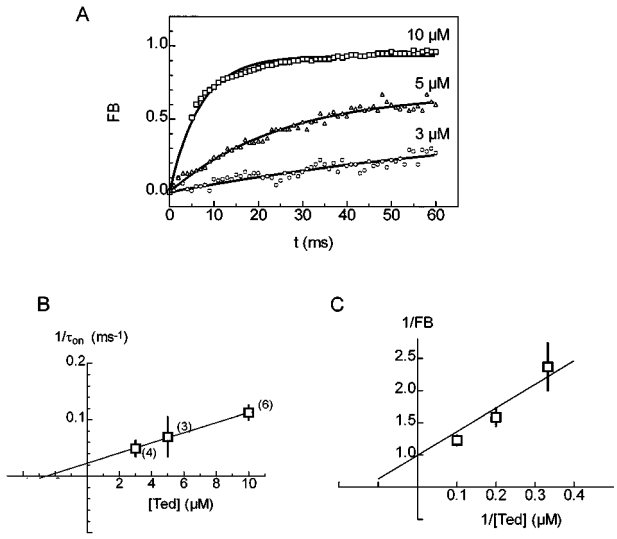

Figure 6 Fractional block (FB) of I_{to} by tedisamil in ventricular human myocytes (A) and graphic estimation of K_D-values (B, C). See text for details.

a simple reversible interaction between tedisamil and the K^+ channel in a one-to-one stoichiometry, the time constants τ_{on} at various concentrations were used to construct a plot of the linear function $1/\tau_{on} = k_1 \times [Ted] + k_2$ (Figure 6B), where k_1 and k_2 are the association and dissociation rate constants, respectively, and [Ted] is the tedisamil concentration in μM. The slope of this function yielded an association rate constant $k_1 = 9 \times 10^6$ mol^{-1} s^{-1} and the intercept at the ordinate gave a dissociation rate constant $k_2 = 23$ s^{-1}. Dividing k_2 by k_1 results in a K_D value of 2.6 μM. Because of the concentration dependence of the maximum fractional block FB_{max}, the K_D value can also be obtained from the slope of the plot of $1/Fb_{max}$ against 1/[Ted] (Figure 6C), the slope gives a K_D of 3.7 μM. These values are in good agreement with the $IC_{50} = 4.4$ μM for tedisamil that was obtained from the reduction in the time constant of inactivation τ_i (see Figure 2).

Discussion

Here we report that tedisamil concentration-dependently reduces I_{to} amplitude and accelerates its apparent inactivation in human myocytes obtained from left ventricular subepicardium. Transient outward current in human myocardium contains two components. The first component I_{to1} is blocked by 4-aminopyridine (4-AP) and is insensitive to Ca^{2+}, whereas the second component I_{to2} is not affected by 4-AP, depends on intracellular Ca^{2+} and is enhanced by caffeine (Coraboeuf & Carmeliet, 1982; Kenyon & Gibbons, 1979; Coraboeuf & Nargeot, 1993). Because of the high Ca^{2+} buffering concentration of EGTA used in the pipette solution, I_{to2} cannot be detected and only the Ca^{2+}-insensitive component I_{to1} is measured, to which I_{to} reported here refers.

In the present work, I_{to} data from subepicardial cells of failing and non-failing hearts have been pooled for the pharmacological characterization of tedisamil effects. This approach seems applicable since I_{to} from non-failing and failing hearts only differs in amplitude, which has been

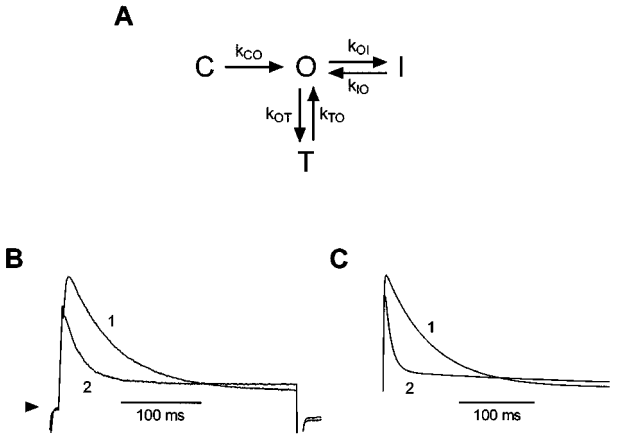

Figure 7 Simulation of tedisamil effects on I_{to} using a kinetic model of four channel states. (A) Diagram of the model, where the channels are supposed to transit from one (or several) resting closed states (C) to an activated open state (O), from where they pass into the inactivated state (I). Binding of tedisamil will result in a blocked state (T). The transitions are reversible and are governed by rate constants. The transitions between the states can be described by the following set of differential equations:

$$dC/dt = -k_{co} * C$$

$$dO/dt = k_{co} * C + k_{TO} * T + k_{IO} * I - k_{OI} * O - [X] * k_{OT} * O$$

$$dT/dt = [X] * k_{OT} * O - k_{TO} * T$$

$$dI/dt = k_{OI} * O - k_{IO} * I$$

where [X] is the concentration of tedisamil, and C, O, I and T are channel states discussed above with the corresponding rate constants. The rate constants have been estimated from the experimental results (B) Original current tracings before and after exposure to tedisamil (10 μM). (C) Simulated current traces as predicted by the model when using the numerical values of the experimentally derived rate constants: k_{CO} 1000 $M^{-1}s^{-1}$; k_{OT} 9×10^6 $M^{-1}s^{-1}$; k_{TO} 23 s^{-1}; k_{IO} 0.5 s^{-1}. For k_{OI}, the numerical value was 16 $M^{-1}s^{-1}$, i.e. the inverse value of the inactivation constant of the original registration. See text for further details.

