Effects of cardioactive drugs on the transmembrane potassium currents in ventricular myocytes

PhD Thesis

László Virág

Department of Pharmacology, Albert Szent-Györgyi Medical University, Szeged, Hungary

1998

Summary

Cardiovascular diseases have a leading role in mortality in the developed countries. One of the most serious problems is the high prevalence and incidence of ischaemic heart disease accompanied by life threatening cardiac arrhythmias. The pharmacological treatment of cardiac arrhythmias has so far remained largely empiric because of our incomplete understanding of the mechanisms by which antiarrhythmic drugs prevent, suppress, and in some cases also induce, arrhythmias. Therefore, it is important to study the mechanism of action of antiarrhythmic drugs at the organ, tissue, cellular and also subcellular levels alike.

In the experiments described in the present thesis single ventricular myocytes isolated from guinea-pig or rabbit heart were used to analyze the effect of cardioactive drugs on transmembrane potassium currents. For cell isolation, an enzymatic dissociation procedure, based on the Langendorff perfusion method, was applied. The transient outward potassium current (I_{to}), the inward rectifier potassium current (I_{k1}) and delayed rectifier potassium current (I_K) were recorded in the whole-cell configuration of the patch-clamp technique. The ATP sensitive potassium current (I_{KATP}) was measured in the inside-out configuration.

The effects of seven antiarrhythmic drugs, quinidine, disopyramide, tedisamil, flecainide, sotalol, amiodarone and GLG-V-13, a new agent with class III antiarrhythmic properties, were compared. All studied drugs decreased the amplitude of I_{Kr} but none of the agents influenced significantly I_{k1}. The amplitude of I_{to} was decreased by quinidine, disopyramide, tedisamil flecainide and not changed by sotalol, GLG-V-13 and acute administration of amiodarone. Among the effective drugs, the inactivation kinetics of I_{to} was significantly accelerated by quinidine, disopyramide and tedisamil decreasing further the densities of I_{to}. During our detailed analysis of disopyramide on I_{to} it was found that the effect of the drug is not use-dependent, the drug binds mainly to the open state of the channel, and the inactivation gating may not be influenced by disopyramide.

The chronic effects of amiodarone were compared to the action of the drug observed after acute administration. Chronic amiodarone treatment decreased substantially the density of I_{Kr} and moderately the amplitude of I_{to} but did not influence the magnitude of I_{k1}. Considering the results of the present and the previous studies it seems reasonable to assume that since acute amiodarone application inhibits both inward and outward currents, the effect of the drug on action potential duration (APD) depends on the relative contribution of these currents to the repolarization in a particular tissue type. Therefore, acute administration of amiodarone may result in lengthening, shortening or no change of the repolarization in cardiac cells. However, after chronic treatment, amiodarone substantially decreases the density of I_K and slightly I_{to} causing lengthening of ADP in ventricular muscle.

The inhibitory effect of glibenclamide (an antidiabetic drug claimed to possess both antiarrhythmic and arrhythmogenic properties) on the activity of ATP sensitive potassium channels (K^+_{ATP} channels) was tested in the presence of ATP and ADP in the intracellular side of the cell membrane. It was found that the blockade of K^+_{ATP} channels by the drug was stronger in the presence of higher concentration of ATP but was weaker if, in addition to ATP, ADP was also applied in the intracellular solution. It was also observed that activity of the channels was decreased by the anion channel blocker stilbene disulfonates. Investigating the mechanism of reactivation of K^+_{ATP} channels by MgATP complex after run-down we conclude that the hydrolysis of the ATP is an important factor for reactivation K_{ATP} channels and the hydrolysis energy appears to be utilized for channel reactivation.

It is concluded that the cardioactive drugs examined in this study exhibit considerable effects on potassium currents in ventricular myocytes which might explain, at least in part, their differential efficacy in various cardiac arrhythmias.

Contents

List of publications related to the subject of the Thesis

Full papers

I. Virág L, Furukawa T, Hiraoka M: Modulation of the effect of glibenclamide on K_{ATP} channels by ATP and ADP. *Mol Cell Biochem* 119:209-215, 1993.

II. Furukawa T, Virág L, Sawanobori T, Hiraoka M: Stilbene disulfonates block ATP-sensitive K^+ channels in guinea pig ventricular myocytes. *J Membrane Biol* 136:289-302, 1993.

III. Furukawa T, Virág L, Furukawa N, Sawanobori T, Hiraoka M: Mechanism for reactivation of ATP-sensitive K^+ channel by MgATP complexes in guinea pig ventricular myocytes. *J Physiol* 479:95-107, 1994.

IV. Varró A, Virág L, Papp J Gy: Comparison of the chronic and acute effects of amiodarone on the calcium and potassium currents in rabbit isolated cardiac myocytes. *Br J Pharmacol* 117:1181-1186, 1996.

V. Németh M, Virág L, Varró A, Papp J Gy: Comparison of the effects of restacorin and flecainide on various cardiac transmembrane potassium currents. *Acta Physiol Hung* 84:317-318, 1996.

VI. Virág L, Varró A, Papp J Gy: Effect of disopyramide on potassium currents in rabbit ventricular myocytes. *Naunyn-Schmiedeberg's Arch Pharmacol* 357:268-275, 1998.

Quotable abstracts

VII. Virág L, Varró A, Papp J Gy: Disopyramide decreases the transient outward current in rabbit isolated cardiac myocytes. *J Mol Cell Cardiol* 26:LXXXVII, 1994.

VIII. Virág L, Varró A, Papp J Gy: Effect of disopyramide on potassium currents in isolated rabbit cardiac myocytes. *J Mol Cell Cardiol* 27:A141, 1995.

IX. Varró A, Virág L, Papp J Gy: The effects of chronic amiodarone treatment on the cardiac calcium and potassium channels. *J Mol Cell Cardiol* 27:A140, 1995.

X. Virág L, Furukawa T, Hiraoka M: Lactate fails to activate ATP-sensitive potassium current in membrane patches excised from guinea pig ventricular myocytes. *J Physiol* 489P:74P, 1995.

XI. Virág L, Iost N, Varró A, Papp J Gy: Comparison of several antiarrhythmic drugs on potassium currents in rabbit ventricular myocytes. *J Mol Cell Cardiol* 28:A58, 1996.

XII. Fazekas T, Virág L, Varró A, Papp J Gy, Berlin KD, Scherlag BJ, Lazzara R: Effects of GLG-V-13, a novel class III antiarrhythmic agent, on cardiac K^+ currents in rabbit ventricular myocytes. *J Mol Cell Cardiol* 29:A118, 1997.

XIII. Virág L, Iost N, Fazekas T, Varró A, Scherlag BJ, Lazzara R, Papp J Gy: Modulation of various cardiac potassium currents by a new class III antiarrhythmic drug, GLG-V-13, in rabbit ventricular myocytes. *Naunyn-Schmiedeberg's Arch Pharmacol* 356(Suppl 1):R7, 1997.

1. Introduction

Cardiovascular diseases have a leading role in mortality in the developed countries. One of the most serious problems is the high prevalence and incidence of ischaemic heart disease accompanied by life threatening cardiac arrhythmias. In spite of the important advances in cardiology, the pharmacological treatment of cardiac arrhythmias remained empiric to a large extent because of our incomplete understanding of both the pathophysiological processes underlying the cardiac rhythm disturbances and the mechanisms by which antiarrhythmic drugs prevent, suppress, and in some cases also induce, arrhythmias. Therefore, in order to develop new more effective agents with less proarrhythmic potency, it is important to understand the mechanism of action of antiarrhythmic drugs at the organ, tissue, cellular and also subcellular levels.

The appearance of cardiac arrhythmias can be explained by abnormal impulse formation or by abnormal impulse propagation, and often by the combination of the two. In acute ischaemia the myocytes become depolarized, mainly due to potassium accumulation in the ischeamic region, which results in slowing of the impulse conduction. The action potential duration (APD) and the effective refractory period (ERP) can be excessively shortened as a result of the opening of ATP sensitive potassium channels (K^+_{ATP} channels). These arrhythmogenic factors can be the source of reentry type cardiac arrhythmias. These disadvantageous changes may be influenced by numerous alternative ways among which we have focussed in this study on the possible antiarrhythmic mechanisms occurring via the blockade of the main potassium currents present in ventricular tissue:

(1) inhibition of the transient outward potassium current (I_{to}),
(2) decrease of the inward rectifier potassium current (I_{k1}),
(3) inhibition of the delayed rectifier potassium current (I_K),
(4) blockade of the ATP sensitive potassium current (I_{KATP}).

In the *C*ardiac *A*rrhythmia *S*uppression *T*rial (CAST I and II) long-term treatment with several class I/C antiarrhythmic drugs (flecainide, encainide and moricizine) significantly

increased postinfarction mortality[1,2]. The failure of these drugs in the treatment of patients with ischaemic heart disease or postinfarction arrhythmias may be associated with the effects of the above-mentioned drugs on the fast sodium current, i.e. with their ability to suppress the impulse conduction at normal heart rate which is a possible proarrhythmic property. As a consequence of the negative results of the CAST studies, the strategy of both treatment of ventricular arrhythmias and development of new drugs turned to the antiarrhythmic agents which selectively lengthen APD and ERP without changing of the conduction velocity, i.e. to the class III antiarrhythmic drugs. The *S*urvival *W*ith *O*ral *d*-sotalol (SWORD) study[3], however, reported not a decrease but rather an increase in mortality after treatment with d-sotalol. The reason is complex but the following features of these drugs must be involved in this failure. First, "pure" class III drugs increase the inhomogeneity of repolarization and consequently that of the refractoriness. Second, the reverse use-dependent effect of these drugs on APD is also disadvantageous because at slow heart rate it may cause early afterdepolarizations (EAD) and consequently torsade de pointes type ventricular arrhythmias.

The failure of the long-term antiarrhythmic therapy with class I/C and III agents shifted the interest toward amiodarone, a drug which seems to reduce postinfarction mortality[4,5]. Amiodarone is a widely used old antiarrhythmic drug. Singh and Vaughan Williams observed first the lengthening of the action potential duration after chronic treatment with the drug for 6 weeks in the rabbit[6]. Since this discovery amiodarone has been termed a class III antiarrhythmic drug. Later it was found that under acute conditions the drug use-dependently blocked the maximal rate of depolarization and the inward sodium current[7,8,9]. It was also reported that amiodarone suppressed the inward calcium current[10]. It is now clear that amiodarone is not a "pure" class III drug, but it has multiple actions including blockade of the α- and β-adrenoceptors as reported by Polster and Broekhuysen in 1976[11]. The most characteristic feature of the electrophysiological effects of amiodarone is that the action of the drug differs markedly after acute and chronic administration[12,13,14]. The influence of amiodarone on various transmembrane ionic current has been studied by several research groups. However, these experiments were carried out after acute administration of the drug[9,10,15,16] and the action of amiodarone on the ionic currents after chronic treatment is still unclear. Therefore, one of the aims of this study was to investigate how amiodarone can

influence the most important transmembrane potassium currents after long-term treatment and to compare it to the effects found after acute administration of the drug.

In 1983, Akinori Noma reported the presence of a new type of potassium channel in single channel recordings from cardiac cell membrane[17]. This channel was characterised by a pronounced blockade of channel activity when millimolar concentration of ATP is present in the intracellular side of the cell membrane. The ATP sensitive potassium channel was then described in the membranes of other tissue types such as skeletal muscle[18] and pancreatic β-cell[19]. It is now generally accepted that these K^+_{ATP} channels are responsible for the action potential shortening that occurs in heart muscle during metabolic blockade. The K^+_{ATP} channels open when the cytosolic ATP level decreases below a critical threshold, and shortening the APD and ERP occurs. However, in intact heart the normal cytosolic ATP level is 5 - 10 mM and only decreases by 25 - 50 % in the first 10 min of ischaemia or hypoxia[20] whereas in excised membrane patches the ATP concentration causing half-maximal blockade of the activity of K^+_{ATP} channels is 15 - 100 μM[17,21,22]. Because of this large discrepancy, it is not clear how K^+_{ATP} channels can be activated early enough to account for shortening of the APD during ischaemia or hypoxia. It has been suggested that activation of only a small fraction of the total K^+_{ATP} channels may be sufficient to produce major electrophysiological effects. This is the so-called "spare channel" hypothesis[23,24]. Nichols *et al.* have shown that APD shortening may be explained by activation of < 1 % of K^+_{ATP} channels caused by modest decreases in cytosolic ATP level[23]. Another possible explanation is that metabolites, such as proton (acidosis)[25], increased intracellular ADP level[21,26] and intracellular lactate[27], generated during ischaemia or metabolic inhibition may markedly reduce the sensitivity of K^+_{ATP} channels to ATP. Therefore, one of the goals of the present study was to investigate how these metabolites, particularly the ATP and ADP, influence the effect of glibenclamide (an antidiabetic drugs claimed to possess both antiarrhythmic[28,29] and arrhythmogenic activity[30,31]) on the activity of K^+_{ATP} channels and thereby to gain more insight into the mechanisms controlling the glibenclamide sensitivity of K^+_{ATP} channels.

1.1. Major specific experimental goals

(1) To investigate the effects of several class I and class III antiarrhythmic drugs and some newly developed agents on the following transmembrane ionic currents:

(a) the transient outward potassium current (I_{to}),

(b) inward rectifier potassium current (I_{k1}),

(c) the rapid component of the delayed rectifier potassium current (I_{Kr}).

(2) To investigate how amiodarone influences the I_{to}, I_{k1} and I_{Kr} transmembrane potassium currents after long-term treatment and to compare it to the effects found after acute administration of the drug.

(3) To investigate how the intracellular ATP and ADP can influence the effect of glibenclamide on the activity of K^+_{ATP} channels

2. Methods

2.1. Animals

Guinea-pigs or New-Zealand white rabbits of either sex (body weights 300 - 500 g and 1.5 - 2 kg, respectively) were used for the study. The animals were untreated, except for the rabbits used to study the chronic effect of amiodarone which were given either 50 mg kg^{-1} day^{-1} amiodarone (Cordarone, Labaz) or its solvent (10 % Tween 80, 10 % ethanol in distilled water; 1 ml kg^{-1}) intraperitonially for 3 - 4 weeks. The investigations were in conformity 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 1985).

