British Journal of Pharmacology (1997) 122, 291–301

The antiarrhythmic agent bertosamil induces inactivation of the sustained outward K^+ current in human atrial myocytes

Sophie Tessier, *Catherine Rücker-Martin, *Loïc Macé, *Edouard Coraboeuf, [1]Jean-Jacques Mercadier & Stéphane N. Hatem

INSERM Unité 460, Faculté de Médecine Xavier Bichat, Paris and *Laboratoire de Cardiologie Moléculaire et Cellulaire, Université de Paris XI - C.N.R.S. ERS 566, Hôpital Marie Lannelongue, Le Plessis Robinson, France

1 In whole-cell patch-clamped human atrial myocytes, the antiarrhythmic agent bertosamil (10 μM) inhibited the sustained component, I_{sus} (38.6±3.1%), and enhanced the inactivating component, I_t (9.1±6.1%), of the outward K^+ current elicited by 750 ms test pulses from −60 mV to +50 mV. Higher concentrations of bertosamil (>10 μM) inhibited both I_t and I_{sus}.

2 Suppression of I_{sus} and stimulation of I_t by 10 μM bertosamil was observed on renewed stimulation following a 2 min rest period during which the drug was applied and persisted after washout, indicating a rest-dependent effect of bertosamil on the outward K^+ current.

3 Cell dialysis with an internal solution containing 10 μM bertosamil increased both I_t (78.0±14.7%) and I_{total} (26.7±8.4%) and inhibited I_{sus} (28.9±6.3%, n=6). In the presence of intracellular bertosamil, external application of the drug inhibited I_t and I_{sus} in a concentration-dependent and use-dependent manner.

4 Following the suppression of I_{sus} by 200 μM 4-aminopyridine (4-AP), bertosamil (10 μM) inhibited I_t. Washout of 4-AP was associated with a larger I_t amplitude than that observed in control conditions. In myocytes characterized by a prominent I_{sus} and lack of I_t, bertosamil (10 μM) induced a rapid and partial inactivation of the current, together with inward rectification of the current measured at the end of the test pulse.

5 In the presence of bertosamil the activation/voltage relationships, steady-state inactivation and recovery from inactivation of I_t were markedly modified, pointing to changes in the conductance underlying I_t.

6 We conclude that bertosamil induces rapid inactivation of sustained outward current which leads to an apparent increase in I_t and decrease in I_{sus}. This effect, which was distinct from the use-dependent inhibition of the outward K^+ current, could represent a new antiarrhythmic mechanism.

Keywords: Outward potassium currents; bertosamil; human atrial myocytes

Introduction

A number of potassium currents control the resting membrane potential and action potential waveform of cardiac myocytes. Voltage and time-dependent outward K^+ currents, which activate at relatively positive potentials, mainly govern the early repolarization phase and the height and duration of the plateau phase, thereby playing an important role in myocardial refractoriness (Shibata *et al*., 1989).

In human atrial myocytes the outward K^+ current is composed of a rapidly inactivating component and a sustained component (Escande *et al*., 1987; Wang *et al*., 1993). Because these two components can be distinguished on the basis of specific electrophysiological and pharmacological properties, it has been proposed that they are carried by at least two distinct types of channel and represent distinct currents (usually named I_{to} or I_t and I_{Kur} or I_{sus}, for the inactivating and sustained components, respectively) (Wang *et al*., 1993). This is also in keeping with molecular cloning studies which revealed a broad diversity of voltage-gated K^+ channels expressed in the heart (Roberds & Tamkun, 1991; Tamkun *et al*., 1991). This diversity may be a source of phenotypic plasticity of K^+ currents, which could contribute to changes in the outward K^+ current observed during development (Escande *et al*., 1985; Crumb *et al*., 1995) and disease conditions like atrial dilatation (Le Grand *et al*., 1994), and might explain the different sensitivity of I_t and I_{sus} to protein kinase C inhibition during growth of human atrial myocytes *in vitro* (Hatem *et al*., 1996). These various cardiac K^+ channels could also provide multiple potential targets for neurotransmitters, hormones and antiarrhythmic drugs. Indeed, expressed K^+ channels exhibit marked differences in sensitivity to drugs such as quinidine, flecainide, diltiazem, (+)-sotalol, clofilium, verapamil and bertosamil (Yatani *et al*., 1993; Grissmer *et al*., 1994; Snyders & Yeola, 1995; Yamagishi *et al*., 1995). However, because of the large number of Kv channel α-subunits and their possibile cooperative interactions in native tissue (Tytgat & Hess, 1992; Po *et al*., 1993), the effects of these pharmacological agents on K^+ currents of cardiac myocytes may be more complex than on currents carried by cloned channels. Moreover, different auxiliary β-subunits can accelerate inactivation of transient K^+ currents (Majumder *et al*., 1995; McCormack *et al*, 1995; Morales *et al*., 1995; Castellino *et al*., 1995) or induce total or partial inactivation of sustained K^+ currents (Rettig *et al*., 1994; Majumder *et al*., 1995; England *et al*., 1995a,b; Leicher *et al*., 1996). The contribution of these different mechanisms to the effects of drugs such as tedisamil and D600 (methoxyverapamil), which accelerate I_t inactivation in rat ventricular myocytes, is unknown (Dukes & Morad, 1989; Lefevre *et al*., 1991).

Tedisamil, a structural analogue of bertosamil, has been found to lengthen the action potential in human atrial myocytes (Németh *et al*., 1996). This effect may also result from modulation of the activation and inactivation of the outward K^+ current, which control the action potential duration in human atrial myocytes. Therefore, the aim of this study was to determine the effects of the antiarrhythmic and bradycardic agent bertosamil on outward K^+ currents in human atrial myocytes. We showed that bertosamil has distinct effects on the two components of the

[1]Author for correspondence at: INSERM Unité 460, Faculté de Médecine Xavier Bichat, 16, Rue Henri Huchard, 75018 Paris, France.

outward K^+ current and the various mechanisms of action responsible for complex changes in the activation and inactivation kinetics of outward K^+ current in human atrial myocytes.

Methods

Myocyte isolation

With approval from the ethics committee of our institution (GREBB, Hôpital de Bicêtre, Université de Paris XI), specimens of human right atrial appendage were obtained from 35 patients (11 to 78 years, mean = 61 ± 3 years) undergoing heart surgery (Table 1). Myocytes were enzymatically isolated as follows: small pieces of atrial appendage were cut up and washed in calcium-free Krebs-Ringer solution containing (in mM): NaCl 35, KCl 4.75, KH_2PO_4 1.19, Na_2HPO_4 16, HEPES 10, glucose 10, $NaHCO_3$ 25, saccharose 134 and 2,3-butanedione oxime (BDM) 30, (pH 7.4), gassed with carbogen and maintained at 37°C. Pieces were reincubated in the same solution without BDM and containing bovine serum albumin (Hoescht-Behring, 5 mg ml^{-1}), 200 iu ml^{-1} collagenase (type IV Sigma Chemical Co., St Louis) and 6 iu ml^{-1} protease (type XXIV, Sigma Chemical). After 30 min of digestion, the enzyme solution was replaced by the same solution containing only collagenase (400 iu ml^{-1}). Isolated myocytes were resuspended in bicarbonate-buffered Tyrode solution containing 2 mM Ca^{2+} and incubated at 37°C, the solution was continuously gassed with 5% CO_2 for at least one hour before use.

Current measurements

Currents were recorded by using the patch-clamp technique in the whole-cell configuration. Borosilicate glass pipettes with a tip resistance of 1.5 to 2 MΩ were connected to the input stage of a patch clamp amplifier (Axoclamp 200A, Axon Instrument). Resistance in series was compensated to obtain the fastest capacity transient current, but the capacitive and leakage currents were not compensated. Currents were filtered at 5 kHz, digitized with the Labmaster (Lab Rac, Scientific Solution) and stored on the hard disk of a personal computer. Data were acquired and analysed on a programme adapted for our laboratory (Acquis 1, G. Sadoc, CNRS UPR 2212, Gif/Yvette, France).

Solutions

Cells were continuously bathed with an external solution containing (in mM): NaCl 137, KCl 5.4, $CaCl_2$2, $MgCl_2$ 1, HEPES 10 and glucose 10, adjusted to pH 7.3 with NaOH. For potassium current measurements (in control conditions), Na^+ was replaced by the equimolar concentration of choline chloride, Ca^{2+} channels were blocked with 0.5 mM Cd^{2+}, and 10^{-2} mM atropine was added to the external solution to prevent activation of muscarinic receptors (Le Grand *et al*. 1994).

Cells were dialysed with an internal solution containing (in mM): K-aspartate 115, KCl 5, MgATP 5, Na-pyruvate 5, $MgCl_2$ 3, EGTA 5 and HEPES 10; pH was adjusted to 7.2 with KOH. In some experiments, an equivalent concentration of KCl replaced K-aspartate in the internal solution. All experiments were carried out at room temperature (22–24°C).