reported to be reduced (Näbauer *et al*., 1996) or unaltered in cells from failing hearts (Wettwer *et al*., 1994). The kinetics of inactivation of I_{to} were unaltered in both of the studies. A reduced current density is best explained by a reduced channel density. Therefore differences in pharmacological effects on I_{to} in cells derived from failing and non-failing hearts are not expected.

The blocking action of tedisamil is use-dependent (Figure 3) and occurs in conjunction with an apparent acceleration of inactivation of I_{to}. These results are strongly suggestive of an open channel block as the mechanism of action of tedisamil. This finding is opposite to the frequency-dependent action of this drug on action potential duration in human papillary muscles (Ohler & Ravens, 1994) where prolongation in action potential duration is largest after a prolonged period of rest suggesting *closed* channel block or a fast open channel block with trapping of the drug. This discrepancy is probably due to the fact that the action potential duration is the sum of all inward and outward currents, and tedisamil affects many other currents besides I_{to} (Faivre *et al*., 1995). For instance, tedisamil may also reduce the delayed rectifier current (Dukes *et al*., 1990; Ohler *et al*., 1994). Although some groups have identified a relatively small delayed rectifier currents in human ventricular myocytes (Beuckelmann *et al*., 1993; Veldkamp *et al*., 1995; Li *et al*., 1996); we were not able to detect this type of K^+ current. Apparently, the procedure used for isolation of myocytes is crucial for preservation of optimal I_K channel activity (Yue *et al*., 1996).

Tedisamil acted on I_{to} as an *open channel blocker*. The reduction of the current amplitude was use-dependent and I_{to} recovered completely, though very slowly, from block at negative membrane potentials where transition of channels towards a closed state are favoured. We have simulated the effects of tedisamil on I_{to} by using a kinetic model (Figure 7A) where the channel can exist in at least four different conformational states: From a closed state (C) at resting potential, it passes into an open state (O) during an activating depolarizing step. During persisting membrane depolarization, the open channel transforms into the inactivated state (I). Binding of tedisamil to the open channel state produces the blocked channel state (T). The channel can only conduct current in the open state, whereas the closed, inactivated and blocked state are non-conducting. The transitions between the different channel states are governed by rate constants and the fraction of channels occupying a certain state at any point of time can be described by differential equations (see legend to Figure 7). By substituting the experimentally obtained rate constants, this model provides a good prediction of the current traces obtained under control conditions and in the presence of tedisamil. The model is able to predict three important properties of the tedisamil block: (i) apparent acceleration of inactivation of I_{to}; (ii) reduction in I_{to} amplitude and (iii) the crossing over of the current traces obtained before and after the addition of tedisamil (Figure 7B and C). This latter finding suggests that tedisamil must unbind and let the channels pass into the open state before they can reach the inactivated state. In rat myocytes, the effects of tedisamil on I_{to} have been described before with a similar model, however, no crossing over has been reported (Dukes *et al*., 1990), probably because the test pulses used were too short in duration. It should be noted that open channel block of I_{to} with association and dissociation rate constants having a similar order of magnitude as reported here have been reported for antiarrhythmic drugs like quinidine, flecainide or propafenone (Slawsky and Castle, 1994) or the local anaesthetic bupivacain (Castle, 1990). The present model calculation is a simple but adequate approach to describe the effects of tedisamil on I_{to}. More elaborate simulations of I_{to} kinetics including potential dependence of activation and recovery from inactivation have been developed by others (Campbell *et al*., 1993, for review see also Campbell *et al*., 1995) but are beyond the scope of this study.

Our results were obtained at room temperature and the kinetics of tedisamil block and unblock may be substantially different at physiological temperature. Furthermore, the tedisamil effects obtained in human subepicardial ventricular myocytes may not be representative for the whole heart. In addition to blocking of potassium currents (with exception of I_{K1}; Dukes *et al*., 1990), tedisamil also inhibits sodium current, albeit only at concentrations above 20 μM. L-type calcium current on the other hand is not influenced with concentrations up to 50 μM (Dukes *et al*., 1990). Tedisamil also inhibits a chloride conductance in guinea pig ventricular myocytes (Faivre, personal communication). This effect may also contribute to the action potential prolonging effect of the substance.

In conclusion, tedisamil is a non-selective potassium channel blocking agent which blocks the potassium current I_{to} in human ventricular myocytes. The open channel block of this K^+-current by tedisamil contributes to the action potential

prolonging effect found in multicellular preparations and hence may explain at least in part the antiarrhythmic effects found *in vivo*. However, blocking effects on other ion channels are very likely to also contribute to the overall action of this new antiarrhythmic agent.

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