2.2. Preparation of guinea-pig ventricular myocytes

After heparin administration (300 i.u./kg I.P.) the animals were anaesthetized with sodium pentobarbitone (30-50 mg/kg, I.P.). The chest was opened under artificial respiration and the aorta was cannulated *in situ* before removal of the heart. Using a Langendorff

apparatus, the dissected heart was then perfused at constant pressure of 100 H_2O cm with Tyrode solution for 3 -5 min in order to flush the blood from the heart. The composition of the Tyrode solution was the following (in mM): NaCl 144, NaH_2PO_4 0.33, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, Glucose 5.5, HEPES 5.0 at pH of 7.4. The perfusate was then changed to nominally Ca^{2+}-free Tyrode solution. After the heart stopped contracting completely, nominally Ca^{2+}-free Tyrode solution with collagenase (0.5 mg/ml, type I, Sigma) was recirculated for 15-20 min. The collagenase was then washed out with high K^+, low Cl^- solution containing (in mM): potassium glutamate 70, taurine 15, KCl 30, KH_2PO_4 10, HEPES 10, $MgCl_2$ 0.5, glucose 11 and EGTA 0.5. The pH of this solution was adjusted to 7.3 by KOH. All perfusates were equilibrated with 100% O_2 at 37 °C. After the ventricles had been cut into small pieces in high K^+, low Cl^- solution and shaken gently in a shaker machine at 37 °C, single cells were separated from tissue pieces by filtering them through a 300 μM mesh. The filtrate was centrifugated at 19 *g* for 5 min and the pellet was finally resuspended and stored in the high K^+, low Cl^- solution at 4 °C for minimum of 60 min before starting the experiment. This method has been developed in the Department of Cardiovascular Diseases, Tokyo Medical and Dental University.

2.3. Preparation of rabbit ventricular myocytes

Based on the above method we decided to adapt this procedure for isolation of rabbit ventricular myocytes to our laboratory conditions.

The animals were sacrificed by cervical dislocation after receiving 400 i.u./kg heparin i.v. The chest was opened and the heart was quickly removed and placed into cold (4 °C) nutrient solution with the following composition (mM): NaCl 135, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, HEPES 10, $NaHCO_3$ 4.4, glucose 10, $CaCl_2$ 1.8, pH 7.2. The heart was then mounted on the basement of a modified, 60 cm high Langendorff column and retrogradely perfused with the previously described solution for washing the blood out of the heart. It took about 3 - 5 min. The heart was then perfused with nominally Ca-free solution using a perfusion pump with an approximate flow rate of 24 ml/min. for 4 min. At the end of this period the heart was completely quiescent and flaccid. The digestion was started by recirculation (approx.

12 ml/min) of the nominally Ca-free solution containing 0.5 mg/ml Collagenase (Sigma type I) and 0.04 mg/ml Pronase E (Sigma) with 0.1 % albumin. We found that when the Ca^{2+} concentration was elevated by 200 μM in the 5th minute of the enzyme perfusion the viability of the myocytes increased. Digestion was allowed to proceed for 15 min, after that the heart was removed from the cannula. The essential part of the isolation process was the following: the ventricles were placed into enzyme free solution containing 1.8 mM $CaCl_2$ and 1 % albumine and were allowed to equilibrate at 37 °C for 10 min. Thereafter the tissue was cut into small fragments. This procedure substantially increased the yield and the quality of the isolated cells. We observed that when we did not wait for 10 min before cutting up the tissue or if we equilibrated the ventricles in Ca^{2+}-free solution the ratio of rod-shape myocytes was far under 50%. Even using a special high K^+, low Cl^- solution was not effective in our hands. The tissue pieces were gently shaken for 5 min, the cells were then separated from the chunks by filtering through a nylon mesh. Sedimentation was used for harvesting the cells; most myocytes reached the bottom of the vessel in 10 minutes after which the supernatant was removed and replaced by Tyrode solution containing 1.8 mM $CaCl_2$. This procedure was repeated 2 times. The cells were stored in HEPES buffered Tyrode solution at 10-15 °C.

2.4. Experimental techniques

2.4.1. Single channel recording

The ATP sensitive potassium current was recorded in the inside-out configuration of the patch-clamp technique[32]. One drop of cell suspension was placed in a transparent recording chamber mounted on the stage of an inverted microscope (NIKON DIAPHOT TMD) and single myocytes were allowed to settle to the bottom of the recording chamber for at least 5 min before starting superfusion with HEPES buffered Tyrode solution. Superfusion was maintained by gravity flow. Micropipettes were fabricated from borosilicate glass capillaries (Clark). In order to reduce the background noise of the recording and to decrease the pipette-bath capacitance, pipette shanks were coated with Sylgard 184 (Dow Corning, USA). After fire polishing of these pipettes, they had a resistance of 5 - 10 MOhm when filled with a pipette (external) solution containing (mM) KCl 140, $CaCl_2$ 1.8, $MgCl_2$ 0.53, HEPES 5, glucose 5.5,

pH 7.3. After establishing high (10 - 20 GOhm) resistance seal, the superfusate was switched to high K^+, low Ca^{2+} solution (internal - bath - solution) with the following composition (mM): KCl 140, HEPES 5, EGTA 2, glucose 5.5, pH 7.3. The concentration of free Ca^{2+} and Mg^{2+} in the bath solution was estimated as 0.2 nM and 5 μM, respectively, using a computer program for calculated metal ion/ligand binding. Establishing the inside-out configuration - excising of the membrane patch - was done by quickly withdrawing the pipette tip from the cell attached to the glass bottom of the bath. The single channel current was recorded using a patch-clamp amplifier (Dagan 8900, USA) at a fixed membrane potential of -60 mV. The current signals were stored on a video cassette recorder via a pulse code modulation converter (RP-880, NF Instruments, Japan). The recorded signals were filtered off line by an 8-pole Bessel low-pass filter (FV-665, NF Instruments) at 500 Hz and digitized at 2500 Hz using an analog to digital converter (TL-1, Axon Instruments, USA) to store on a disc of a computer (IBM PC/AT) for analysis. The experiments were performed at room temperature (23 - 25 °C).

A "50 % threshold criterion" was used to detect events with the help of manual confirmation. The open probability (P_o) was calculated using the equation:

$$P_0 = (\sum_{j=1}^{N} t_j j) / (T_d N)$$

where t_j is the time spent at current levels corresponding to j=0,1,2,.....N channels in the open state. T_d is the duration of the recording which was usually 30 sec and N is the number of channels active in the patch. The number of available channels was estimated as the maximum open channels in the bath solution containing no ATP. Spontaneous run-down of the ATP sensitive potassium channels is common in cell-free configuration. In order to minimize the influence of run-down we monitored the activity of the K_{ATP} channels in ATP free solution for 1 - 2 minutes after establishment of inside-out patches. If P_o did not decrease more than 10 % of the initial value we then used these patches for further experiments. Furthermore, P_o of the channels at the end of each experiment was measured after washing out of ATP, ADP, and/or glibenclamide. If the fraction of the P_o at the end of the experiment was less than 0.8 of the control value the experiment was discarded from the analysis.

2.4.2. Experiments in whole-cell configuration

The transient outward, inward rectifier and delayed rectifier potassium currents were recorded in whole-cell configuration of the patch-clamp technique[32]. Micropipettes were fabricated from glass capillaries (Clark, UK) using a computer controlled horizontal puller (Mecanex, Switzeland). These pipettes had a tip resistance of 1.5 - 2.5 MOhm and were used without fire polishing or Sylgard coating. A typical pipette solution was the following (in mM): KCl 140, $MgCl_2$ 4, K_2ATP 5, HEPES 10, EGTA 1. The pH of the solution was adjusted to 7.2 by KOH. HEPES buffered Tyrode solution (PH 7.4) was used as normal superfusate which contained 0.25 mM $CdCl_2$ in order to block the slow calcium current (I_{Ca}) completely. After establishing a high resistance seal (1 - 10 GOhm) by gentle suction, the cell membrane beneath the tip of the electrode was ruptured by further suction or by applying 1.5 V electrical pulses for 1 - 5 ms. Recordings were obtained by using an Axopatch 1D patch-clamp amplifier (Axon Instruments, USA) and a 333 KHz analogue-to-digital converter (Digidata 1200, Axon Instruments, USA) under software control (PClamp 6, Axon Instruments, USA). The experiments were carried out at 35 - 37 °C provided by a TC-1 type temperature controller (Cell Microcontrol, USA). The cell capacitance was measured by applying 10 mV hyperpolarizing voltage step from the holding potential of -10 mV. The capacity was calculated by integration of the capacitive transient divided by the amplitude of the voltage step (10 mV). The series resistance (R_s) was typically 4 - 8 MOhm. In those experiments in which large and/or rapidly activating - inactivating currents were measured (transient outward current, inward rectifying potassium current) the series resistance was carefully compensated (usually 50 - 80 % depending on the voltage protocols) and the experiments in which R_s was high or substantially increased during the measurement were discarded from the analysis.

2.5. Statistical analysis

Statistical analysis was performed using Student's t-test for paired or unpaired data. The results were considered to be significant at $P < 0.05$ level. Numerical data are expressed as means ± S.E.M.

3. Results

3.1. Effect of antiarrhythmic drugs on the transient outward potassium current

It is generally accepted that I_{to} consists of two components[33,34,35]. The first component is a potassium current which is sensitive to the K^+ channel blocker 4-aminopyridine. The second component may be a Ca^{2+} sensitive chloride current[36] which therefore depends on the Ca^{2+} release from the sarcoplasmic reticulum. In this study we focussed our attention on the 4-aminopyridine sensitive component of the transient outward current. In order to abolish the latter Ca^{2+} dependent component, the pipette solution contained 1mM EGTA, a Ca^{2+} chelator, which buffered the intracellular Ca^{2+} concentration.

It is impossible to separate I_{to} and I_K completely. However, the amplitude of I_K is much smaller than that of I_{to} and also, the inactivation kinetics of I_{to} are considerably faster than the activation of I_K, which suggests that I_K does not interfere significantly with the measurement of I_{to}. Nevertheless, it cannot be ruled out that I_K contaminated the measurement of I_{to} in some negligible degree.

It is also possible theoretically that the fast Na^+ current influences the measurements, though both activation and inactivation of I_{Na} are faster than those of I_{to}. Applying less negative holding potential (-40 mV) could have inactivated I_{Na}, but in this case the amplitude of I_{to} would have been largely reduced because of partial inactivation. Since application of TTX throughout the measurements to block I_{Na} would have greatly increased the costs of this study, we had performed preliminary studies testing the effect of 50 μM TTX on I_{to} in five separate experiments. Application of TTX did not affect significantly the amplitude of I_{to} in the voltage range of -10 - +50 mV, suggesting that influence of I_{Na} is negligible.

The effect of seven antiarrhythmic drugs; quinidine, disopyramide, flecainide, tedisamil, sotalol, GLG-V-13, a new class III agent, and amiodarone on the transient outward potassium current were compared. The current was activated by 400 ms long depolarizing voltage pulses from the holding potential of -90 mV to test potentials ranging from 0 to +60 mV with a pulse frequency of 0.33 Hz as Figure 1 shows in the case of disopyramide. The amplitude of I_{to} was measured as the difference between the peak and the sustained current at the end of the voltage

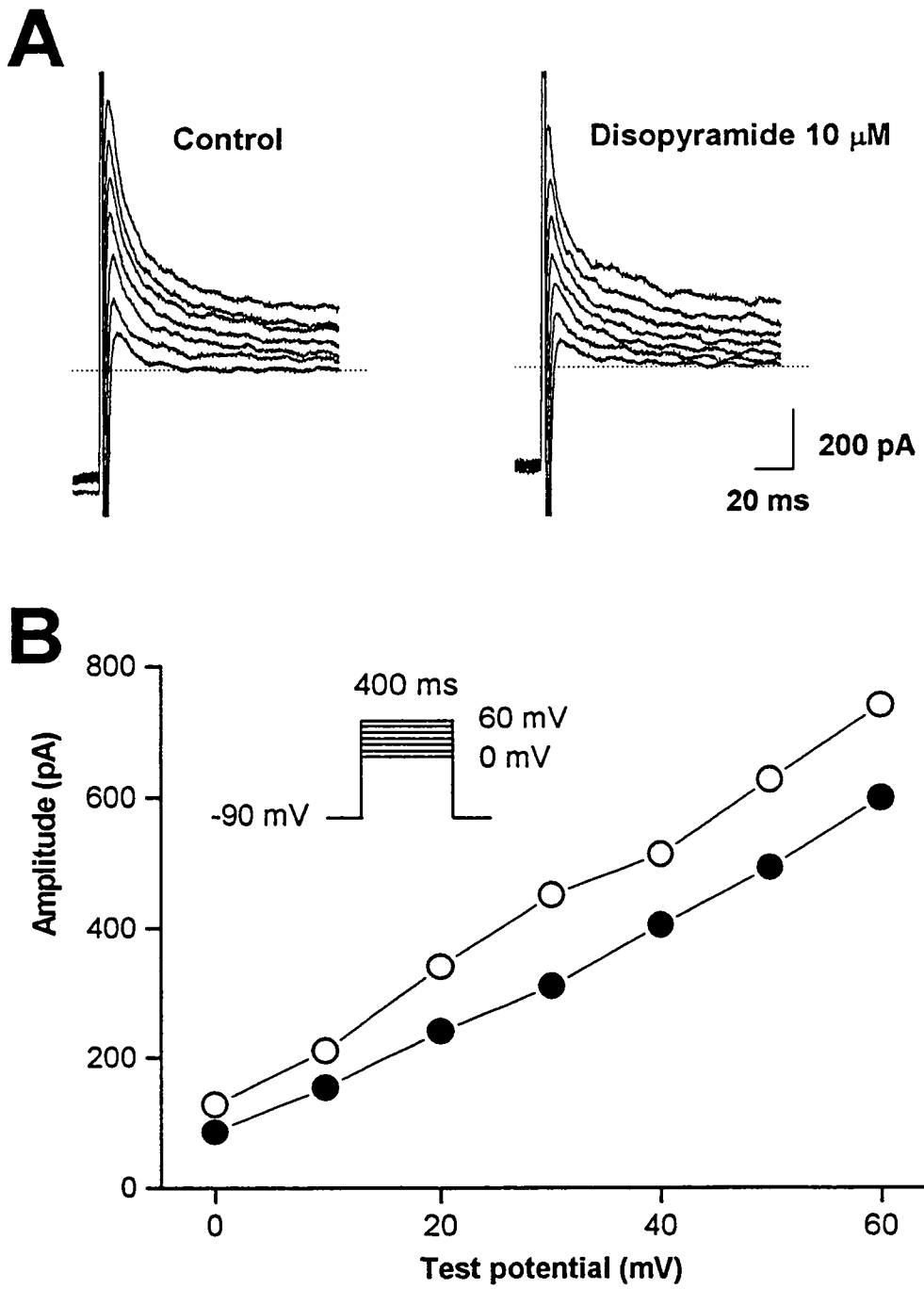

Figure 1. Effect of 10 μM disopyramide on I_{to} in a representative experiment. Panel **A**: original current records under control conditions (*left*) and after application of 10 μM disopyramide (*right*). Panel **B**: the current-voltage relationship in control conditions (*open circles*) and in the presence of 10 μM disopyramide (*closed circles*). The inset shows the voltage protocol applied during the measurement.

pulse. The amplitude of the current was decreased by quinidine, flecainide, disopyramide and tedisamil and not changed by sotalol, GLG-V-13 and acute administration of amiodarone (Fig. 2). The decay of the current was well fitted by a double exponential function. Among the effective drugs quinidine, disopyramide and tedisamil influenced not only the amplitude of the current but the fast component of the current decay was also accelerated (Table 1). None of the studied drugs altered the slow phase of the decay of I_{to}.