Drugs

Bertosamil (3-isobutyl-7-isopropyl-9,9-pentamethylene-3,7-diazabicyclo-(3.3.1) nonane sesquihydrogenfumarate; KC 10784) was a gift from Dr Ziegler (Solvay Pharma Deutschland, Hannover, Germany). Since bertosamil is a water-soluble

Table 1 Clinical characteristics of the patients

Sex	*Diagnosis*	*Age*	*Previous medication*
M	CI	47	None
M	CI	75	Molsidomine, amiodarone, heparin
M	CI	48	Diltiazem, molsidomine, nifedipine, metoprolol, heparin
F	CI	64	Amiodarone, diltiazem, atenolol, nitrate, heparin
F	CI	75	Diltiazem, molsidomine, nicardipine, aspirin
M	CI	54	Verapamil, perindopril, nitrate
F	CI	74	Nifedipine, ramipril, nitrate, heparin
F	CI	76	Molsidomine, amlodipine, acebutolol, aspirin
M	CI	69	Amlodipine, atenolol
M	CI	68	Molsidomine, atenolol, diltiazem, aspirin, heparin
F	CI	78	Amiodarone, nifedipine, captopril, furosemide, heparin
M	CI	56	Molsidomine, diltiazem, nicorandil, heparin
M	CI	60	Molsidomine, diltiazem, atenolol, aspirin
F	CI	73	Molsidomine, atenolol, diltiazem, hydroxyzine
M	CI	60	Amiodarone, digoxin, adalate, furosemide, lisinopril, aspirin
M	CI	73	Amiodarone, digoxin, nicardipine, lisinopril, furosemide, aspirin
M	CI	60	Atenolol, molsidomine
F	CI	40	None
M	CI	48	Diltiazem, molsidomine, trinitrine
F	CI	64	Amiodarone, diltiazem, aspirin
M	CI	62	Amiodarone, atenolol, nitrendipine
M	CI, MI	54	Diltiazem, acebutolol, heparin
M	CI, MI	61	None
F	CI, AI	73	Amiodarone, captopril, hydrochlorothiazide
F	AI	72	Molsidomine, trinitrine, diltiazem, dipyridamole
F	AI	74	Atenolol, molsidomine, aspirin
F	AI	72	None
F	AI, MI	70	None
F	MI	67	Furosemide
F	MI	71	Captopril, digoxin, furosemide, heparin
F	MI	57	Altizide, spironolactone
F	ASD	50	Amiodarone, captopril, hydrochlorothiazide
M	ASD	21	None
M	VSD	46	Propranolol
F	VSD	11	Paracetamol

CI: Coronary insufficiency; AI: aortic insufficiency; MI: mitral insufficiency; ASD: atrial septal defect; VSD: ventricular septal defect.

drug, it was dissolved in the external or internal solution. 4-Aminopyridine was obtained from Sigma and was dissolved in the extracellular solution the pH of which was adjusted to 7.3 with HCl.

Data analysis

The amplitude of the rapidly inactivating current (I_t) was measured as the difference between the peak of the outward K^+ current and the current measured at the end of 750 ms test pulses. The amplitude of the slowly inactivating current (I_{sus}) was measured as the difference between the amplitude of the current measured at the end of the 750 ms test pulses and the zero current. The amplitude of the total peak current (I_{total}) was measured by the difference between the amplitude of the peak and the zero current. The time course of the transient outward K^+ current inactivation was fitted with a double exponential function: $y(t) = Aexp(-t/\tau1) + Bexp(-t/\tau2) + C$ (equation 1), where t is time, A and B are amplitude terms, $\tau1$ and $\tau2$ time constants of the fast and the slow inactivation phases, respectively, and C is the amplitude of a steady-state component. The effect of bertosamil on the outward K^+ current was quantified by using concentration-response curves which were fitted with an equation of the form: $Y = [(A-B)/(1 + (C/IC_{50})^K)] + B$ (equation 2) where Y is the percentage of current, A is the response in the absence of bertosamil, B is the maximal response induced by the drug, C is the concentration of bertosamil and k the slope factor, in order to estimate the IC_{50}. Data on the conductance/voltage activation curves were best fitted with a Boltzmann distribution equation: $G/G_{max} = 1/[1 + exp((V_{1/2} - V)/k)]$ (equation 3) where G is the conductance at a conditioning potential of V, G_{max} is the maximal conductance, $V_{1/2}$ is the potential at which half of the K^+ channels are activated, and k is the slope factor. Normalized conductance values for steady-state inactivation curves at all potentials were fitted with the Boltzmann function: $I/I_{max} = 1/[1 + exp((V_{1/2} - V)/k)]$ (equation 4), where I is the amplitude of the current at a conditioning potential of V, and I_{max} is the maximal current amplitude, and $V_{1/2}$ is the potential at which half of the K^+ channels are inactivated. Recovery from inactivation was well fitted by a two exponential function: $y(t) = 100\text{-}(Aexp(-t/\tau1) + Bexp(-t/\tau2))$ (equation 5), where A and B are amplitudes, t is time, $\tau1$ and $\tau2$ are time constants.

Statistical analysis

Values are expressed as means ± s.e.mean. The decrease in the density of the two components of the outward K^+ current after bertosamil-exposure was analysed by using Student's two-tailed t test. Percentages forming were submitted to angular transformation and the resulting data were analysed by Student's two-tailed t test. P values <0.05 were considered significant.

Results

Concentration-dependent inhibitory effects of bertosamil on I_t and I_{sus}

Most of the human atrial myocytes studied (88%, $n = 103$) contained an outward K^+ current composed of rapidly inactivating (I_t) and sustained (I_{sus}) components, in keeping with previous studies from our group (Hatem *et al.*, 1996) and other laboratories (Wang *et al.*, 1993). This is illustrated in Figure 1a, which shows representative traces of currents elicited by

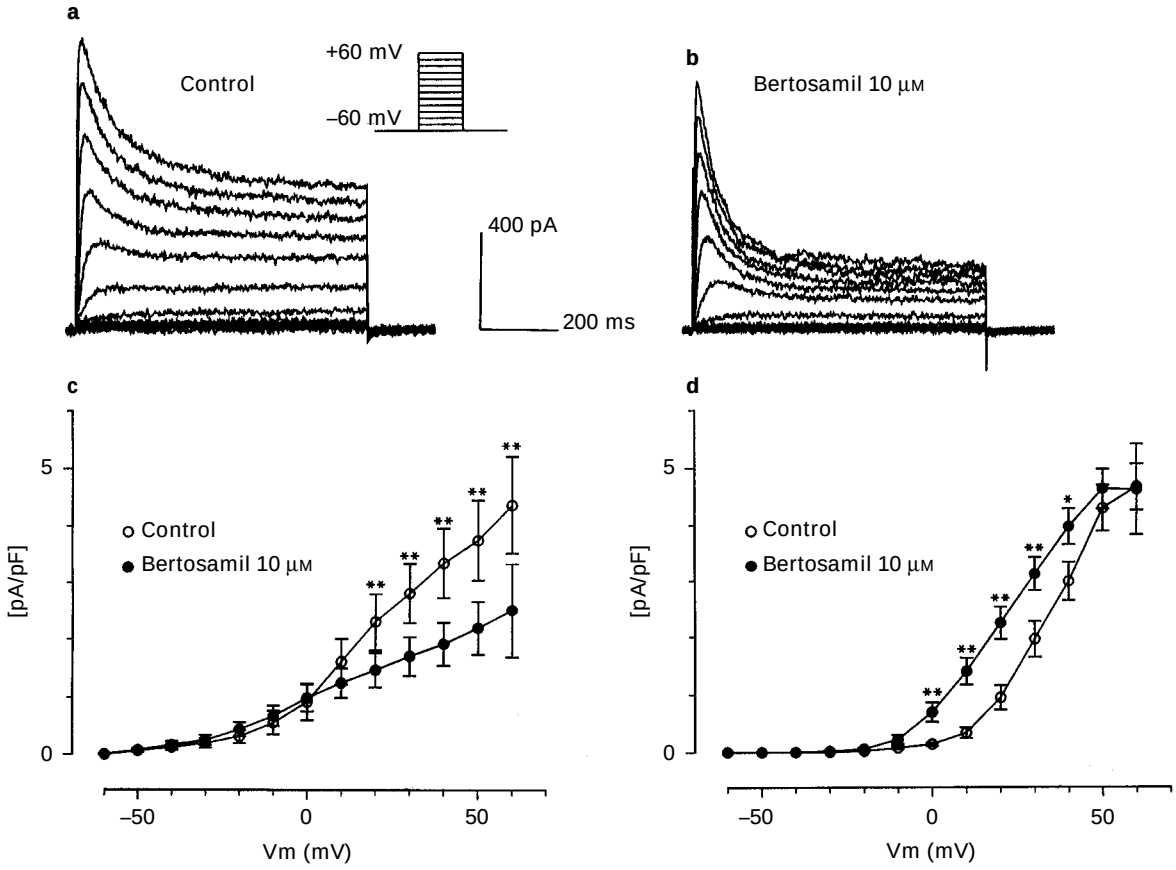

Figure 1 Effects of external application of 10 μM bertosamil on the outward K^+ current of human atrial myocytes. Traces of currents elicited by 10 mV incremental 750 ms test pulses from −60 mV to +60 mV (at 0.2 Hz) in control conditions (a) and after the addition of 10 μM bertosamil (b). Cell capacitance: 99 pF. Current density-voltage relations of I_{sus} (c) and I_t (d) in control conditions and during bertosamil exposure. Each point is the average current density from 13 cells (*$P<0.05$ and **$P<0.01$).