Table 1. The fast time constant of I_{to} decay (ms)

	control	drug
quinidine 10 μM	8.4±0.7	3.3±0.6
disopyramide 10 μM	6.3±0.2	3.9±0.3
tedisamil 1 μM	9.4±0.7	3.5±0.3

3.1.1 Detailed analysis of the effect of disopyramide on the transient outward potassium current

The effect of disopyramide on the decay of I_{to} was studied by applying 300 ms

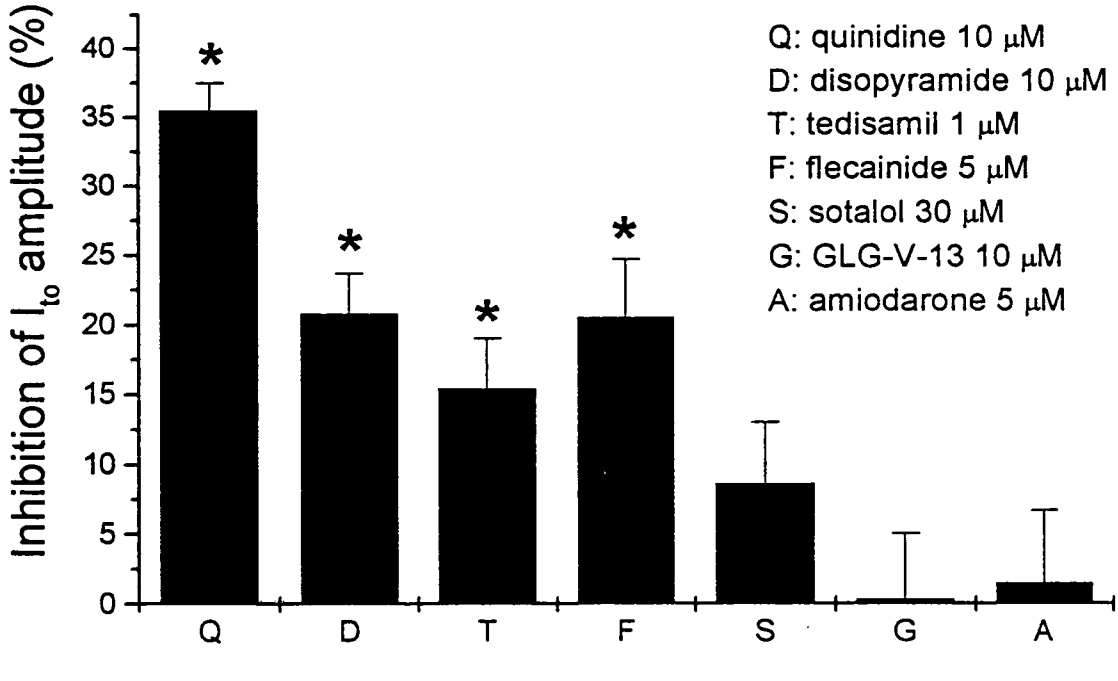

Figure 2. Effect of seven different antiarrhythmic drugs on the amplitude of I_{to} at 50 mV. Asterisks represent the P values of less than 0.05, n=4-8.

depolarizing voltage pulses from holding potential of -90 mV to +50 mV in every 3 seconds. The time constant of the fast component decreased as a function of increasing disopyramide concentration (Fig. 3A). The acceleration of the decay kinetics of I_{to} is an important drug effect as reflected in the dose-response curve in Figure 3B. This shows the effect of the drug on the total charge movement through the channels as a function of disopyramide concentration. The total charge movement was calculated by integrating the current traces from the peak to the end of the pulse taking the steady-state current as baseline. The estimated EC_{50} value for the effect of disopyramide on I_{to} was 14.1 μM.

The possible use-dependent effect of 10 μM disopyramide on I_{to} was tested with a series of 400 ms long depolarizing pulses to +50 mV applied at 1 Hz after at least 1 min resting at the holding potential of -90 mV. Figure 4A shows that the disopyramide (10 μM) evoked I_{to}

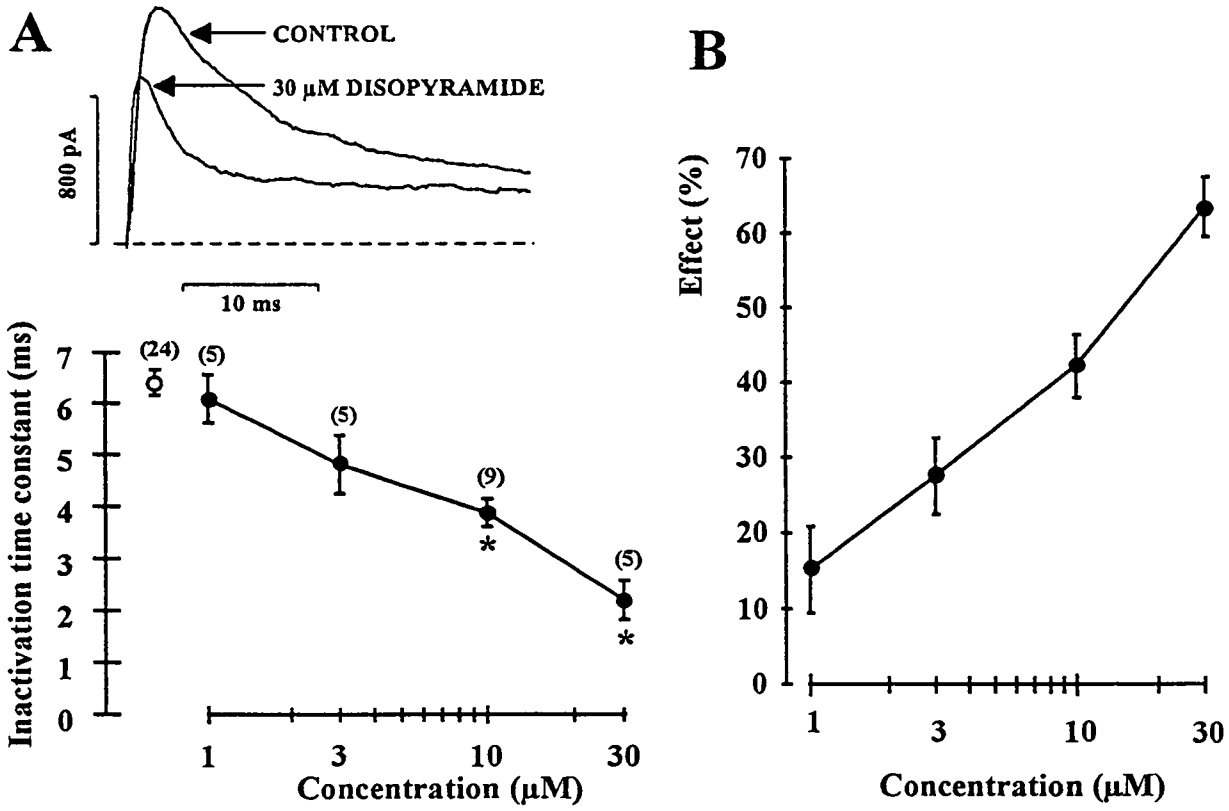

Figure 3. A Effect of disopyramide on the decay of I_{to}. *Upper panel*: original current traces under control conditions and in the presence of 30 μM disopyramide. *Lower panel*: dose-response curve for the effect of disopyramide on the fast component of the current decay (*open circle*: pooled control, n = 24; *closed circles*: drug, n = 5 - 9). Asterisks represent the P values of less than 0.05. **B** Dose-response curve for the inhibition of I_{to} by disopyramide, calculated from the charge movement through the channels (n = 4 - 8).

depression was not use-dependent. The effect of the drug on the recovery from inactivation was also measured with conventional double-pulse protocol. The recovery process was well fitted by a single exponential function. Disopyramide did not influence the reactivation of I_{to} (the time constant was 1080 ± 259 ms for control and 1112 ± 239 ms, n=5, in the presence of disopyramide, n.s.) suggesting that the offset kinetics of the drug are faster than the recovery

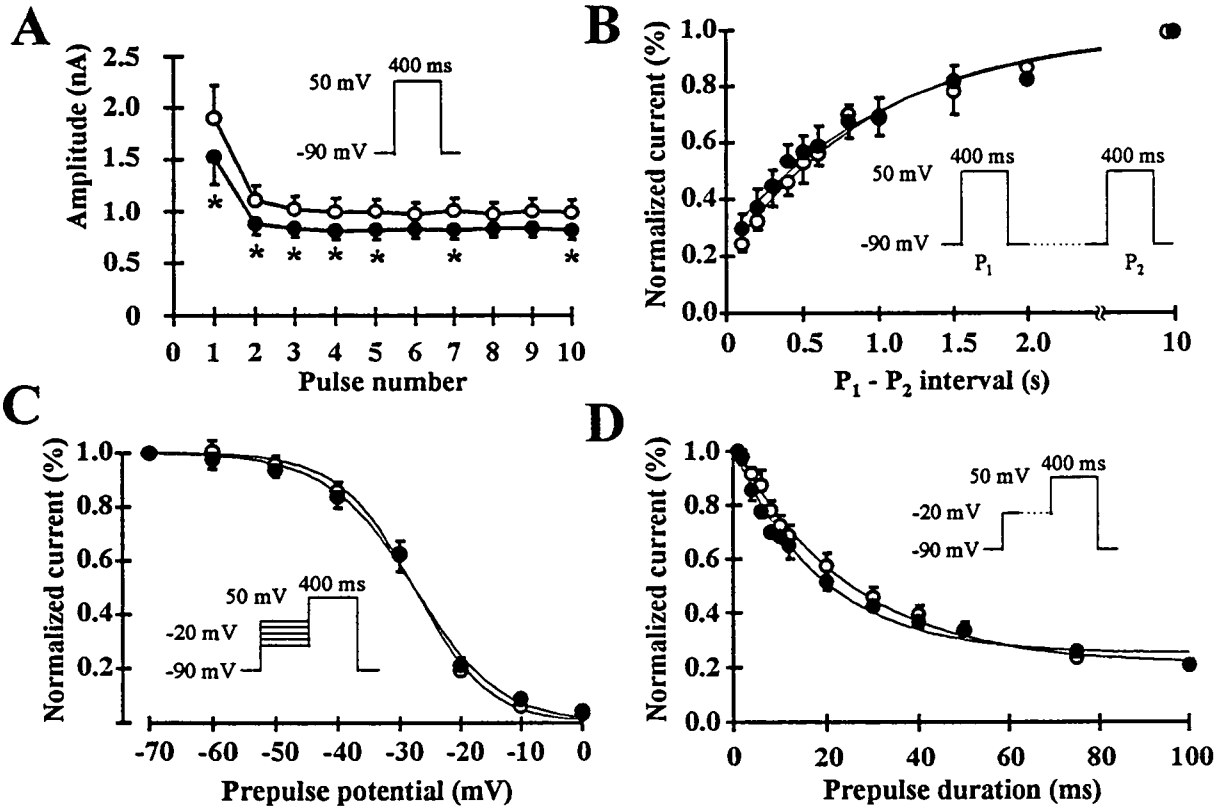

Figure 4. B Lack of use-dependent effect of 10 μM disopyramide on I_{to}. The illustration shows current amplitude as a function of pulse number (*open circles*: control; *closed circles*: 10 μM disopyramide n = 6). Asterisks represent the P values of less than 0.05. **B** Recovery of I_{to} from inactivation under control condition (*open circles*) and after application of 10 μM disopyramide (*closed circles*, n = 5). The double-pulse protocol used (see *inset*) consisted of two identical 400 ms long depolarizing pulses (P_1, P_2) to +50 mV from the holding potential of -90 mV. The P_1 - P_2 interval was 0 - 10 s. The normalized current (P_2/P_1) was plotted as a function of P_1 - P_2 interval. C Voltage dependence of the steady-state inactivation of I_{to} under control conditions (*open circles*) and in the presence of 10 μM disopyramide (*closed circles*, n = 6). Prepulses (500 ms long) to potentials ranging from -70 to 0 mV were applied before 400 ms long depolarizing test pulses to +50 mV (see *inset*). The holding potential was -90 mV. **D** Onset of inactivation of I_{to} under control conditions (*open circles*) and after application of 30 μM disopyramide (*closed circles*) at -20 mV.

of I_{to} from inactivation (Fig. 4B). Figure 4C shows that 10 μM disopyramide did not shift the steady-state inactivation curve suggesting that the drug does not bind preferentially to the inactivated state of the channel, or at least does not influence the voltage dependence of the function of inactivation gate.

The increased rate of decay of the current in the presence of disopyramide may result either from the blockade of the channels in the open state or from the modification of inactivation gating i.e. increasing the rate of inactivation. To test the latter possibility, the onset of inactivation was studied under control conditions and in the presence of 30 μM disopyramide by a prepulse protocol (see inset in Figure 4D); the test pulse to +50 mV was

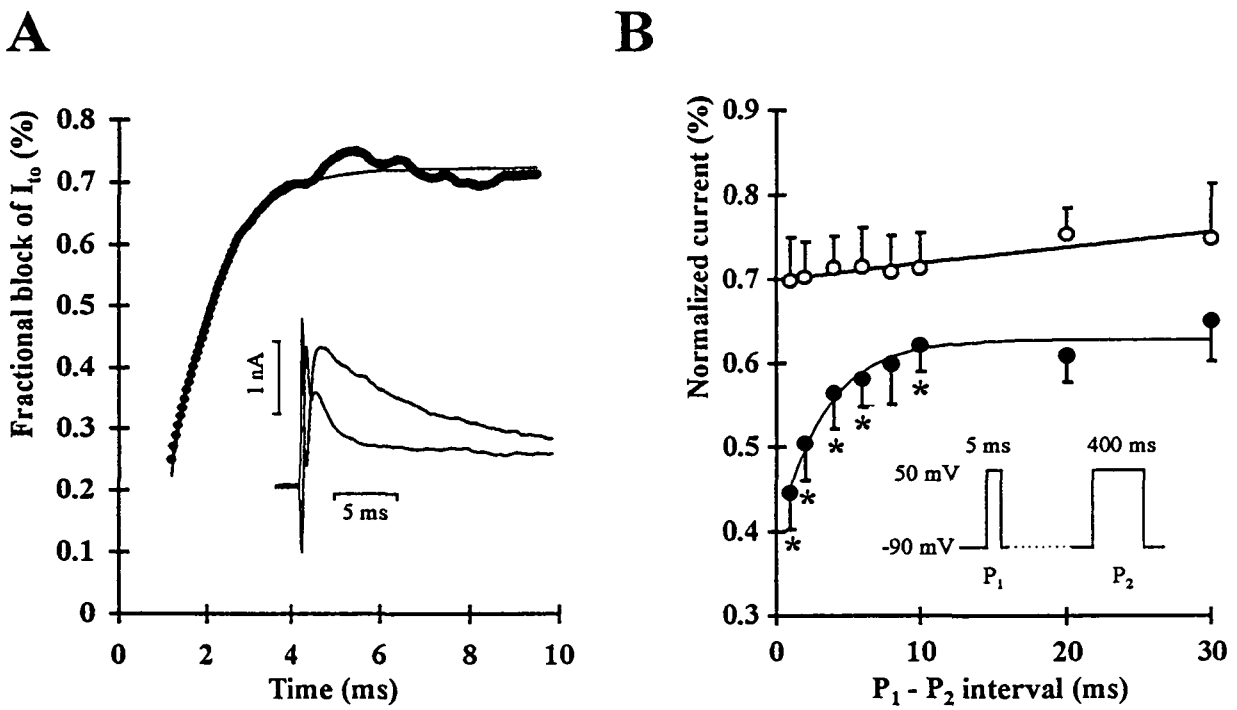

Figure 5. A Fractional block (drug-induced current reduction divided by control current) of I_{to} as a function of time after clamping the cell to the test potential of +50 mV in a representative experiment. The cell was clamped to +50 mV at time t = 0 ms. The *inset* shows the original current records from which the individual values of fractional block were calculated. This relationship was well fitted by a single exponential function. Data obtained during the 1st ms were omitted from the fitting process because of distortion by capacitive transients. **B** Double-pulse protocol was used for estimating the offset kinetics of disopyramide (see *inset*). The first pulse (P_1) was a 5 ms long depolarizing voltage pulse from the holding potential of -90 mV to +50 mV. The duration of the test pulse (P_2) was 400 ms. The current amplitude, normalized to the current amplitude activated by P_1, is shown as a function of the P_1 - P_2 interval under control conditions (*open circles*) and after application of 30 μM disopyramide (*closed circles*). Asterisks represent the P values of less than 0.05 (n = 3).