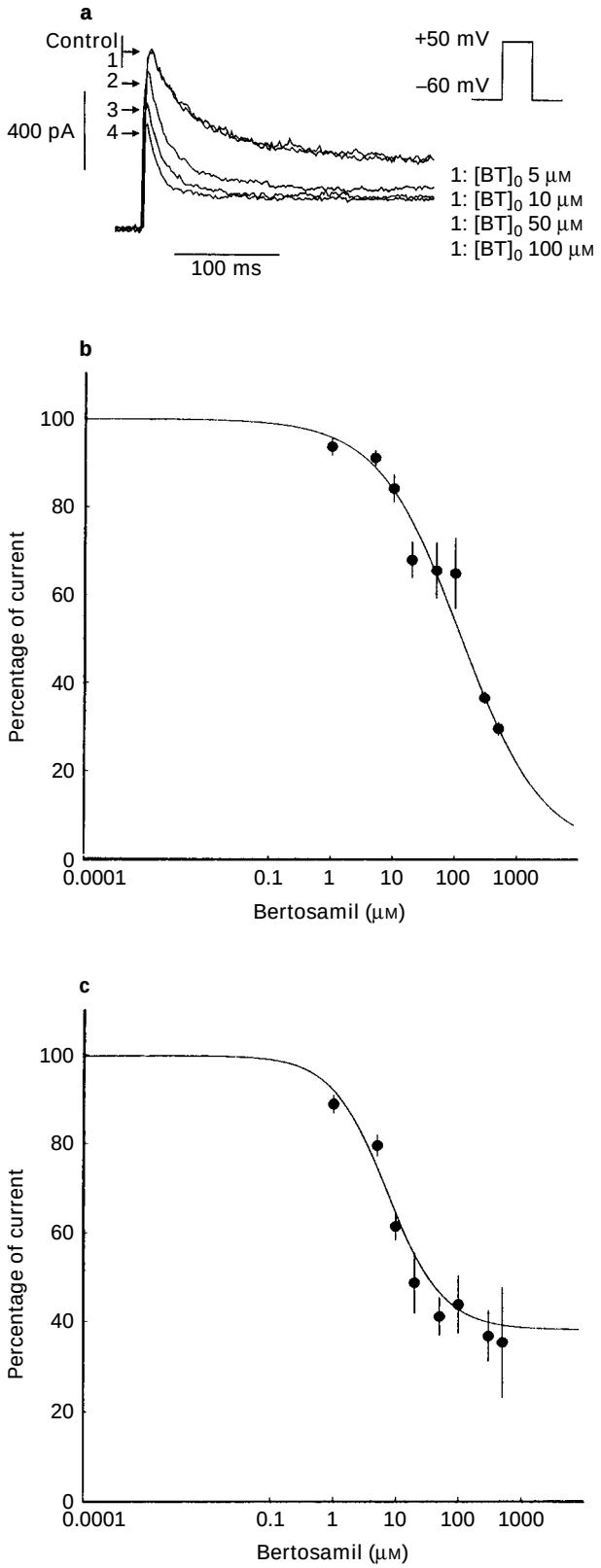

Figure 2 Concentration-dependent effects of external bertosamil on outward K^+ current. (a) Superimposition of current traces elicited by 750 ms depolarizing test pulses from a holding potential of −60 mV to +50 mV upon application of increasing concentrations of bertosamil. Cell capacitance: 136 pF. Concentration-dependence of effect of bertosamil on I_{total} (b) and I_{sus} (c), represented as the ratio of current amplitude before and after application of various concentrations of bertosamil. Except for the concentration of 200 μM bertosamil, each point is the average value of 3 to 33 measurements; vertical lines show s.e.mean.

10 mV incremental 750 ms test pulses from a holding potential of −60 mV. As normal myocytes are rich in large non permeant anions and, as a result, are much poorer in Cl^- ions than is the extracellular medium, cells were dialysed with K-aspartate-containing instead of KCl-containing internal solution. Figure 1b shows traces of the outward K^+ current recorded in the same cell as in Figure 1a, upon external application of 10 μM bertosamil. Bertosamil (10 μM) markedly inhibited I_{sus} but not I_t. The density-voltage relationships of I_{sus} in the absence and presence of bertosamil are shown in Figure 1c. The two curves seemed to cross around 0 mV and the inhibitory effect of bertosamil on I_{sus} was significant only at potentials above +10 mV (at +60 mV, I_{sus} density was 4.4 ± 0.9 pA/pF vs 2.5 ± 0.8 pA/pF in the absence and presence of bertosamil, respectively; $n = 13$, $P < 0.01$; Figure 1c). The large s.e.mean for I_{sus} reflect variations in the density of this current among the cells studied (from 1.2 pA/pF to 9.4 pA/pF at +60 mV in control conditions). In contrast to its depressive effect on I_{sus}, bertosamil enhanced the amplitude of I_t at potentials between 0 mV and +40 mV (at +40 mV, 3.0 ± 0.3 pA/pF vs 4.0 ± 0.3 pA/pF, in control and bertosamil conditions, respectively, $n = 13$, $P < 0.05$; Figure 1d) and shifted its voltage-dependence of activation towards negative potentials (Figure 1d), a shift which could explain, in part, why the enhancing effect of bertosamil on I_t no longer occurred at potentials more positive than +40 mV. Bertosamil also accelerated both the activation (at +60 mV, time to peak: 14.0 ± 1.8 ms vs 8.8 ± 0.1 ms; $n = 10$, $P < 0.05$) and inactivation (at +60 mV, $\tau 1$: 55.7 ± 8.9 ms vs 23.7 ± 0.9 ms, see equation 1 in Methods; $n = 10$, $P < 0.01$) of the outward current. Figure 2a

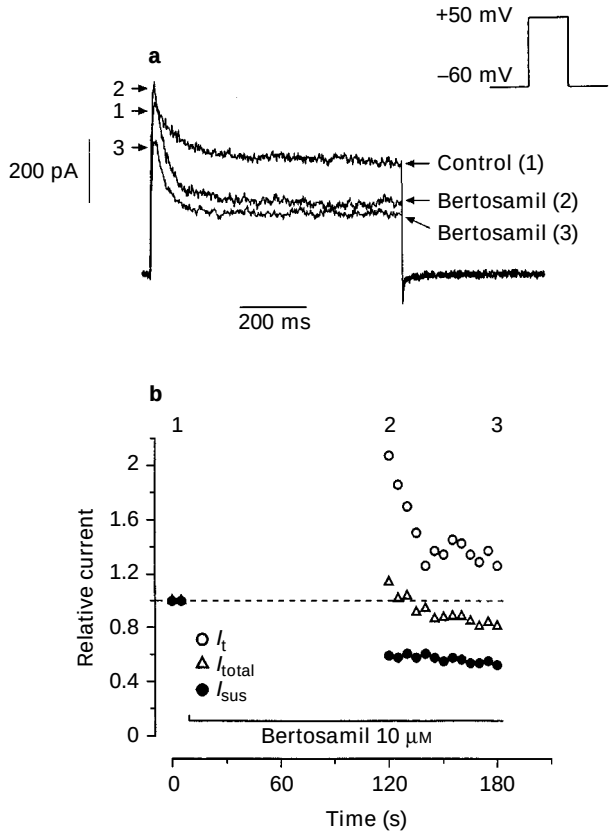

Figure 3 Post-rest effects of external bertosamil on the outward K^+ current. (a) Superimposition of current traces elicited by test pulses from −60 mV to +50 mV, in control conditions (trace 1), after a 2 min rest period during which 10 μM bertosamil was applied (trace 2), and after resumption of the stimulation in the presence of the drug (trace 3). Cell capacitance: 117 pF. (b) Normalized amplitudes of I_t, I_{total} and I_{sus} obtained in control conditions (1), on resumption of stimulation following bertosamil exposure during the rest period (2) and in the continuous presence of the drug (3).

shows a typical example of a concentration-dependent effect of bertosamil on the outward K^+ current. Whereas almost total inhibition of the sustained current was obtained with 10 μM bertosamil, 100 μM was necessary to inhibit around half of the total outward K^+ current (I_{total}) (IC_{50} of $\sim$120 μM and $\sim$7 μM for I_{total} and I_{sus}, respectively, see equation 2 in Methods and Figure 2b–c). Therefore, depending on the concentration used, bertosamil increased or decreased the amplitude of I_t, whereas it inhibited I_{sus} at all the concentrations tested. These opposite effects of 10 μM bertosamil on the two components of the outward K^+ current suggest either (i) an open-channel block resulting in time-dependent inhibition of the outward K^+ current, (ii) accelerated inactivation of the outward current, or (iii) specific stimulating and inhibitory effects on channels carrying inactivating and non inactivating outward K^+ currents, respectively.

The effects of bertosamil on the outward K^+ current depend on channel state

We first examined if the effects of bertosamil depended on the channel state (open or closed) by studying the effects of the drug when applied during a 2 min rest period. In Figure 3a, the first trace of current was obtained before drug application. The current recorded at the resumption of stimulation in the presence of 10 μM bertosamil (trace 2) was characterized by a marked reduction in I_{sus} and an increase in the amplitude of I_t, together with an acceleration of its rate of inactivation, which resulted in a higher amplitude of I_{total} than that obtained in control conditions. Further depolarizations were associated with a slow inhibition of I_t until steady-state was reached (trace 3), and a slight additional decrease in I_{sus} (Figure 3b). Moreover, the rest-dependent effects of bertosamil persisted after drug washout. This is illustrated in Figure 4, which shows that return to the control solution was associated with a significant slow increase in I_t (23.1 $\pm$ 10.8%) and in the total outward current (I_{total}) (19.8 $\pm$ 6.5%) (n = 3), the latter reaching values higher than in controls (Figure 4a), whereas I_{sus} remained inhibited. The persistence of the inhibition of I_{sus} after drug washout was further demonstrated by recording current at least 30 min after the end of cell pretreatment with 10 μM bertosamil (applied for 20 min) (Figure 4c–d). Figure 4c shows currents elicited by 10 mV incremental test pulses in pretreated cells, which were characterized by marked suppression of I_{sus}, whereas a large and rapidly inactivating current component still activated. The density of I_{sus} measured in pretreated cells was not significantly different from that obtained in cells superfused with bertosamil (Figure 4d, at +50 mV: 3.8 $\pm$ 0.7 pA/pF, 2.2 $\pm$ 0.4 pA/pF and 1.8 $\pm$ 0.2 pA/pF, in control conditions, during bertosamil exposure and in pretreated cells, respectively; $P < 0.01$). Taken together, our results point to different mechanisms of action of this drug on the outward current.

Different sites of action of bertosamil

To determine if the rest-dependent effects of bertosamil arise from entry of the drug into the cell and its binding to an internal site, cells were dialysed with an internal solution containing 10 μM bertosamil. Figure 5a shows the superimposition of currents elicited by a test pulse from −80 mV to +70 mV just after the break of the patch (trace labelled 'control') and at the steady-state effect of internal dialysis with 10 μM bertosamil (labelled '$[BT]_i$') observed after $\sim$10 min. Internal dialysis of bertosamil resulted mainly in a marked inhibition of I_{sus} (35.4 $\pm$ 3.5%, n = 6,

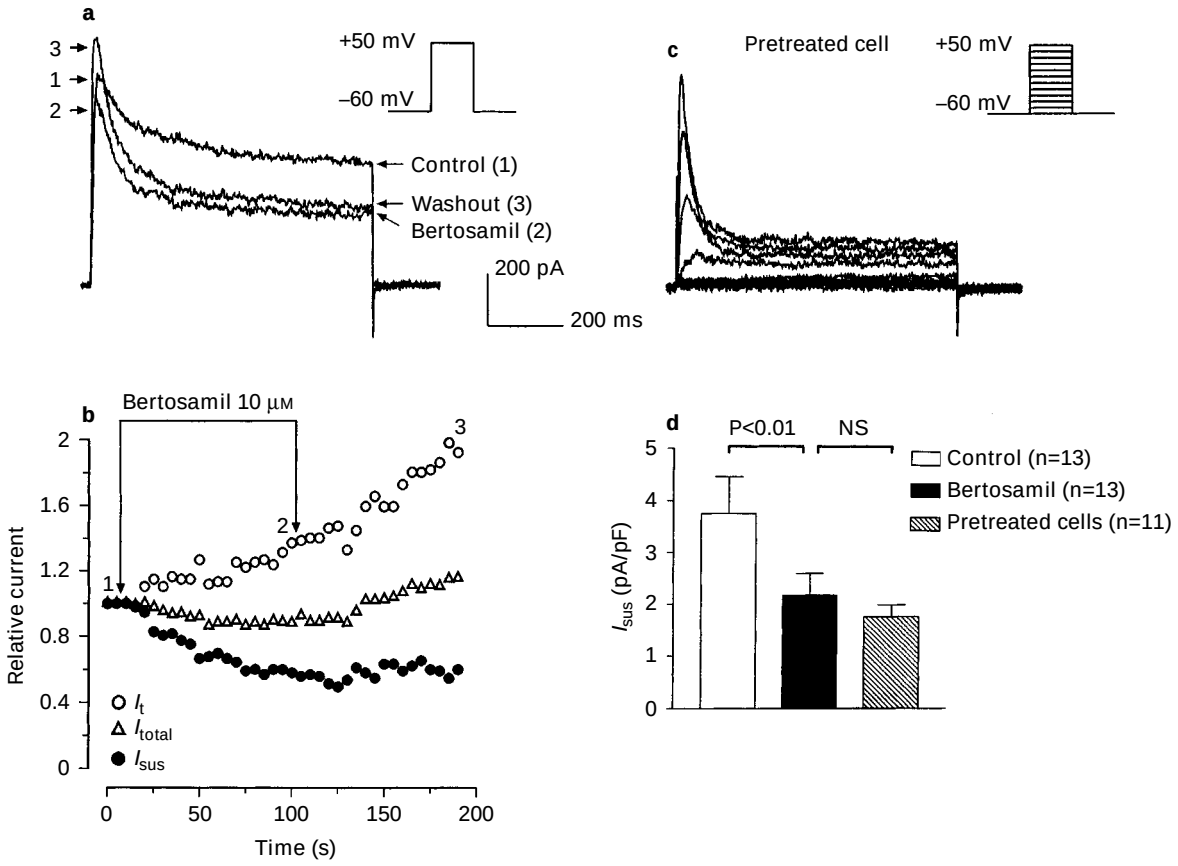

Figure 4 Effects of bertosamil washout on the outward K^+ current. (a) Superimposition of current traces elicited by a 750 ms test pulse from −60 mV to +50 mV before (trace 1), during (trace 2) and after the application of 10 μM bertosamil (trace 3). Cell capacitance: 109 pF. (b) Time course of changes in the normalized amplitude of I_t, I_{total} and I_{sus} upon external application of 10 μM bertosamil and during drug washout. (c) Current traces elicited by 10 mV depolarization steps recorded 30 min after pretreating cells with 10 μM bertosamil. Cell capacitance: 147 pF. (d) Density of I_{sus} in control conditions, on acute bertosamil exposure and in cells pretreated with bertosamil. Values are means $\pm$ s.e.mean.

Figure 5b) associated with an increase in I_t (38.3±21.3%, $n=6$) and an acceleration of its inactivation (at +70 mV, $\tau 1$: 31.7±7.7%, $n=7$, Figure 5c). Similar results were obtained with 50 μM bertosamil (not shown). To check that these alterations did not simply result from prolonged dialysis, a similar protocol (i.e. current measurements performed just after the break of the patch and at the end of the experiment) was repeated with the usual control internal solution, which did not induce significant changes in either the amplitude of I_{sus} (Figure 5b) or the rate of inactivation of the outward K^+ current ($\tau 1$) (Figure 5c). In addition, the slight difference in series resistance measured just after the break of the patch and at the end of the experiments cannot account for the current changes observed during internal dialysis of bertosamil (Table 2). We then examined if the inhibitory effect of externally applied bertosamil on the outward K^+ current (Figure 2a) was observed when the drug was also present inside the cell. Figure 6a shows the superimposition of current traces elicited by test pulses from −80 mV to +70 mV obtained at the steady-state of the internal dialysis (labelled '$[BT]_i$') together with external application of various concentrations of the drug. External application of bertosamil in the presence of intracellular drug inhibited both I_t and I_{sus} in a concentration-dependent manner (for instance at +50 mV, I_t was inhibited by 19.9±4.8% and 56.1±13.3% with 10 and 100 μM bertosamil, respectively). The effects of external bertosamil on both I_t and I_{sus} in cells internally dialysed with the drug were partly reversible on washout (Figure 6b). These data suggest that bertosamil binds to an external site and an internal site responsible for the use-dependent and rest-dependent effects on the outward K^+ current, respectively.

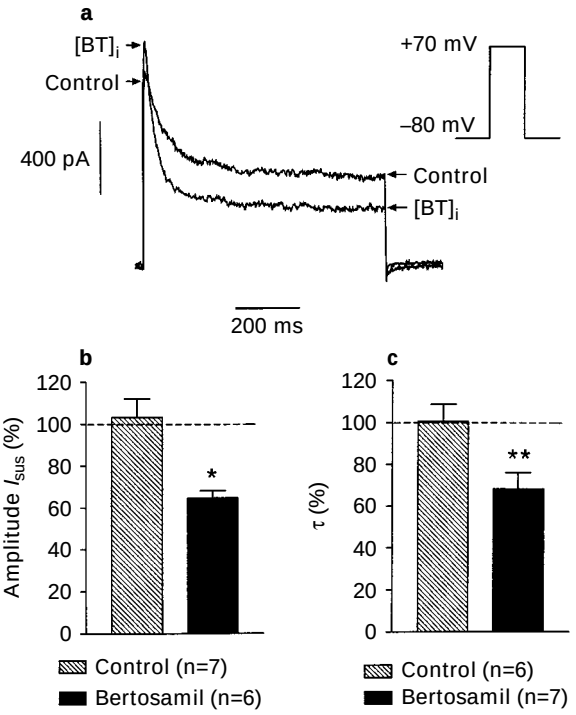

Figure 5 Effects of intracellular bertosamil on outward K^+ current. (a) Superimposition of currents elicited by test pulses from −80 mV to +70 mV obtained just after breaking the patch and after a 10 min dialysis of the cell with a 10 μM bertosamil-containing internal solution $[BT]_i$. Cell capacitance: 95 pF. (b) Percentage changes in the amplitude of I_{sus} and (c) in the rate of inactivation ($\tau 1$) during prolonged intracellular dialysis with a control solution and 10 μM bertosamil-containing internal solution. Values are means±s.e.mean. (*$P<0.05$ and **$P<0.01$).

Table 2 Series resistance (R_s)

	R_s *at time 0*	R_s *after 10 min dialysis*
Control	28.8±12.1 (7)	21.2±8.6 (7) NS
Bertosamil 10 μM	36.7±11.5 (5)	27.3±7.2 (5) NS

Values are presented as the mean±s.e.mean and compared with Student's paired t test (NS: non significant).
Numbers in parentheses indicate the number of experiments.