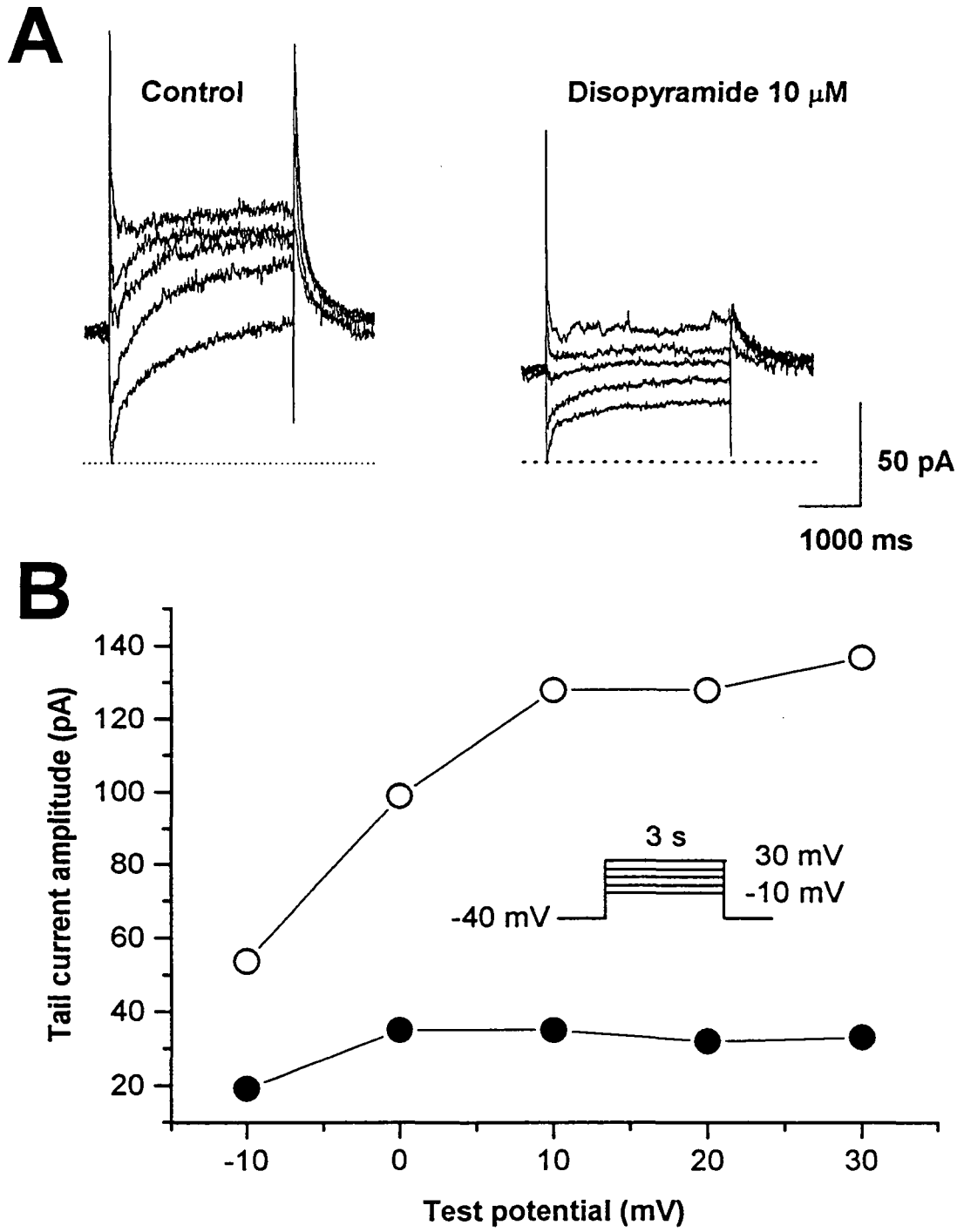

Figure 6. Effect of 10 μM disopyramide on I_{Kr} in a representative experiment. Panel **A**: original current records under control conditions (*left*) and after application of 10 μM disopyramide (*right*). Panel **B**: the current-voltage relationship in control conditions (*open circles*) and in the presence of 10 μM disopyramide (*closed circles*). The inset shows the voltage protocol applied during the measurement.

preceded by a conditioning pulse to -20 mV lasting for 0 - 100 ms. The holding potential was -90 mV. The decline of the current amplitude with increasing duration of the prepulse was well fitted by a single exponential function with a time constant of 25.5 ± 3.6 ms for control and 18.7 ± 2.0 ms in the presence of the drug (n.s., n=5) suggesting that the inactivation gating at -20 mV is not influenced by disopyramide (Fig. 4D).

The onset kinetics of the drug was estimated by calculating the fractional block of 30 μM disopyramide as a function of time after clamping the cell to +50 mV from the holding potential of -90 mV. The time constant of this relationship was 1.48 ± 0.18 ms (n=5) which reflects the binding kinetics of disopyramide to the open state of the channel (Fig. 5A). The offset kinetics of the drug were measured by using a double-pulse protocol (see inset in Figure 5B). In control conditions the amplitude of the current was just slightly changed when the interpulse interval increased representing the recovery of I_{to} from inactivation. This is too slow to be resolved in the present time scale. However, in the presence of 30 μM disopyramide the normalized current amplitude - interpulse interval relationship was well fitted by a single

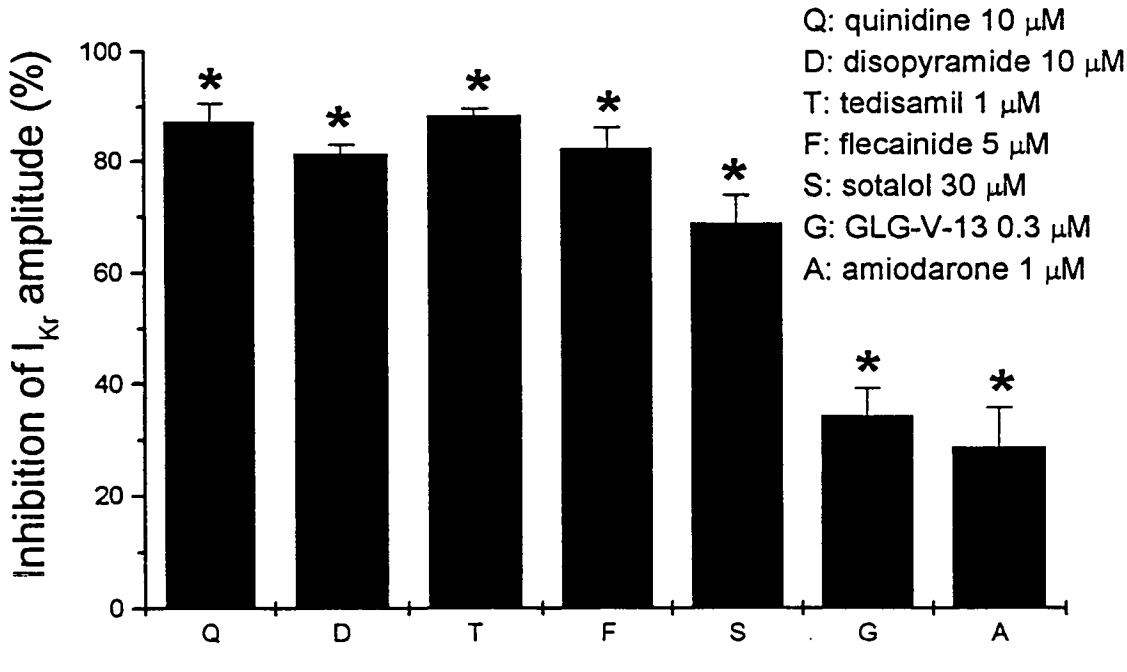

Figure 7. Effect of seven different antiarrhythmic drugs on the amplitude of I_{Kr} tail current at 30 mV. Asterisks represent the P values of less than 0.05, n=4-10.

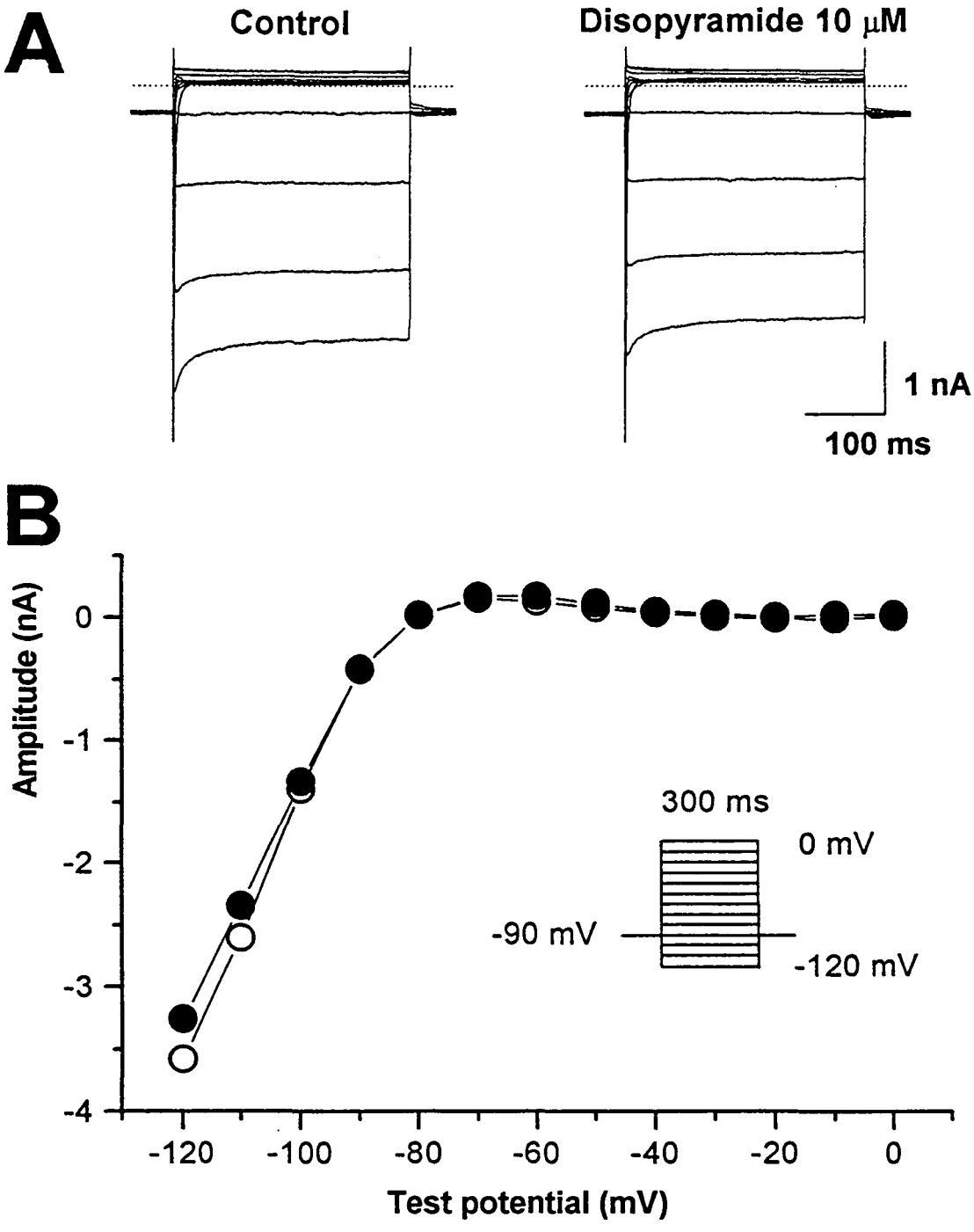

Figure 8. Lack of effect of 10 μM disopyramide on I_{k1} in a representative experiment. Panel **A**: original current records under control conditions (*left*) and after application of 10 μM disopyramide (*right*). Panel **B**: the current-voltage relationship in control conditions (*open circles*) and in the presence of 10 μM disopyramide (*closed circles*). The inset shows the voltage protocol applied during the measurement.

exponential function with time constant of 3.9 ± 0.5 ms (n=3) which reflects the rate of unbinding of the drug from the channel (Fig. 5B).

3.2. Effect of antiarrhythmic drugs on the delayed rectifier potassium current

The delayed rectifier potassium current (I_K) in guinea-pig ventricular myocytes can be separated into two components; a rapid (I_{Kr}) and a slow component (I_{Ks}) as reported by Sanguinetti and Jurkiewicz[37] while I_K appears to have only one component in rabbit ventricular myocytes which is similar to I_{Kr}[38,39]. The effects of seven antiarrhythmic drugs; quinidine, disopyramide, flecainide, tedisamil, sotalol, GLG-V-13 and amiodarone on this current (I_{Kr}) were therefore measured in rabbit ventricular myocytes by applying 3 s long depolarizing voltage pulses to various test potentials ranging from -10 to +30 mV in 10 mV increments at a pulse frequency of 0.2 Hz as Figure 6 shows in the case of disopyramide. On clamping the cells back to the holding potential of -40 mV, an outward tail current was observed which was attributed to I_{Kr}. All of these drugs decreased the amplitude of the tail current (see Figure 7 for

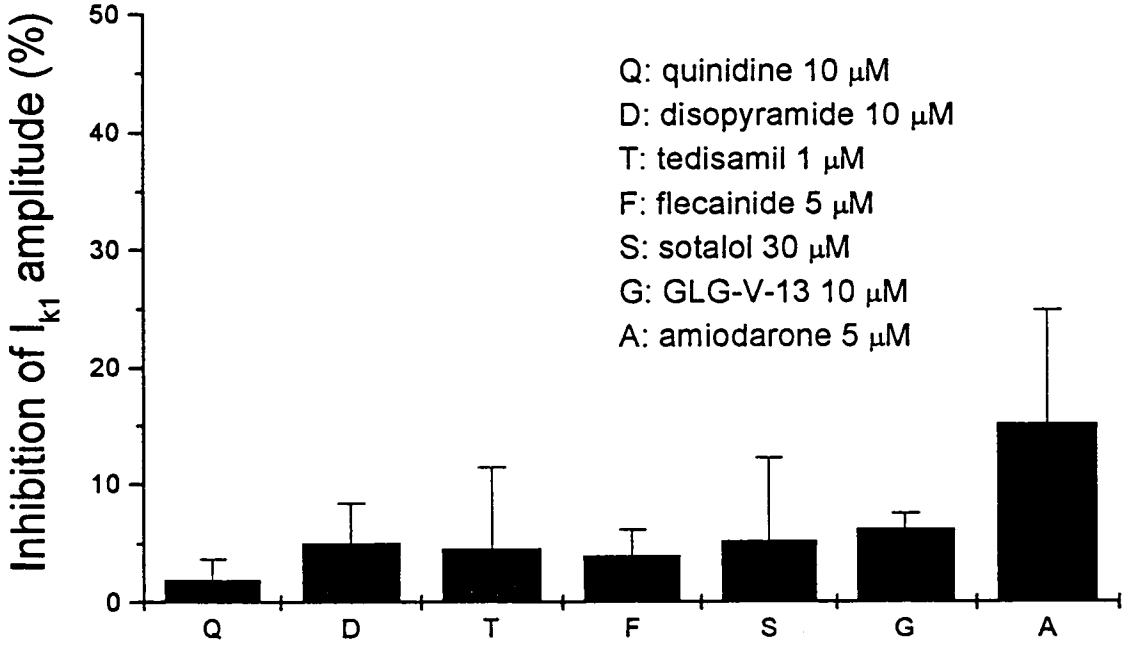

Figure 9. Lack of effect of seven different antiarrhythmic drugs on the amplitude of I_{k1} at -100 mV (n=4-8, N.S.).

the effects of the drugs at a test potential of +30 mV) which suggested that the studied drugs inhibited I_{Kr}. The estimated EC_{50} value for the effect of disopyramide on I_{Kr} was 1.8 μM (data not shown).

3.3. Effect of antiarrhythmic drugs on the inward rectifier potassium current

The possible effect of seven antiarrhythmic drugs; quinidine, disopyramide, flecainide, tedisamil, sotalol, GLG-V-13 and amiodarone on the inward rectifier potassium current was

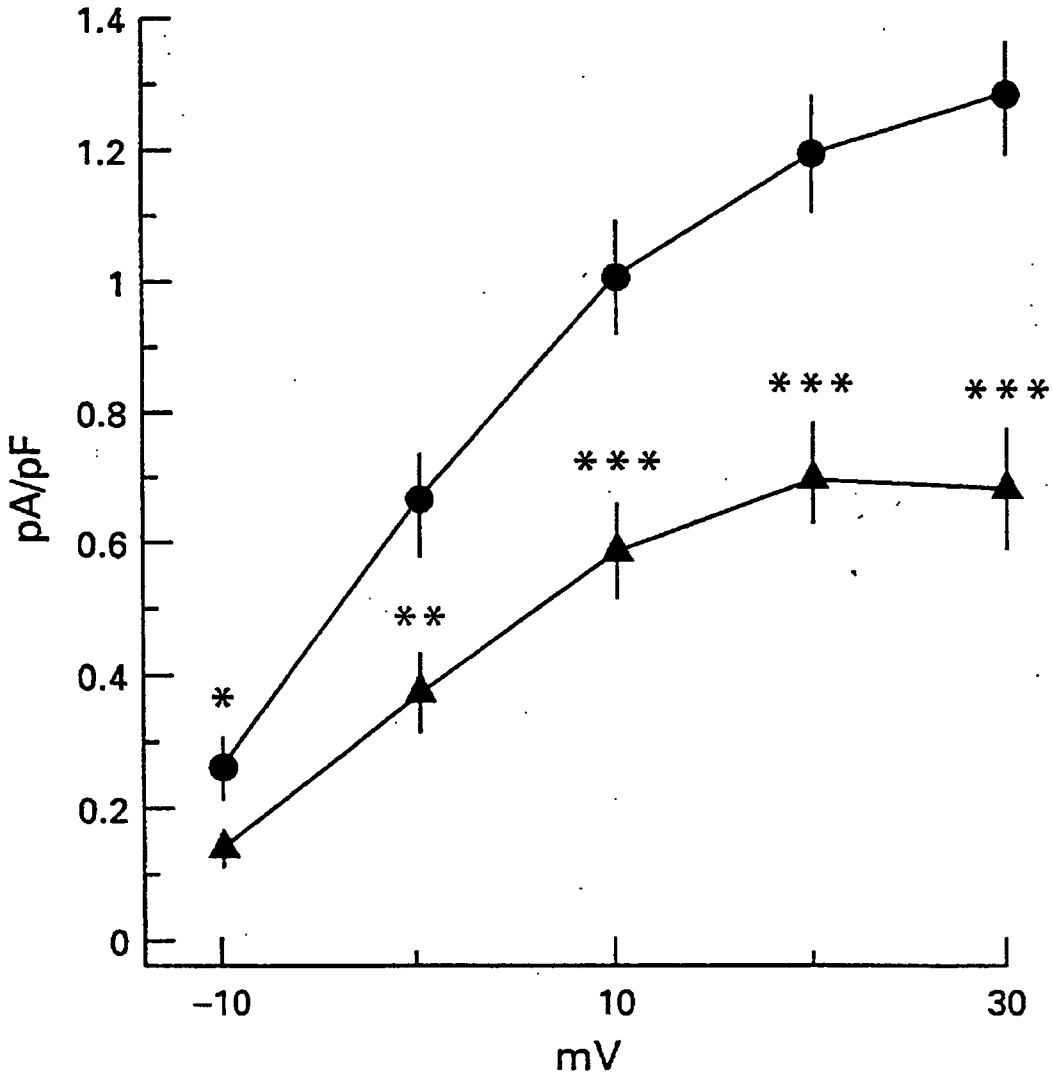

Figure 10. Effect of chronic amiodarone treatment (50 mg kg^{-1} day^{-1} for 3 - 4 weeks) on the peak outward tail current (I_K) amplitude in rabbit ventricular myocytes. Tail current was measured at -40 mV holding potential after applying 3 s long depolarizing pulses to -10 - +30 mV (* **P < 0.05**; ** P < 0.01; *** P < 0.001).

studied by measuring the steady-state current level at the end of the 400 ms long voltage pulse in the voltage range of -120 to 0 mV with a pulse frequency of 0.33 Hz as Figure 8 shows in the case of 10 μM disopyramide. The holding potential was -90 mV. The studied drugs did not influence significantly the steady-state current voltage relation (see Figure 9 for the effect of these drugs at -100 mV), suggesting the lack of effect of the drugs on I_{k1}.

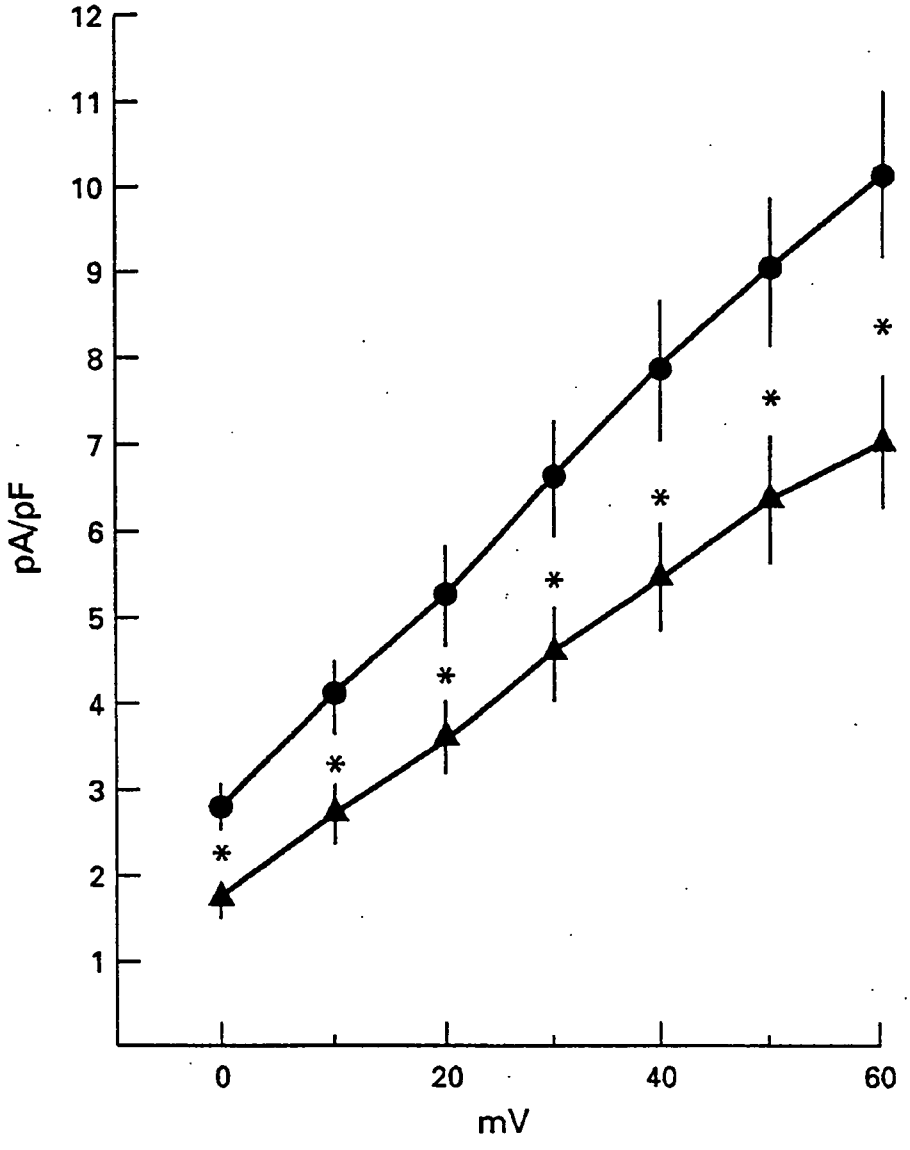

Figure 11. Effect of chronic amiodarone treatment (50 mg kg^{-1} day^{-1} for 3 - 4 weeks) on the peak transient outward current (I_{to}) in rabbit ventricular myocytes. Holding potential: -90 mV. Pulse frequency: 0.3 Hz (* $P < 0.05$). *Circles*: control, 5 animal, 15 cells; *triangles*: amiodarone, 5 animals, 15 cells.

3.4. Chronic effect of amiodarone on potassium currents

Rabbit ventricular myocytes were isolated from 5 amiodarone and from 5 solvent-treated animals. Successful current records were obtained in 17 cells (5 rabbits) in amiodarone and in 17 cells (5 rabbits) in solvent-treated groups.

The rapid component of the delayed rectifier potassium current was recorded as described previously. The time course of the outward tail current, which represents the deactivation process of I_{Kr}, was fitted by a single exponential function. Current records were

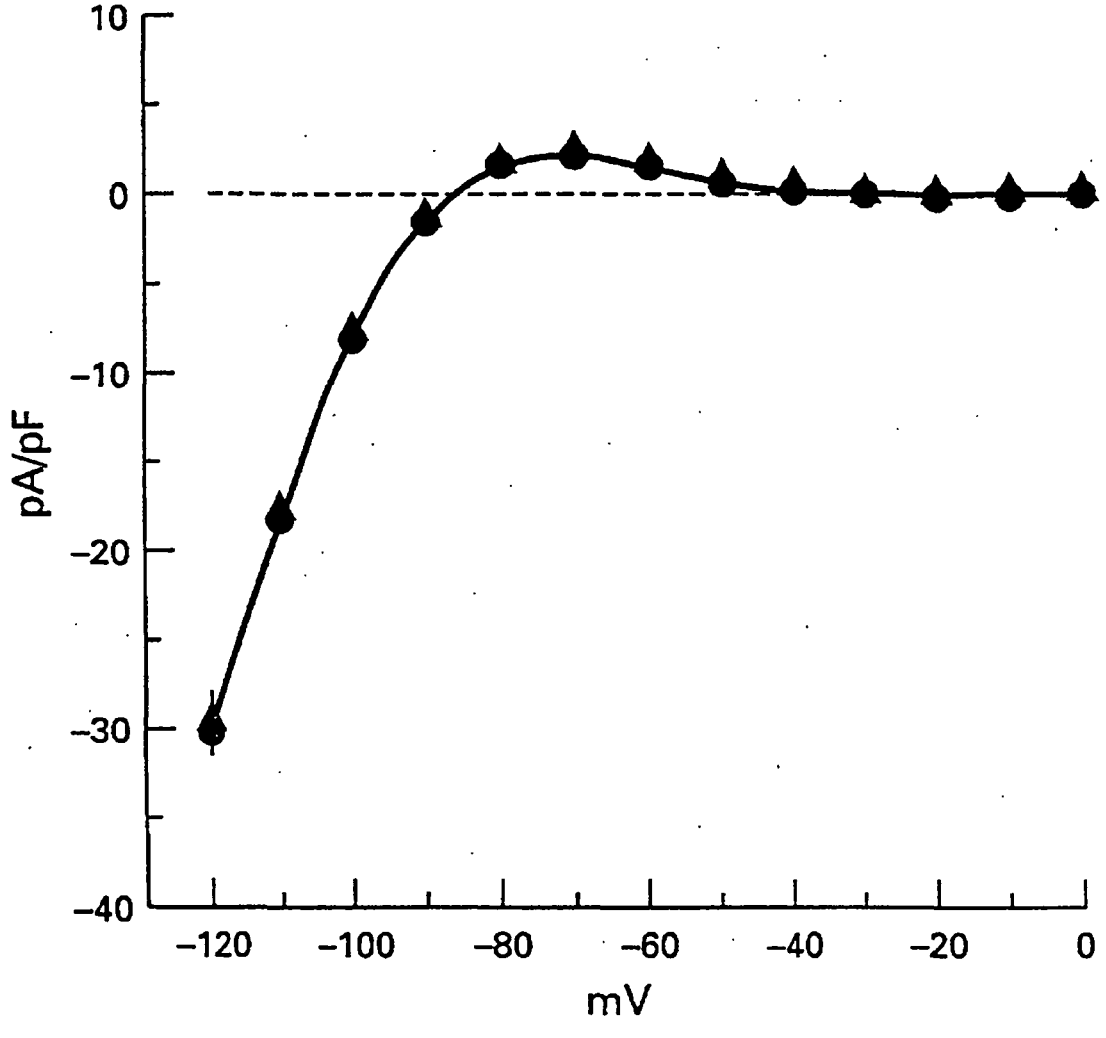

Figure 12. Lack of effect of chronic amiodarone treatment (50 mg kg^{-1} day^{-1} for 3 - 4 weeks) on the steady-state current-voltage relation (inward rectifier potassium current, I_{k1}) in the voltage range of -120 - 0 mV in rabbit ventricular myocytes. Holding potential: -90 mV. Pulse duration 400 ms. Pulse frequency: 0.3 Hz. *Circles*: control, 5 animal, 17 cells; *triangles*: amiodarone, 5 animals, 17 cells.

obtained in 16 cells isolated from 5 animals treated chronically with amiodarone and in 15 cells from the solvent-treated group (5 animals). The results are shown in Figure 10. There were significant differences between the amiodarone and the solvent-treated groups in the amplitude of the current. However, the deactivation of I_{Kr} was not influenced by amiodarone treatment (the deactivation time constant at +30 mV was 104.5 ± 7.7 ms in 15 cells from 5 control rabbits and 98.3 ± 12.0 ms in 15 cells from 5 amiodarone-treated animals).