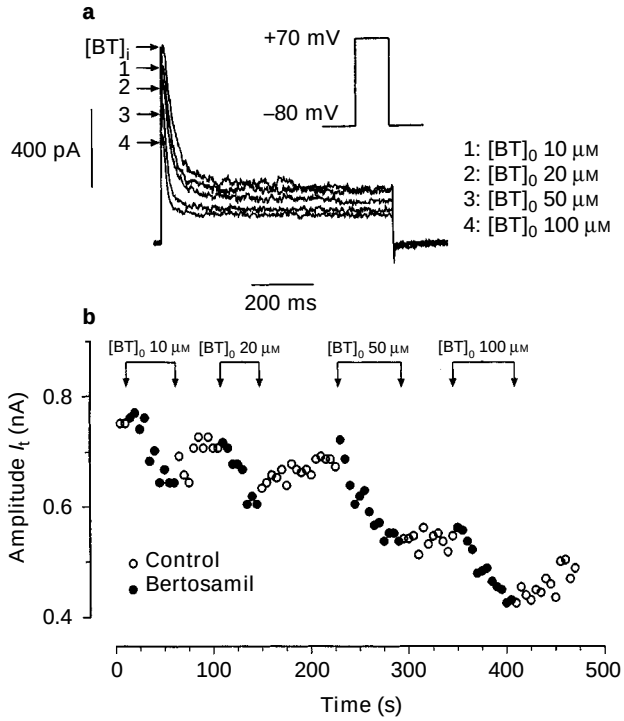

Figure 6 Effects of external bertosamil on outward K^+ current in the presence of intracellular bertosamil. (a) Superimposition of current traces elicited by test pulses from −80 mV to +70 mV recorded after internal dialysis of 10 μM bertosamil ($[BT]_i$) and in the presence of increasing extracellular concentrations of the drug (traces 1 to 4). Cell capacitance: 111 pF. (b) Time course of changes in the amplitude of I_t during external application of increasing concentrations of bertosamil separated by washout periods (control).

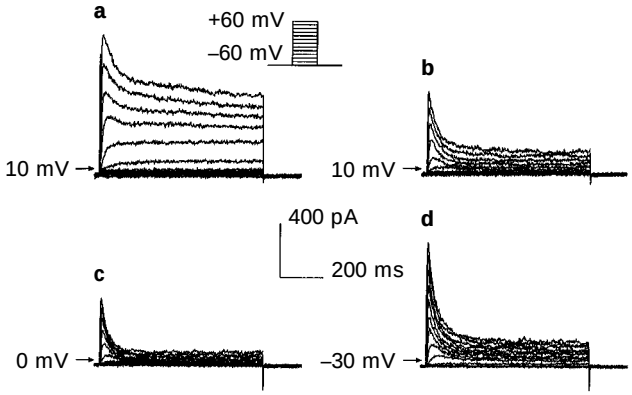

Figure 7 Effects of external application of 4-AP and bertosamil on I_t. Family of currents elicited by 10 mV step depolarizations from a holding potential of −60 mV in control conditions (a), on 200 μM 4-AP exposure (b), 200 μM 4-AP and 10 μM bertosamil exposure (c) and following 4-AP and bertosamil washout (d). The activation potentials of the outward K^+ current is indicated. Cell capacitance: 74 pF.

Bertosamil inactivates I_{sus}

Previous studies have shown that in human atrial myocytes I_t and I_{sus} can be distinguished by their sensitivity to the K^+ channel blocker 4-aminopyridine (4-AP), which reversibly suppresses I_{sus} at micromolar concentrations, whereas millimolar concentrations are necessary to suppress I_t (Wang *et al*., 1993; Hatem *et al*., 1996). This is in keeping with the high sensitivity to 4-AP ($IC_{50} = 50$ μM) of expressed Kv1.5 channels which are believed to carry I_{sus} (Bouchard & Fedida, 1995). Thus, in an attempt to study the effects of bertosamil on I_t independently of I_{sus}, the latter was inhibited by 200 μM 4-AP which is 4 times the IC_{50} for I_{sus} but one order of magnitude below that for I_t (Wang *et al*., 1993). Figure 7a–b shows families of currents elicited by 10 mV incremental test pulses from −60 mV to +60 mV in control conditions (Figure 7a)

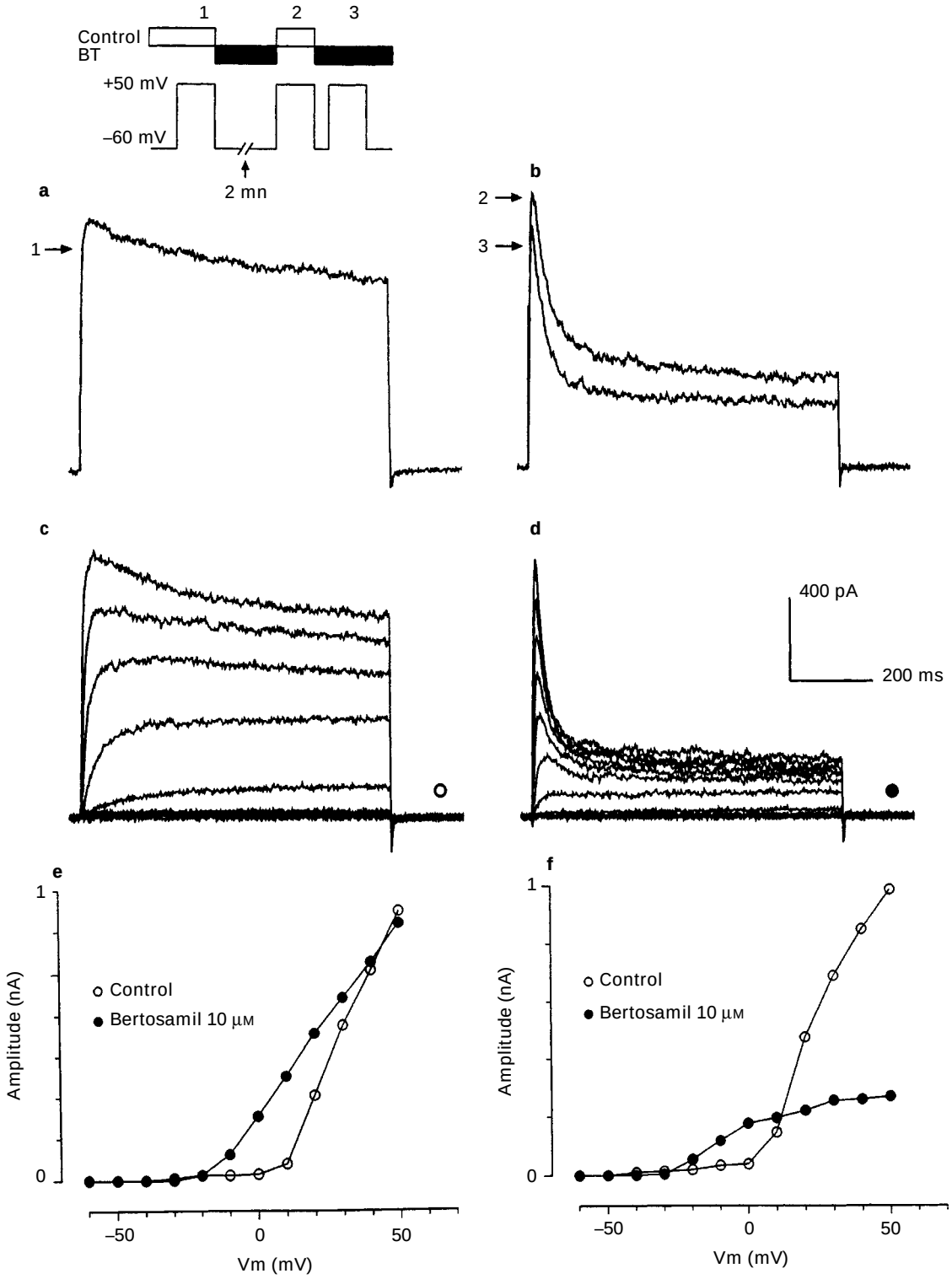

Figure 8 Effects of bertosamil on I_{sus} in myocytes lacking I_t. (a) Traces of current elicited by test pulses from −60 to +50 mV obtained in control conditions (trace 1), (b) on resumption of stimulation following application of bertosamil only during the rest period (trace 2) and on the reintroduction of bertosamil (trace 3). Family of currents elicited by 10 mV depolarization steps (c) in control conditions and (d) following bertosamil exposure. Cell capacitance: 186 pF. Current-voltage relationships of the current measured at the maximum peak amplitude (I_{total}, e) and at the end of 750 ms test pulses (I_{sus}, f) in control conditions and following bertosamil exposure.

and upon 4-AP exposure (Figure 7b). 4-AP markedly inhibited I_{sus} at all potentials at which this current activated (at +60 mV, 66.2±13.9%, $n=4$), whereas the amplitude of I_t was slightly decreased (at +60 mV, 8.9±7.6%, $n=4$). In the presence of 200 μM 4-AP, 10 μM bertosamil slightly inhibited I_t and I_{sus} (at +60 mV, 30.7±9.0% and 76.1±12.3%, respectively, $n=4$; Figure 7c). Washout of bertosamil and 4-AP was associated with a marked increase in the inactivating component, which reached values higher than in control conditions, whereas I_{sus} remained largely inhibited. Note that a shift of the activation of the outward K^+ current towards negative potentials was observed following bertosamil washout.

The relative amplitude of I_t and I_{sus} can vary greatly from cell to cell in human atria. Wang *et al.* (1993) even showed that the inactivating current was lacking in about 10% of myocytes. We took advantage of this situation to analyse further the effect of bertosamil in cells exhibiting a prominent I_{sus} (i.e. a slowly, monoexponentially decaying outward current) (Figure 8a). Figure 8b shows that application of bertosamil (10 μM) during a 2 min rest period (application stopped just before resuming stimulation) induced a large initial rapidly inactivating component and markedly inhibited the current remaining at the end of the test pulse (see trace 2, Figure 8b). Both the inactivating and sustained components were inhibited at the reintroduction of the drug (see trace 3, Figure 8b). Current traces elicited by a 10 mV incremental test pulse protocol before and after application of bertosamil are illustrated in Figure 8c and d, respectively. In control conditions a rapidly slowly inactivating current began to activate at +10 mV, while upon bertosamil exposure a rapidly inactivating began to activate at −20 mV. Figure 8 shows the current-voltage relationships of the current measured at the time of its maximum amplitude (Figure 8e) and at the end of the test pulse (Figure 8f) in control conditions and in the presence of bertosamil. In the presence of the drug the peak current-voltage relationships was shifted to the left by ~20 mV (Figure 8e) and the relationship of the current measured at the end of the pulse was characteristic of an inward rectifying process (Figure 8f). By means of a double test-pulse protocol, we checked that the drug-induced inactivating component of I_{sus} could be inhibited during a second test pulse separated from the first by an interval (10 ms) too short to allow normal recovery of the current from inactivation (Figure 9). Taken together, these data indicate that bertosamil induces an inactivation of I_{sus} which contributes to the increase in I_t.