The transient outward potassium current was measured as described previously. The amplitude of I_{to} recorded in the myocytes isolated from chronically amiodarone-treated animals (15 cells, 5 rabbits) showed moderate but significant depression compared to the solvent-treated group (15 cells, 5 rabbits). The results are shown in Figure 11. However, the inactivation process of I_{to} was not altered by the amiodarone treatment; the time constant for the fast component of I_{to} inactivation was 8.13 ± 0.31 ms in the control group and 8.23 ± 0.48 ms in the amiodarone-treated group, the slow time constant was 55.7 ± 5.45 ms for control (16 cells, 5 animals) and 51.6 ± 6.3 ms for the amiodarone-treated group (17 cells, 5 animals).

The effect of amiodarone treatment on the inward rectifier potassium current was also investigated. The current was measured as described previously. The results are shown in Figure 12; there were no significant differences between the solvent-treated and amiodarone-treated groups in the amplitude of I_{k1} (17 - 17 cells, 5 - 5 rabbits), suggesting lack of effect of amiodarone on the inward rectifier potassium current.

3.5. Effect of glibenclamide on the ATP sensitive potassium current

After establishing inside-out patches in ATP-free solution a population of K^+ channels with unitary current of 75 pS appeared. The activity of these channels was inhibited by application of ATP in dose-dependent manner indicating that these channels are K_{ATP} channels. When the intracellular side of the membrane was exposed to ATP-free internal solution the activity of the channels decreased spontaneously and gradually with time, called as "run-down" phenomenon. The activity of the channels could be reactivated if the intracellular side of the membrane was superfused with solution containing 2 mM ATP in the presence of Mg^{2+} for 3 min and thereafter MgATP was washed out (Fig. 13). Studying the nature of the run-down

process, our data suggested that the hydrolysis of the ATP was an important factor for reactivation of K_{ATP} channels. Since no products of ATP hydrolysis could reproduce MgATP induced channel reactivation and since the degree of the channel recovery was dependent upon the duration of MgATP application, the hydrolysis energy appears to be utilized for channel reactivation (for detailed data see *J Physiol* 479:95-107, 1994[40] in Annex).

Effects of stilbene disulfonates, widely used anion channel blockers, on the activity of K_{ATP} channels were studied in inside-out and outside-out membrane patches excised from guinea-pig ventricular myocytes. All the tested drugs, 4,4'-diisothiocyanatostilbene,2,2'-disulfonic acid (DIDS), 4-acetamido-4'- isothiocyanatostilbene,2,2'-disulfonic acid (SITS), 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS), and 4,4'-diaminostilbene-2,2'-disulfonic acid (DADS), blocked the K_{ATP} current when they were applied to the intracellular but not extracellular side of the membrane patch. The order of potency of the drugs was as follows: DIDS > SITS ≈ DNDS > DADS. The inhibitory actions of DNDS and DADS were reversible, whereas those induced by DIDS and SITS were irreversible (for detailed data see *J Membrane Biol* 136:289-302, 1993[41] in Annex).

The effect of glibenclamide on K_{ATP} channels was measured in the bath solution containing two different concentrations of ATP (100 μM and 200 μM). Panel *a* and *b* in Figure 14A shows original current records in the presence of 100 μM ATP and the tracings in panel *e* and *f* were recorded in bath solution containing 200 μM ATP. Figure 14B displays the

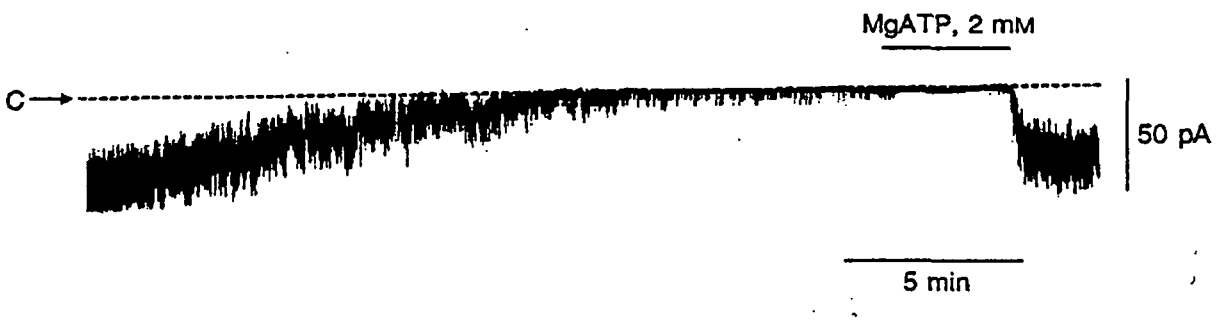

Figure 13. Spontaneous run-down and reactivation of K_{ATP} channels by transient (3 min) exposure to 2 mM MgATP. The record was made from an inside-out membrane patch at a fixed membrane potential of -50 mV. C and an arrow represent closed level of K_{ATP} channels, the current record was filtered at 1 kHz for display. Inward current is shown as downward deflection.

corresponding amplitude histograms. The figures clearly indicate that the inhibition by 10 nM glibenclamide (panel *b* and *f*) was stronger at a concentration of 200 μM ATP than at 100 μM ATP. In order to perform a more quantitative analysis, the effects of glibenclamide at three different concentrations (1 nM, 10 nM and 100 nM) on the activity of K_{ATP} channels were studied in the presence of both 100 μM and 200 μM ATP. Our results are summarized in Figure 15. The open probability in the presence of 10 nM glibenclamide relative to the control value was 0.674 ± 0.037 at 100 μM ATP and 0.335 ± 0.058 at 200 μM ATP ($p < 0.05$). One nM glibenclamide tended to inhibit the channel activity with a greater magnitude at 200 μM ATP than at 100 μM, the difference, however, did not reach a statistically significant level (0.944 ± 0.028 for 100 μM ATP versus 0.734 ± 0.115 for 200 μM ATP, n.s.). The block of

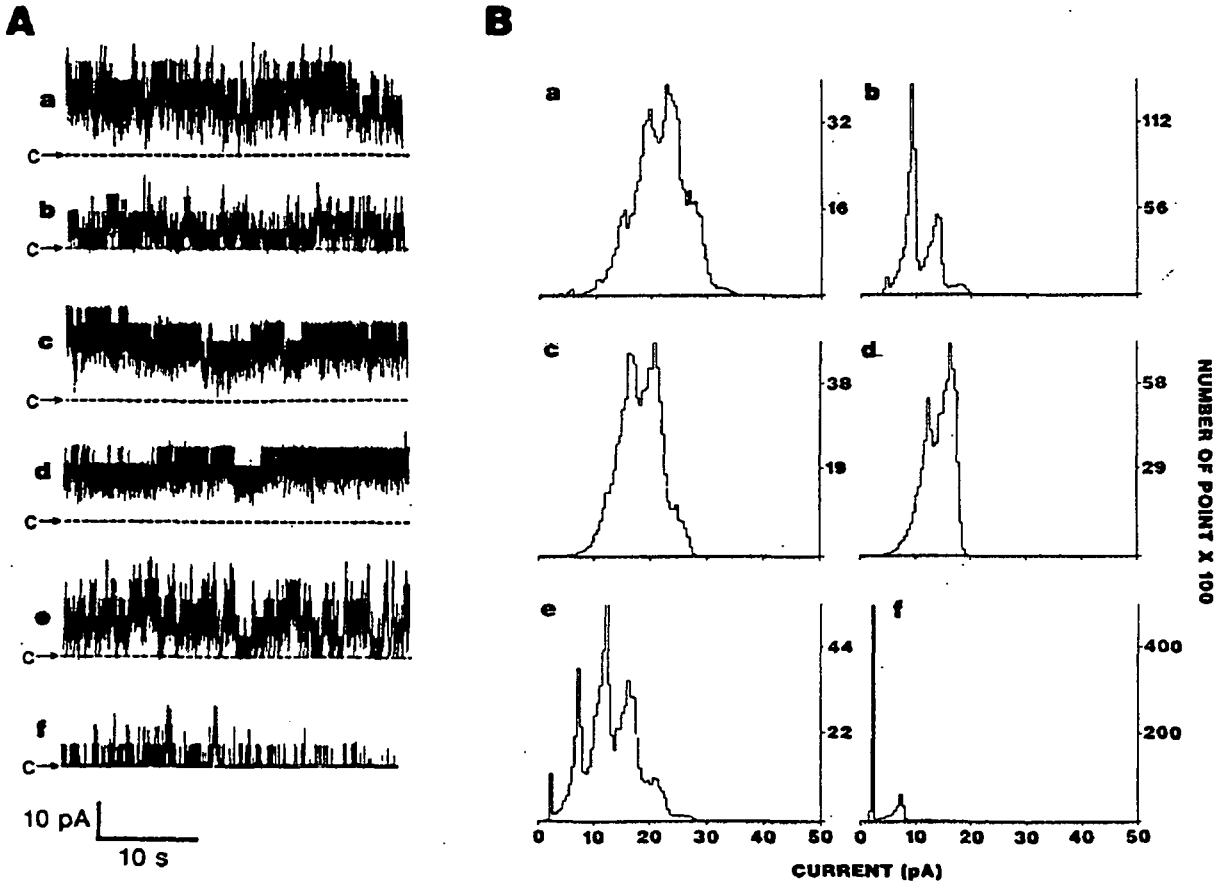

Figure 14. Effect of 10 nM glibenclamide on the activity of K_{ATP} channels in the presence of 100 μM ATP (*a* for control, *b* for test), 100 μM ATP and 100 μM ADP (*c* for control, *d* for test), and 200 μM ATP (*e* for control, *f* for test). **A** Original current traces, **B** amplitude histograms from the same patches as **A**. In each panel, *c* and an arrow represent the closed level. The holding potential of the patches was -60 mV.

K_{ATP} channel activity by 100 nM glibenclamide was very strong, therefore, we did not find any significant difference between at 100 μM and at 200 μM ATP in the effect of the drug.

In order to study how the presence of ADP at the intracellular side of the cell membrane affects the glibenclamide induced inhibition of the activity of K_{ATP} channels, the effect of glibenclamide on K_{ATP} currents was compared between in the presence and absence of 100 μM ADP. Panels *c* and *d* in Figure 14A display original current records indicating the effect of 10 nM glibenclamide in the presence of 100 μM ATP and 100 μM ADP. Figure 14B

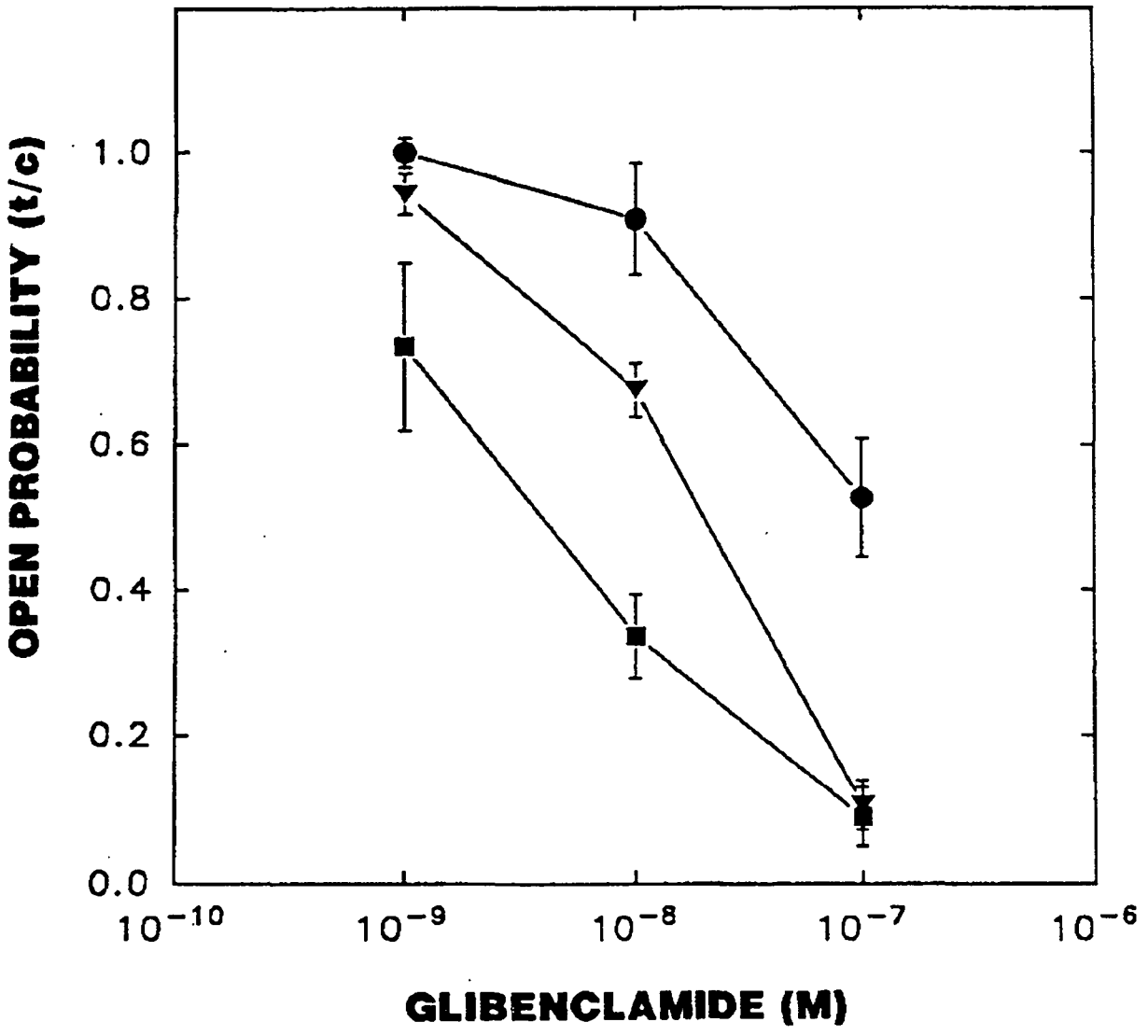

Figure 15. Effect of glibenclamide on the relative open probability of the K_{ATP} channels in the presence of 100 μM ATP (*reverse triangles*), 100 μM ATP and 100 μM ADP (*circles*), and 200 μM ATP (*squares*) as a function of glibenclamide concentration. The relative open probability was defined as the ratio of the channel activity with glibenclamide to that without the drug (n = 2 - 5).

indicates the corresponding amplitude histograms in panel *c* and *d*. It appeared that in the solution containing both 100 μM ATP and 100 μM ADP the inhibition of K_{ATP} channels by 10 nM glibenclamide was not strong. Comparing the blockade of K_{ATP} channels by glibenclamide in the presence of 100 μM ATP alone and in the presence of both 100 μM ATP and 100 μM ADP, 100 nM glibenclamide decreased the activity of the channels significantly stronger if the intracellular solution contained only ATP, compared to that containing both ATP and ADP (0.105 ± 0.033 versus 0.527 ± 0.081, $p < 0.05$). Although, at concentrations of 1 nM and 10 nM glibenclamide tended to inhibit K_{ATP} current to a lesser degree in the presence of ADP than in its absence, these differences were not statistically significant (Fig. 15).