Bertosamil modifies gating characteristics of I_t

In the presence of bertosamil the inactivating component of the outward K^+ current probably resulted from the fusion of I_t recorded before drug application and the inactivating component of the current resulting from drug-induced inactivation of I_{sus}. Consequently, the gating characteristics of the inacti-

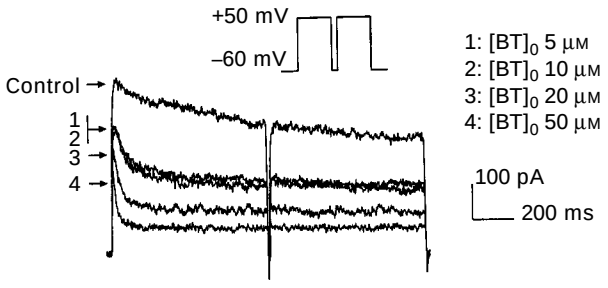

Figure 9 Traces of current elicited by a double-test pulse protocol separated by 10 ms intervals in myocyte with a prominent I_{sus}. At all concentrations tested, bertosamil induced a rapid initial inactivating component and a fall in the sustained component of current elicited by the first pulse, whereas the second pulse only elicited a decreased non inactivating current. Cell capacitance: 92 pF.

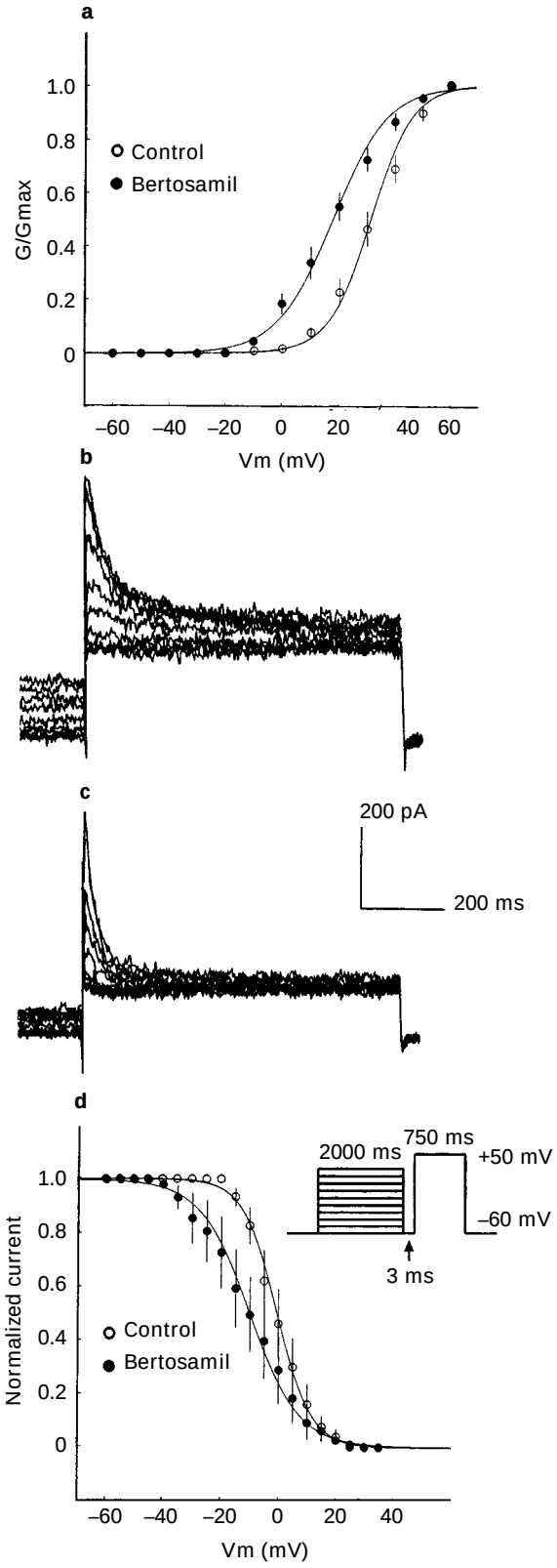

Figure 10 Changes in the gating characteristics of the voltage-activated outward K^+ current induced by external application of bertosamil. (a) Activation curve of I_t in control conditions and on 10 μM bertosamil exposure. Normalized conductances are plotted as a function of test potential and given an equilibrium potential for K^+ of −85 mV in our experimental conditions ($n=13$). Traces of currents elicited by the second pulse of the protocol shown in the inset in the absence (b) and presence of 10 μM bertosamil (c). Cell capacitance: 117 pF. (d) Inactivation curve of I_t in control and bertosamil conditions obtained with the protocol shown in the inset. Data were normalized to the largest amplitude of current (I/I_{max}) ($n=8$). Values are means and vertical lines show s.e.mean.

Table 3 Gating characteristics of I_t and I_{sus} during external application of bertosamil

	Activation			*Inactivation*	
	$V_{1/2}$ (mV)	k (mV)	G_{max} (nS)	$V_{1/2}$ (mV)	k (mV)
I_t					
Control	31.3±2.1 (13)	7.5±0.4 (13)	3.2±0.3 (13)	−0.6±2.8 (8)	−4.1±0.2 (8)
Bertosamil 10 μM	17.7±2.5 (13)**	9.7±0.4 (13)**	3.5±0.4 (13) NS	−10.6±5.2 (8)**	−4.7±0.2 (8) NS
I_{sus}					
Control	8.7±2.7 (13)	12.0±0.9 (13)	2.9±0.5 (13)		
Bertosamil 10 μM	−3.6±3.6 (13)**	13.3±1.3 (13) NS	1.7±0.4 (13)**		

Values are presented as the mean±s.e.mean and compared with Student's paired t test; $^{**}P<0.01$; NS: non significant. Numbers in parentheses indicate the number of experiments.

vating component of the outward current should be altered upon bertosamil exposure. Figure 10a shows activation-voltage relationships of I_t in the absence (control) and presence of 10 μM bertosamil. Data were best fitted by a single Boltzmann distribution (see equation 3 in Methods). Bertosamil induced a marked shift of both I_t and I_{sus} towards negative potentials, as indicated by the variation of $V_{1/2}$ (Table 3). Furthermore, bertosamil significantly inhibited the maximal conductance (G_{max}) of I_{sus} (Table 3). Steady-state inactivation of the outward K^+ current was induced by using a double-pulse protocol (2 s prepulse followed 3 ms later by a 750 ms pulse at +50 mV) in control and bertosamil conditions (Figures 10b–c). Figure 10d shows inactivation curves of I_t, which were best fitted by a Boltzmann distribution (see equation 4 in Methods). Bertosamil shifted the curve towards negative potentials, as indicated by the significant variations in $V_{1/2}$ (Table 3). The kinetics of I_t recovery from inactivation were studied by using a double-pulse protocol (two 750 ms test pulses from −60 mV to +50 mV separated by variable intervals shown in the inset of Figure 11). The analysis was done in control conditions (Figure 11a) and in the presence of external 10 μM bertosamil (Figure 11b). The amplitudes of I_t at the different intervals were normalized to I_t elicited by the conditioning pulse, and the values were plotted against the interpulse interval (Figure 11c). The curves of the mean data were fitted by a two-exponential function (see equation 5 in Methods), resulting in the time constants of recovery and current amplitudes indicated in Table 4. The reactivation kinetics of I_t were markedly slowed by 10 μM bertosamil, as indicated by the increased amplitude of the slow phase, whereas that of the rapid phase was decreased (Table 4). These data indicate a much larger contribution of the slow phase to the recovery process of I_t induced by bertosamil which, together with changes in its activation and steady-state inactivation, point to major changes in the conductance underlying the inactivating component of the outward K^+ current.

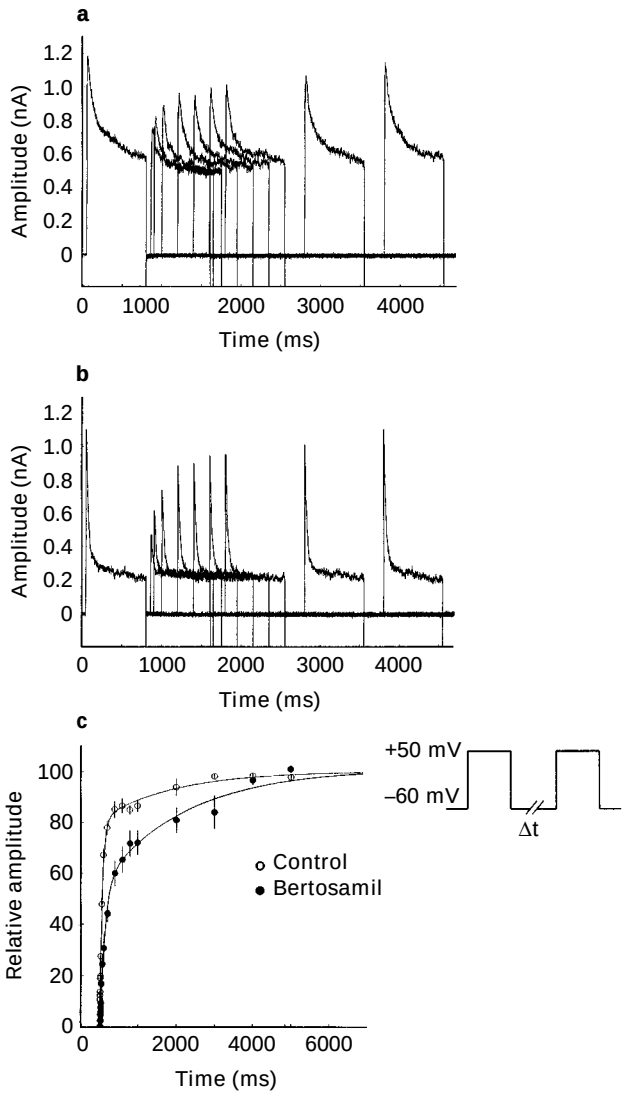

Figure 11 Effects of bertosamil on reactivation kinetics of I_t. Representative current traces of the recovery from inactivation of the inactivating current in control conditions (a) and after 10 μM bertosamil exposure (b) with a protocol consisting of two identical 750 ms pulses from −60 mV to +50 mV with interpulse interval of varying duration (from 4 to 1000 ms). Cell capacitance: 154 pF. (c) Graph showing time course of recovery from inactivation of I_t in the absence (control) and presence of 10 μM bertosamil ($n=7$). Values are means and vertical lines show s.e.mean.

Discussion

To our knowledge, this is the first description of the effects of the antiarrhythmic agent bertosamil on the outward K^+ current of human atrial myocytes. We found two effects of bertosamil: (i) inhibition of I_{sus} associated with accelerated inactivation and increased amplitude of I_t when the drug was applied at rest or internally, and (ii) a concentration- and use-dependent inhibition of I_t and I_{sus} during external application of the drug. Our main conclusion is that, in addition to a use-dependent inhibitory effect on the outward K^+ current, bertosamil induces a rapid inactivation of I_{sus}. The latter effect could constitute a new mechanism by which antiarrhythmic agents modulate the outward K^+ current in human atrial myocytes.

Several mechanisms may explain the different effects of 10 μM bertosamil on the two components of the outward K^+ current: (i) inhibition of I_{sus} associated with a stimulation of I_t, and (ii) changes in the inactivation kinetics of the outward K^+ current, which could be caused by either an open channel block resulting in time-dependent inhibition of the current or alterations in the gating mechanism of K^+ channels. A stimulating effect of bertosamil on I_t is inconsistent with the observation that, following inhibition of I_{sus} by 4-AP (Hatem *et al*., 1996), 10 μM bertosamil inhibited I_t. Time-dependent inhibition of the outward K^+ current caused by an open-

Table 4 Time course of recovery from inactivation of I_t

	τ *fast* (ms)	*Amplitude fast* (%)	τ *slow* (ms)	*Amplitude slow* (%)
Control	69.4±6.2	79.0±4.9	1878.9±151.7	19.3±3.8
Bertosamil 10 μM	127.6±31.8 NS	54.3±6.6*	2165.5±255.9 NS	45.9±6.8*

Values are presented as the mean±s.e.mean and compared with Student's paired *t* test; *$P<0.05$; NS: non significant ($n=7$).

channel block can be ruled out, as the effects of 10 μM bertosamil on I_t and I_{sus} were observed when the drug was applied during a resting period or in bertosamil-pretreated cells (Figure 4c), indicating that bertosamil binds to the channels in the resting (closed) state. Therefore, the most likely explanation for our results is that bertosamil accelerates the kinetics of inactivation of the outward current, which appears to result mainly from a drug-induced inactivation of I_{sus}. This is deduced from the observation that in myocytes exhibiting a prominent I_{sus} and almost no I_t, bertosamil, applied only during rest, transformed the slowly inactivating current into a rapidly inactivating I_t-type current. Furthermore, when I_{sus} was suppressed by pretreating cells with 200 μM 4-AP, bertosamil inhibited I_t with little change in its rate of inactivation. Following washout of both 4-AP and bertosamil, step depolarizations elicited a much larger inactivating component than that recorded in control conditions, with a marked suppression of the current remaining at the end of the test pulses and a shift of the activation of the outward current towards negative potentials. This indicates that removal of 4-AP blockade of I_{sus} unmasked the inactivation of I_{sus} induced by bertosamil. This drug-induced inactivation of I_{sus} is clearly different from the use-dependent inhibition of I_{sus} by bertosamil, which persists after intracellular dialysis or external application of the drug during rest. This may explain why a larger inhibition of I_{sus} was obtained upon external than internal bertosamil exposure: in the former condition the inhibition of I_{sus}, probably results from the addition of a drug-induced inactivation and inhibition of I_{sus}. The inactivation of I_{sus} induced by bertosamil contributes, to a large extent, to the apparent increase in I_t, its accelerated inactivation and changes in its steady-state inactivation (Figure 10) and its rate of recovery from inactivation (Figure 11). These alterations, which indicate marked modifications in the gating and conductance properties of channels carrying I_t, very likely reflect the fact that, in the presence of bertosamil, the inactivating component of the outward current results from the fusion of I_t recorded before drug application and the inactivating component of I_{sus} induced by bertosamil.

Various mechanisms have been shown to modulate the inactivation kinetics of rapidly activating non-inactivating voltage-gated outward K^+ currents. For instance, coexpression of cloned β-subunits with α-subunits of Kv1 channels confers rapid A-type inactivation to non inactivating delayed rectifier currents (Rettig *et al*., 1994; Majumder *et al*., 1995; England *et al*., 1995a,b; Leicher *et al*., 1996). In addition, coexpression of the hKvβ1.3-subunit with hKv1.5 *Shaker* channels results in a shift toward negative potentials of the voltage-dependent activation of the current, together with rapid and partial inactivation of the current at positive potential, resulting in an inward rectification (England *et al*., 1995a). It has also been shown that, in addition to transitions of the Hodgkin-Huxley type involved in the activation of voltage-dependent *Shaker* channels, another transition precedes the opening of the pore in channels from which the inactivation gate has been deleted. This transition can be selectively blocked by a potassium channel blocker such as 4-AP, which binds to an internal site and stabilizes the closed pore structure (McCormack *et al*., 1994). Other K^+ channel blockers such as quaternary ammonium accelerate C-type inactivation of cloned *Shaker* channels without N-type inactivation (Baukrowitz & Yellen, 1996a,b). No firm conclusion can be drawn as to the exact mechanism by which bertosamil modulates the inactivation kinetics of I_{sus} on the basis of our results. Nevertheless, our data underline a clear analogy between the effects of the hKvβ1.3-subunit on hKv1.5 (England *et al*., 1995a) and of bertosamil on I_{sus}, i.e. a shift of activation to more negative potentials, partial inactivation and inward rectification with depolarization, suggesting that the drug might modulate the interactions between β- and α-subunits. If this is the case, the binding site of the drug should be easily accessible at the cytoplasmic face of the sarcolemma (where β-subunits are located), in keeping with the marked effect of intracellular dialysis of bertosamil described here.

It is generally believed that I_t and I_{sus} are distinct currents characterized by specific electrophysiological and pharmacological properties. As Kv1.5 *Shaker* channels are abundantly expressed in human atrial myocardium (Bouchard & Fedida, 1995; Mays *et al*., 1995) and carry a rapidly activating slowly inactivating current highly sensitive to 4-AP (Bouchard & Fedida, 1995; Grissmer *et al*., 1994), it is assumed that this channel type carries I_{sus} in human atrial myocytes. Furthermore, cloned Kv1.5 *Shaker* channels are highly sensitive to tedisamil (Honoré *et al*., 1994), which induces an open-channel block in keeping with the use-dependent inhibitory effect of bertosamil on I_{sus}. However, our result indicate that other mechanisms account for the effects of bertosamil on I_{sus} in human atrial myocytes, possibly involving complex interactions between the various channel subunits in cardiac myocytes. The present observation that bertosamil modifies I_t through the inactivation of I_{sus} is another illustration of the difficulty in analysing the effects of a drug on global currents of intact cells only in terms of distinct sensitivity of channels to the drug.

In conclusion, bertosamil-induced inactivation of rapidly activating slowly inactivating outward current in human atrial myocytes may be an important new mechanism by which currents involved in the control of the shape and duration of the action potential are modulated. I_t is more strongly depressed than I_{sus} during atrial dilatation secondary to chronic haemodynamic overload in man. This alteration of the outward K^+ current participates, together with the reduction in the Ca^{2+} current, in the disappearance of the early repolarizing phase and the shortening of the action potential (Le Grand *et al*., 1994), which could contribute to the high incidence of arrhythmias during atrial dilatation (Boutdjir *et al*., 1986). Thus, the ability of bertosamil to modulate differentially the two voltage-gated outward K^+ currents and to restore an inactivating component may be an original property of potential interest in the treatment of atrial arrhythmias.

We thank the surgical team headed by Professor Jean-Yves Neveux for providing atrial samples. We are also indebted to David Young for his help in restyling the manuscript and Dr Ziegler (Solvay Pharma Deutchland, Hannover, Germany) for generous gift of bertosamil. This work was supported by grants from Institut National de la Santé et de la Recherche Médicale (INSERM, CRE 930410), Ministère de la Recherche et de l'Enseignement Supérieur (ACC-SV9), Fondation de l'Avenir, and Hôpital Marie Lannelongue. S.T. was supported by a grant from Ministère de l'Enseignement Supérieur et de la Recherche and S.N.H. by a grant from Institut National de la Santé et de la Recherche Médicale (poste d'accueil).