4. Discussion

4.1. The transient outward potassium current

The presence and the magnitude of I_{to} in cardiac cells greatly varies between the different regions of the heart and shows considerable species dependency as well. The existence of I_{to} has been reported in ventricular muscle in various species; including rat[42], rabbit[35,38], dog[43], and man[44,45] but it was not found or its amplitude was very small in guinea-pig[42] and cat[46]. Differences in the distribution of the I_{to} channels between endocardium and epicardium have also been revealed[47]. In ventricle, I_{to} primarily affects the phase 1 repolarization, therefore, blocking of this current influences the early portion of the plateau phase potential which in turn may modulate other important transmembrane ion currents, such as I_{Ca} and I_K, influencing cardiac repolarization. Since I_{to} in atrial tissue, which is larger than I_{to} measured in ventricular myocardium, is a major current responsible for repolarization, inhibition of this current causes lengthening of repolarization and refractoriness. Therefore, blockade of I_{to} in ventricular muscle may not represent an important antiarrhythmic mechanism, however, it may have great significance in the treatment of atrial fibrillation.

It has been reported that I_{to} has two components which differ from each other in amplitude, voltage dependent kinetics, frequency dependence and drug sensitivity. The first component of I_{to} is a 4-aminopyridine sensitive K^+ current while the second (4-aminopyridine

insensitive) component is dependent on the intracellular Ca^{2+} concentration and the major ion carrier for this current is most likely Cl^{-}[33,34,35,36]. The present work has focussed on the 4-aminopyridine sensitive component of I_{to}.

The effect of disopyramide on I_{to} has not yet been extensively studied. Coraboeuf *et al.* was the only investigator who reported that disopyramide, at concentration as high as 60 μM, decreased the amplitude of I_{to} in sheep Purkinje fibres measured by the two microelectrode voltage clamp technique[48]. In the present study[49,50,51], in addition to confirming this previous finding in ventricular myocytes, we also showed that disopyramide, at therapeutically relevant concentrations, depressed the current. The block of disopyramide on I_{to} is not use-dependent without any apparent effect on the recovery from inactivation and the steady-state inactivation of the current. These facts imply that the occupation by disopyramide of its binding site does not influence the operation of the inactivation gate. We found in this study that the current decay increased with increasing disopyramide concentration. One possibility that might explain this phenomenon is the increased rate of inactivation, though this seems unlikely because disopyramide did not affect significantly the onset of inactivation at -20 mV, supposing that the inactivation process is weakly voltage dependent. The acceleration of the onset of inactivation in the presence of disopyramide proved to be not significant statistically, but a weak drug induced increase in the rate of inactivation, at least at higher drug concentration, cannot be ruled out. This assumption seems to be supported by the findings presented in Figure 5B. The figure shows that in control conditions about 30% of the channels become inactivated during the first 5 ms long depolarizing pulse (see first open circle in Figure 5B). After application of the drug this value is higher (see the last closed circle), though it is not significantly different from the control value, indicating that the inactivation process might be faster in the presence of the drug. Therefore, the observed increase in the rate of decay of the current in the presence of disopyramide can be due to drug binding to the open state of the channel, thus causing open channel block, however, the peak amplitude of the current is also decreased by the drug. Although decline of the current amplitude might also be due to the increased rate of current decay, minor binding of disopyramide to the closed state of the channel, i.e. weak tonic block in the presence of the drug, cannot be ruled out.

Comparing the action of disopyramide to the effects of other studied antiarrhythmic drugs on I_{to}, it was found that quinidine, tedisamil and flecainide inhibited the current while sotalol, GLG-V-13 and acute administration of amiodarone did not affect I_{to}[52,53,54,55]. In case of quinidine and tedisamil, similarly to disopyramide, the decay of the current was also accelerated by these drugs, which may be due to open channel block of I_{to}. There is no previous information about the action of GLG-V-13 on I_{to}, and the present work revealed first the ineffectiveness of the drug on this current[53,54]. Acceleration in the current decay in the presence of quinidine was observed previously which was interpreted as consistent with open channel block. However, quinidine slowed the recovery of I_{to} from inactivation and caused use-dependent inhibition of the current[56,57]. The lack of change in I_{to} reactivation kinetics in the case of disopyramide suggests that the unblocking kinetics of quinidine is slower than that of disopyramide. Similar properties have been noted for I_{to} block with tedisamil in rat ventricular cells[58]. In contrast with our study an increased rate of inactivation was observed in the presence of flecainide in atrial myocytes and in rat ventricular cells[59,60]. This discrepancy may be due to the species and tissue dependency of I_{to} channels. The lack of effect of sotalol found in our study is in agreement with the results reported by others in atrial and ventricular myocytes, however block of I_{to} by sotalol was observed in Purkinje fibres[61,62].

In conclusion, some class I antiarrhythmic drugs block I_{to} which may influence the repolarization phase of the action potential in the ventricle. However, they cause substantial changes in repolarization in atrial tissues, explaining the effectiveness of the examined class I antiarrhythmic agents in treatment of atrial fibrillation.

4.2. The delayed rectifier potassium current

There is a general agreement that I_K has at least two current components[37]. One test for this was performed by measuring the time-dependent and the tail current amplitudes at various pulse durations. This envelop of tail test revealed that the ratio of the time-dependent and the tail currents changed with pulse duration, indicating the presence of more than one channel type. Pharmacological studies using the compound E-4031 further showed that the I_K in guinea-pig ventricular myocytes consists of at least two channel types. The so called rapid

component (I_{Kr}), which is sensitive to E-4031, exhibits prominent rectification and activates rapidly (τ_{act}=15 ms at +20 mV) compared to the slowly activating, E-4031 insensitive component (I_{Ks}). The current is not inactivating. The deactivation kinetics of I_{Kr} are also relatively rapid (τ_{deact}=140 ms at -40 mV). However, the amplitude of I_{Ks} is about 10 times larger than that of I_{Kr}. It was suggested that the two currents due to the relatively fast activation of I_{Kr} play equally important role in initiating repolarization in guinea pig ventricular myocytes[37].

It has been reported that the characteristics of I_K show considerable species differences. Measurements of the two I_K components in dog ventricular myocytes revealed that although the activation and rectification properties of I_{Kr} and I_{Ks} are very similar to those found in guinea pig, the deactivation kinetics were considerably different - in the dog the E-4031 sensitive component (I_{Kr}) deactivated slowly whereas the decay kinetics of the slow (E-4031 insensitive) component was relatively rapid (τ_{deact}=2 to 3 sec for I_{Kr}, and 150 ms for I_{Ks} at -35 mV)[63]. In contrast to guinea-pig and dog, the delayed rectifier in rabbit ventricular myocytes has been reported to consist of only one rapidly activating component which is similar to that characterised in guinea-pig and dog as I_{Kr}[38,39]. Quite recently Salata *et al.* have demonstrated two kinetically distinct components of I_K in rabbit, describing I_{Ks} during elevated sympathetic tone[64]. In human ventricle there have only been few data available about I_K. There is no information on this current, especially in undiseased myocardium. In human ventricular myocytes only a small and rapidly activating component of I_K has been found which was attributed to I_{Kr}[65]. However, Li *et al.* recently reported the presence of I_{Ks} in human ventricular cells[66]. Thus, further studies are necessary to characterise the delayed rectifier potassium current in human heart.

Numerous antiarrhythmic drugs produce more lengthening in the duration of the action potential at slower stimulation frequency than at fast heart rate. This phenomenon, termed as reverse use-dependent effect, is disadvantageous since at fast heart rate APD can be less affected by these drugs. At normal heart rate, however, or during bradycardia the repolarization can be excessively lengthened causing early afterdepolarizations which is most likely associated with torsade-de pointes arrhythmias. Sanguinetti and Jurkiewicz[37,67] assumed that this reverse use-dependent effect might be due to the fact that these antiarrhythmic agents

inhibit predominantly I_{Kr} but do not influence I_{Ks} because accumulation of I_{Ks} during fast heart rate, due to its slow deactivation rate, would attenuate the APD lengthening effect of I_{Kr} blockade. This speculation may be valid in guinea pig but in other species, including man, it does not seem likely, because I_{Ks} is relatively small and the deactivation process of I_{Kr} and I_{Ks} is different from that found in guinea pig (see above). Therefore, the role of I_{Ks} in formation of torsade de pointes arrhythmias is questionable.

In our study, therefore, the effects of disopyramide on I_{Kr} was measured and compared to other antiarrhythmic drugs belonging to different groups of the Vaughan-Williams classification scheme[50,51,52,53,54,55]. Kotake *et al.* reported first that disopyramide blocked I_K in sinus node preparation using the two microelectrode voltage-clamp technique[68]. Later Hiraoka *et al.* reported that 11 μM disopyramide decreased I_K in guinea-pig ventricular myocytes[69]. Carmeliet found that disopyramide use-dependently depressed the rapid component of I_K (I_{Kr}) in rabbit ventricular myocytes[16]. Our results, in addition to confirming these previous findings in rabbit ventricular cells, provide evidence that this effect is present at relatively low concentrations; the estimated EC_{50} value of 1.8 μM calculated from the dose-response relationship is considerably lower than the corresponding value for I_{to} block.

GLG-V-13 is a new bispidine-analogue (3,7-diheterobicyclo[3.3.1]nonane) with heart rate lowering, ERP prolonging and antiarrhythmic properties. In terms of prolonging the monophasic action potential duration in anaesthetized guinea pigs, GLG-V-13 is approximately 10 times more potent than d-sotalol as reported earlier[70]. In the present study, in addition to confirming the previous finding that this drug strongly inhibits I_{Kr} in rabbit myocytes, we also showed that GLG-V-13 is a "pure" class III antiarrhythmic agent as it influences neither I_{to} nor I_{k1} (discussed later)[53,54].

Comparing the action of disopyramide to the effects of other antiarrhythmic drugs on I_K two interesting observations have been emerged. Firstly, flecainide, despite the fact that this drug can produce only small and variable effect on repolarization and refractoriness, blocked I_{Kr} in the rabbit. A similar effect of flecainide has also been reported in cat ventricular myocytes by Follmer *et al*[71]. Secondly, d-sotalol, a well known class III antiarrhythmic agent with repolarization and refractoriness prolonging properties, exerted relatively moderate depressing effect, comparing it to the other studied drugs, on I_{Kr} tail current in rabbit ventricular cells.

Others reported similar findings or described the effect of sotalol at relatively high concentrations[72,61]. The impact of flecainide and d-sotalol on the action potential is difficult to interpret. The well known effects of flecainide on the fast Na^+-current and the Ca^{2+}-current which tend to shorten APD may compensate the repolarization prolongation, that would have been caused by the blockade of I_K. In the case of d-sotalol, without altering the depolarizing inward currents, the repolarization of the action potential may be lengthened by some I_{Kr} blockade exerted by the drug.

In conclusion, the block of I_{Kr} may be a common feature of the antiarrhythmic drugs that increase APD (class IA and III drugs) or have little effect on APD (class IC drugs).

4.3. The inward rectifier potassium current

The primary role of the inward rectifier potassium current is to maintain the resting or diastolic membrane potential in cardiac ventricular muscle and Purkinje fibres. At potentials more negative than the reversal potential I_{k1} shows linear current-voltage relation with high conductivity which provides larger permeability for K^+ at rest than for other ions, thereby setting the resting membrane potential of the cell close to the K^+ equilibrium potential according to the Goldman-Hodgkin-Katz model. The current shows strong inward rectification due to the voltage-dependent inhibition of the I_{k1} channels by intracellular Mg^{2+}. In consequence of the n-shape current-voltage relation at voltage range more positive than the K^+ equilibrium potential but more negative than -40 mV, I_{k1} carries a repolarizing current contributing to the final repolarization[73,74,75]. Therefore, blockade of I_{k1} decreases the resting potential and would lengthen not the plateau phase but mainly the final repolarization.

In numerous earlier reports it was presented and therefore it is generally accepted that several antiarrhythmic drugs block I_{k1}[76,48,77]. The effect of disopyramide on I_{k1} is rather controversial. Coraboeuf *et al.*[48] were the first who studied the effect of disopyramide on the current in sheep Purkinje fibres using the two microelectrode voltage-clamp technique, however, the concentration of the drug (60 μM) used in their study was several times higher than the upper limit of the therapeutic concentration. In these circumstances they reported reduction of the instantaneous background current in the presence of disopyramide. Martin *et*

al.[78] studied the influence of disopyramide on I_{k1} at single channel level and found no significant effect of the drug on the open probability of inward rectifier potassium channels in cell-attached patches of rabbit ventricular cells at room temperature. The blockade of I_{k1} by quinidine and propafenone has also been reported[76,77], however, propafenone inhibited the current in supratherapeutic concentration.

Our experiments with disopyramide support the results of Martin *et al.*[78], since the drug did not influence I_{k1} even in relatively high concentration (30 μM, data not shown). Similarly to disopyramide neither quinidine, in contrast to the results of Salata and Wasserstrom[76], nor the other studied drugs - flecainide, tedisamil, sotalol, GLG-V-13 and amiodarone - were found to block I_{k1}, thereby suggesting the lack of effect of these drugs on the inward rectifier potassium current. The discrepancy between our experiments and the other studies with disopyramide and quinidine might be explained by the run-down of channels or change of passive electrical properties - increasing of the series resistance - of the cells during measurement of the current.

Recently, more or less specific I_{k1} inhibitors are under development, such as terikalant[79] and MS-551[80]. However, it is not clear whether I_{k1} blockade is a useful antiarrhythmic mechanism. One possible disadvantage is that diminution of I_{k1} leads not only to lengthening of the final repolarization but it may also cause depolarization, which is undesirable[81]. On the other hand, a possible advantage of an I_{k1} blocker would be that, since an elevated extracellular potassium concentration increases I_{k1}, the repolarization shortening effect of the enhanced K^+ concentration induced by ischaemia or high frequency might be more effectively attenuated by I_{k1} blockade than by inhibiting other outward repolarizing currents[79]. Therefore, well-founded discussion of the possible antiarrhythmic mechanism of I_{k1} blockade needs further more detailed studies, including in-depth analysis of the effect of the drug in the whole heart.