References

BAUKROWITZ, T. & YELLEN, G. (1996a). Use-dependent blockers and exit rate of the last ion from the multi-ion pore of a K^+ channel. *Science*, **271** 653–656.

BAUKROWITZ, T. & YELLEN, G. (1996b). Two functionally distinct subsites for the binding of internal blockers to the pore of voltage-activated K^+ channels. *Proc. Natl. Acad. Sci. U.S.A.*, **93,** 13357–13361.

BOUCHARD, R. & FEDIDA, D. (1995). Closed- and open-state binding of 4-aminopyridine to the cloned human potassium channel Kv1.5. *J. Pharmacol. Exp. Ther.*, **275,** 864–876.

BOUTJDIR, M., LE HEUZEY, J.-Y., LAVERGNE, T., CHAUVAUD, S., GUIZE, L., CARPENTIER, A. & PERONNEAU, P. (1986). Inhomogeneity of cellular refractoriness in human atrium: a factor of arrhythmias? *Pace*, **9,** 1095–1100.

CASTELLINO, R.C., MORALES, M.J., STRAUSS, H.C. & RASMUSSON, R.L. (1995). Time- and voltage-dependent modulation of a Kv1.4 channel by a β-subunit (Kvβ3) cloned from ferret ventricle. *Am. J. Physiol.*, **269,** (*Heart Circ. Physiol.*, **38**), H385–H391.

CRUMB, W.J., PIGOTT, J.D. & CLARKSON, C.W. (1995). Comparison of I_{to} in young and adult atrial myocytes: evidence for developmental changes. *Am. J. Physiol.*, **268,** (*Heart Circ. Physiol.*, **37**), H1335–H1342.

DUKES, I.D. & MORAD, M. (1989). Tedisamil inactivates transient outward K^+ current in rat ventricular myocytes. *Am. J. Physiol.*, **257** (*Heart Circ. Physiol.*, **26**), H1746–H1749.

ENGLAND, S.K., UEBELE, V.N., KODALI, J., BENNETT, P.B. & TAMKUN, M.M. (1995a). A novel K^+ channel β-subunit (hKvβ1.3) is produced via alternative mRNA splicing. *J. Biol. Chem.*, **270,** 28531–28534.

ENGLAND, S.K., UEBELE, V.N., SHEAR, H., KODALI, J., BENNETT, P.B. & TAMKUN, M.M. (1995b). Characterization of a voltage-gated K^+ channel β subunit expressed in human heart. *Proc. Natl. Acad. Sci. U.S.A.*, **92,** 6309–6313.

ESCANDE, D., COULOMBE, A., FAIVRE, J.F., DEROUBAIX, E. & CORABOEUF, E. (1987). Two types of transient outward currents in adult human atrial cells. *Am. J. Physiol.*, **252,** (*Heart Circ. Physiol.*, **21**), H142–H148.

ESCANDE, D., LOISANCE, D., PLANCHE, C. & CORABOEUF, E. (1985). Age-related changes of action potentials plateau shape in isolated human atrial fibres. *Am. J. Physiol.*, **249**, (*Heart Circ. Physiol.*, **18**), H843–H850.

FEDIDA, D., WIBLE, B., WANG, Z., FERMINI, B., FAUST, F., NATTEL, S. & BROWN, A.M. (1993). Identity of a novel delayed rectifier current from human heart with a cloned K^+ channel current. *Circ. Res.*, **73,** 210–216.

GRISSMER, S., NGUYEN, A.N., AIYAR, J., HANSON, D.C., MATHER, R.J., GUTMAN, G.A., KARMILOWICZ, M.J., AUPERIN, D.D. & CHANDY, K.G. (1994). Pharmacological characterization of five cloned voltage-gated K^+ channels, types Kv1.1, 1.2, 1.3, 1.5 and 3.1, stably expressed in mammalian cell lines. *Mol. Pharmacol.*, **45,** 1227–1234.

HATEM, S.N., BENARDEAU, A., RÜCKER-MARTIN, C., SAMUEL, J.-L., CORABOEUF, E. & MERCADIER, J.-J. (1996). Differential regulation of voltage-activated potassium currents in cultured human atrial myocytes. *Am. J. Physiol.*, **271** (*Heart Circ. Physiol.*, **40**), H1609–H1619.

HONORÉ, E., BARHANIN, J., ATTALI, B., LESAGE, F. & LAZDUNSKI, M. (1994). External blockade of the major cardiac delayed-rectifier K^+ channel (Kv1.5) by polyunsaturated fatty acids. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 1937–1944.

LEFEVRE, I.A., COULOMBE, A. & CORABOEUF, E. (1991). The calcium antagonist D600 inhibits calcium-independent transient outward current in isolated rat ventricular myocytes. *J. Physiol.*, **432,** 65–80.

LE GRAND, B., HATEM, S., DEROUBAIX, E., COUETIL, J.P. & CORABOEUF, E. (1994). Depressed transient outward and calcium currents in dilated human atria. *Cardiovasc. Res.*, **28,** 548–556.

LEICHER, T., ROEPER, J., WEBER, K., WANG, X. & PONGS, O. (1996). Structural and functional characterization of human potassium channel subunit β1 (KCNA1B). *Neuropharmacology*, **35,** 787–795.

MAJUMDER, K., DE BIASI, M., WANG, Z. & WIBLE, B.A. (1995). Molecular cloning and functional expression of a novel potassium channel β-subunit from human atrium. *FEBS Lett.*, **361,** 13–16.

MAYS, D.J., FOOSE, J.M., PHILIPSON, L.H. & TAMKUN, M.M. (1995). Localization of the Kv1.5 K^+ channel protein in explanted cardiac tissue. *J. Clin. Invest.*, **96,** 282–292.

MCCORMACK, K., JOINER, W.J. & HEINEMANN, S.H. (1994). A characterization of the activating structural rearrangements in voltage-dependent *Shaker* K^+ channels. *Neuron*, **12,** 301–315.

MCCORMACK, K., MCCORMACK, T., TANOUYE, M., RUDY, B. & STÜHMER, W. (1995). Alternative splicing of the human *Shaker* K^+ channel β1 gene and functional expression of the β_2 gene product. *FEBS Lett.*, **370,** 32–36.

MORALES, M.J., CASTELLINO, R.C., CREWS, A.L., RASMUSSON, R.L. & STRAUSS, H.C. (1995). A novel β subunit increases rate of inactivation of specific voltage-gated potassium channel α subunits. *J. Biol. Chem.*, **270,** 6272–6277.

NÉMETH, M., VIRAG, L., HALA, O., VARRO, A., KOVACS, G., THORMAHLEN, D. & PAPP, J.G. (1996). The cellular electrophysiological effects of tedisamil in human atrial and ventricular fibers. *Cardiovasc. Res.*, **31,** 246–248.

PO, S., ROBERDS, S., SNYDERS, D.J., TAMKUN, M.M. & BENNETT, P.B. (1993). Heteromultimeric assembly of human potassium channels. Molecular basis of a transient outward current? *Circ. Res.*, **72,** 1326–1336.

RETTIG, J., HEINEMANN, S.H., WUNDER, F., LORRA, C., PARCEJ, D.N., DOLLY, J.O. & PONGS, O. (1994). Inactivation properties of voltage-gated K^+ channels altered by presence of β-subunit. *Nature*, **369,** 289–294.

ROBERDS, S.L. & TAMKUN, M.M. (1991). Cloning and tissue-specific expression of five voltage-gated potassium channel cDNAs expressed in rat heart. *Proc. Natl. Acad. Sci. U.S.A.*, **88,** 1798–1802.

SHIBATA, E.F., DRURY, T., REFSUM, H., ALDRETE, V. & GILES, W. (1989). Contribution of a transient outward current to repolarization in human atrium. *Am. J. Physiol.*, **257,** (*Heart Circ. Physiol.*, **26**), H1773–H1781.

SNYDERS, D.J. & YEOLA, S.W. (1995). Determinants of antiarrhythmic drug action. Electrostatic and hydrophobic components of block of the human cardiac hKv1.5 channel. *Circ. Res.*, **77,** 575–583.

TAMKUN, M.M., KNOTH, K.M., WALBRIDGE, J.A., KROEMER, H., RODEN, D.M. & GLOMER, D.M. (1991). Molecular cloning and characterization of two voltage-gated K^+ channel cDNAs from human ventricle. *FASEB J.*, **5,** 331–337.

TYTGAT, J. & HESS, P. (1992). Evidence for cooperative interactions in potassium channel gating. *Nature*, **359,** 420–423.

WANG, Z., FERMINI, B. & NATTEL, S. (1993). Sustained depolarization-induced outward current in human atrial myocytes. Evidence for a novel delayed rectifier K^+ current similar to Kv1.5 cloned channel currents. *Circ. Res.*, **73,** 1061–1076.

YAMAGISHI, T., ISHII, K. & TAIRA, N. (1995). Antiarrhythmic and bradycardic drugs inhibit currents of cloned K^+ channels, Kv1.2 and Kv1.4. *Eur. J. Pharmacol.*, **281,** 151–159.

YATANI, A., WAKAMORI, M., MIKALA, G. & BAHINSKI, A. (1993). Block of transient outward-type cloned cardiac K^+ channel currents by quinidine. *Circ. Res.*, **73,** 351–359.

(Received March 6, 1997
Revised May 9, 1997
Accepted June 11, 1997)