4.4. Comparison of the effects of chronic and acute administration of amiodarone

In this study[82,83] the effects of chronic administration and acute application of amiodarone on I_{Kr}, I_{to} and I_{k1} were compared. The findings were as follows: (1) both chronic administration and acute application of amiodarone depressed I_K, (2) chronic amiodarone

treatment slightly depressed I_{to} while it was not changed after acute administration of amiodarone, (3) neither chronic treatment nor acute amiodarone administration influenced I_{k1}.

In vivo investigation[12] and in vitro experiments[13,14] have also shown that the acute and chronic electrophysiological effects of amiodarone substantially differ from each other. After acute amiodarone administration an increase of PQ interval is the dominant ECG change[12], while QT_c lengthening was usually observed only after chronic amiodarone treatment[12,84,85]. Acute amiodarone exposure in the tissue bath either does not change[13,14,86] or slightly increases[87,7,88] or decreases[14] the duration of the action potential (APD) in various cardiac preparations while chronic amiodarone treatment always markedly increases APD in ventricular muscle fibres[6,89] and does not[14] or only moderately lengthens[90] APD in Purkinje fibres. These results suggest that there are also differences in the effect of the drug on the transmembrane ionic currents between acute and chronic amiodarone administration.

The acute effects of amiodarone on various transmembrane ionic current including I_{Na}, I_{Ca}, and I_K, have been well characterised by other research groups[9,10,15]. It is well established that acute administration of amiodarone inhibits I_{Na}[9], I_{Ca}[10,91] and I_K[15,16,92] in guinea pig, rabbit and cat single myocytes. However, very little information is available about the action of acutely applied amiodarone on I_{to} and I_{k1}. It has been reported that a high concentration of amiodarone (20 μM) inhibited I_{k1}[93]. Only preliminary data are available concerning the acute effect of the drug on I_{to} and generally, very little is known about the possible influence of chronic administration of amiodarone on the various transmembrane ionic currents[94]. These preliminary data are in agreement with our results regarding potassium currents. Considering the results of the present and the previous studies it seems reasonable to assume that since acute amiodarone application inhibits both inward and outward currents, the effect of the drug on APD depends on the relative contribution of these currents to the repolarization of the action potential in a particular tissue type. Therefore, acute administration of amiodarone may result in lengthening, shortening or no change of the action potential repolarization. However, after chronic treatment, amiodarone decreases further the density of I_K and I_{to} causing lengthening of ADP in ventricular muscle.

The blockade of the transmembrane ionic currents after acute application of amiodarone can be best explained by direct state dependent interactions of the drug with the

ion channels. However, the mechanism of the effect of chronic amiodarone treatment seems to be more complex. Singh and Vaughan-Williams, as early as in 1970, observed the effect of long-term amiodarone treatment on thyroid function[6]. Later it was demonstrated that experimental hypothyroidism attenuated the repolarization lengthening effect of chronic amiodarone treatment[95]. These results suggest that the effects of long-term amiodarone treatment are mediated, at least partly, via thyroid action.

In summary, it is concluded that acute application of amiodarone in the tissue bath and chronic amiodarone treatment can influence transmembrane ionic currents differently. This may help to understand the marked differences in the amiodarone-induced electrophysiological changes observed after acute and long-term treatments with the drug in patients.

4.5. Effect of glibenclamide on the ATP sensitive potassium current

The ATP sensitive potassium channels were first described in cardiac muscle by Noma[17]. The activation of K^+_{ATP} current plays a major role in the decrease of the action potential duration seen under ischaemic conditions. The shortening of action potential is accompanied by a decrease in contraction which is a major mechanism consuming ATP. Therefore, activation of the ATP sensitive potassium current prevents further depletion of ATP and protects the cell from irreversible impairment of its energy metabolism[17].

The most potent inhibitor of the K^+_{ATP} channel is the sulfonylurea glibenclamide. Our results[96] suggest that there is some positive interaction between the binding of ATP to its binding site and the inhibitory effect of glibenclamide. Application of higher ATP concentration in the intracellular side of the cell membrane results in shift of the dose-response curve toward lower glibenclamide concentrations, i.e. the block of K^+_{ATP} channels by glibenclamide becomes stronger. Some observations of other research groups seem to support this findings. It has been reported that using inside-out membrane patches in the absence of ATP, glibenclamide inhibited the activity of the K^+_{ATP} channels with an EC_{50} of 6 μM and the Hill coefficient was 0.35. Complete channel inhibition was not observed, even in the presence of 300 μM glibenclamide[97]. Under similar conditions another research group found an EC_{50} value of 0.5 μM for glibenclamide inhibition and the Hill coefficient was 0.5 in this case[98]. The estimated

EC_{50} and the Hill coefficient were 18 nM and 1.2, respectively, in our experiments in the presence of 100 μM ATP which is similar to that measured by Findaly[99]. The substantial difference in the Hill coefficient in the presence and absence of nucleotides and the high EC_{50} values in ATP free conditions suggest that ATP may have important role in the regulation of glibenclamide sensitivity of K^+_{ATP} channels.

In our experiments the blocking effect of glibenclamide on the activity of K^+_{ATP} channels was significantly weaker if, in addition to ATP, ADP was applied in the intracellular side of the patch membrane. This finding appears to be consistent with other previous reports[97,98]. The mechanism by which ADP attenuates the ability of glibenclamide to inhibit the activity of the K^+_{ATP} channels is unknown but may be similar to the effect of ADP on the ATP sensitivity of the K^+_{ATP} current, i.e. in the presence of ADP K^+_{ATP} channels become less sensitive to ATP as reported previously by others[21,26]. It has been reported that not only ADP but intracellular lactate, accumulating in ischaemic condition, has also attenuated the effect ATP on the channels[27], though our experiments in excised patches could not confirm these findings[100].

These results well correlate with the studies in which it has been found that although sulfonylurea drugs may be very effective in cell free conditions or when the ATP-sensitive K^+ current has been activated by potassium channel openers, such as pinacidil or cromakalim, their effect may be lost when the current has been induced by metabolic inhibition[98,101]. The loss of effect of glibenclamide may be explained if we consider that the intracellular ADP, which rises during metabolic stress, and the declining intracellular ATP allosterically influence the sulfonylurea receptor. However, the factors responsible for the loss of glibenclamide blockade on K^+_{ATP} channels during metabolic inhibition is still unclear.

It has been reported that several class I antiarrhythmic drugs, such as quinidine, cibenzoline, disopyramide, procainamide and flecainide, are able to block the K^+_{ATP} current[102,103]. The inhibitory action of these drugs on K^+_{ATP} current may be advantageous, since in ischaemic conditions the marked shortening of the time for repolarization, caused by opening of K^+_{ATP} channels, may be the source of life threatening ventricular arrhythmias. On the other hand it was observed that inhibition of ATP sensitive potassium channels by glibenclamide during ischaemia enhances the myocardial damage caused by subsequent

reperfusion[30]. Therefore, the inhibitory effect of antiarrhythmic drugs on the activity of K^+_{ATP} channels may be useful in the early phase of ischaemia but may be harmful in view of the protection of the cells from calcium overload induced by ischaemia/reperfusion injury[103].

5. References

[1] The CAST Investigators: Preliminary report: effect of encainide and flecainide on mortality in randomized trial arrhythmia supression after myocardial infarction. *N Engl J Med* 321:406-412, 1989.

[2] The CAST II Investigators: Effect of the antiarrhythmic agent moricizine on survival after myocardial infarction. *N Engl J Med* 327:227-233, 1992.

[3] The SWORD Investigators: Problems for BMS' D-sotalol. *SCRIP* No 1973, 23, 1994.

[4] Pfisterer M, Kiowski W, Burckhardt D, Follath F, Burkart F: Beneficial effect of amiodarone on cardiac mortality in patients with asymptomatic complex ventricular arrhythmias after acute myocardial infarction and preserved but not impaired left ventricular function. *Am J Cardiol* 69:1399-1402, 1992.

[5] The CASCADE Investigators: Randomized antiarrhythmic drug therapy in survivors of cardiac arrest (the CASCADE study). *Am J Cardiol* 72:280-287, 1993.

[6] Singh BN, Vaughan-Williams EM: The effect of amiodarone, a new anti-anginal drug, on cardiac muscle. *Br J Pharmacol* 39:657, 1970.

[7] Mason JW, Hondeghem LM, Katzung BG: Block of inactivated sodium channels and of depolarization-induced automaticity in guinea pig papillary muscle by amiodarone. *Circ Res* 55:277-285, 1984.

[8] Varro A, Nakaya Y, Elharrar V, Surawicz B: Use-dependent effects of amiodarone on V_{max} in cardiac Purkinje and ventricular muscle fibers. *Eur J Pharmacol* 112:419-422, 1985.

[9] Follmer CH, Aomine M, Yeh JZ, Singer DH: Amiodarone-induced block of sodium current in isolated cardiac cells. *J Pharmac Exp Ther* 243:187-194, 1987.

[10] Nishimura M, Follmer CH, Singer DH: Amiodarone blocks calcium current in single guinea pig ventricular myocytes. *J Pharmacol Exp Ther* 251:650-659, 1989.

[11] Polster P, Broekhuysen J: The adrenergic antagonism of amiodarone. *Biochem Pharmacol* 25:131-134, 1976.

[12] Wellens HJJ, Brugada P, Abdollah H, Dassen WR: A comparison of the electrophysiologic effects of intravenous and oral amiodarone in the same patient. *Circulation* 69:120-124, 1984.

[13] Kadoya M, Konishi T, Tamamura T, Ikeguchi S, Hashimoto D, Kawai C: Electrophysiological effects of amiodarone on isolated rabbit heart muscle. *J Cardiovasc Pharmacol* 7:643-648, 1985.

[14] Varro A, Nakaya Y, Elharrar V, Surawicz B: The effects of amiodarone on repolarization and refractoriness of cardiac fibers. *Eur J Pharmacol* 154:11-18, 1988.

[15] Balser JR, Bennett PB, Hondeghem LM, Roden DM: Suppression of time-dependent outward current in guinea pig ventricular myocytes. Action of quinidine and amiodarone. *Circ Res* 69:519-529, 1991.

[16] Carmeliet E: Use-dependent block of the delayed K^+ current in rabbit ventricular myocytes. *Cardiovasc Drugs Ther* 7(Suppl 3):599-604, 1993.

[17] Noma A: ATP-regulated K^+ channels in cardiac muscle. *Nature* 305:147-148, 1983.

[18] Spruce AE, Standen NB, Stanfield PR: Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. *Nature* 316:736-738, 1985.

[19] Cook DL, Hales CN: Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. *Nature* 311:271-273, 1984.

[20] Rovetto MJ, Whitmer JT, Neely JR: Comparison of the effects of anoxia and whole heart ischaemia on carbohydrate utilization in isolated working rat hearts. *Circ Res* 32:699-711, 1973.

[21] Findaly I: Effects of ADP upon the ATP-sensitive K^+ channel in rat ventricular myocytes. *J Membr Biol* 101:83-92, 1988.

[22] Nichols CG, Lederer WJ: The regulation of ATP-sensitive K^+ channel activity in intact and permeabilized rat ventricular myocytes. *J Physiol* 423:91-110, 1990.

[23] Nichols CG, Ripoll C, Lederer WJ: ATP-sensitive potassium channel modulation of the guinea pig ventricular action potential and contraction. *Circ Res* 68:280-287, 1991.

[24] Findaly I, Faivre JF: ATP-sensitive K^+ channels in heart muscle: spare channels. *FEBS Lett* 279:95-97, 1991.

[25] Cuevas J, Bassett AL, Cameron JS, Furukawa T, Myerburg RJ, Kimura S: Effect of H^+ on ATP-regulated K^+ channels in feline ventricular myocytes. *Am J Physiol* 261:H755-H761, 1991.

[26] Lederer WJ, Nichols CG: Nucleotide modulation of the activity of rat heart ATP-sensitive K^+ channels in isolated membrane patches. *J Physiol* 419:193-211, 1989.

[27] Keung EC, Li Q: Lactate activates ATP-sensitive potassium channels in guinea pig ventricular myocytes. *J Clin Invest* 88:1772-1777, 1991.

[28] Wolleben CD, Sanguinetti MC, Siegl PK: Influence of ATP-sensitive potassium channel modulators on ischemia-induced fibrillation in isolated rat hearts. *J Mol Cell Cardiol* 21:783-788, 1989.

[29] Leprán I, Baczkó I, Varró A, Papp J Gy: ATP-sensitive potassium channel modulators: both pinacidil and glibenclamide produce antiarrhythmic activity during acute myocardial infarction in conscious rat. *J Pharmacol Exp Ther* 277:1215-1220, 1996.

[30] Cole WC, McPherson CD, Sontag D: ATP-regulated K^+ channels protect the myocardium against ischemia/reperfusion damage. *Circ Res* 69:571-581, 1991.

[31] Bernauer W: Concerning the effect of the K^+ channel blocking agent glibenclamide on ischaemic and reperfusion arrhythmias. *Eur J Pharmacol* 326:147-156, 1997.

[32] Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* 391:85-100, 1981.

[33] Coraboeuf E, Carmeliet E: Existance of two transient outward currents in sheep cardiac Purkinje fibers. *Pflügers Arch* 392:352-359, 1982.

[34] Escande D, Coulombe A, Faivre JF, Deroubaix E, Coraboeuf E: Two types of transient outward currents in adult human atrial cells. *Am J Physiol* 252:H142-H148, 1987.

[35] Hiraoka M, Kawano S: Calcium-sensitive and insensitive transient outward current in rabbit ventricular myocytes. *J Physiol* 410:187-212, 1989.

[36] Zygmunt AC, Gibbons WR: Calcium-activated chloride current in rabbit ventricular myocytes. *Circ Res* 68:424-437, 1991.

[37] Sanguinetti MC, Jurkiewicz NK: Two components of cardiac delayed rectifier K^+ current: Differential sensitivity to block by Class III antiarrhythmic agents. *J Gen Physiol* 96:195-215, 1990.

[38] Giles WR, Imaizumi Y: Comparison of potassium currents in rabbit atrial and ventricular cells. *J Physiol* 405:123-145, 1988.

[39] Carmeliet E: Voltage- and time-dependent block of the delayed K^+ current in cardiac myocytes by dofetilide. *J Pharmacol Exp Ther* 262:809-817, 1992.

[40] Furukawa T, Virág L, Furukawa N, Sawanobori T, Hiraoka M: Mechanism for reactivation of ATP-sensitive K^+ channel by MgATP complexes in guinea pig ventricular myocytes. *J Physiol* 479:95-107, 1994.

[41] Furukawa T, Virág L, Sawanobori T, Hiraoka M: Stilbene disulfonates block ATP-sensitive K^+ channels in guinea pig ventricular myocytes. *J Membrane Biol* 136:289-302, 1993.

[42] Josephson IR, Sanchez-Chapula J, Brown AM: Early outward current in rat single ventricular cells. *Circ Res* 54:157-162, 1984.

[43] Simurda J, Simurdova M, Cupera P: 4-aminopyridine sensitive transient outward current in dog ventricular fibres. *Pflügers Arch* 411:442-449, 1988.

[44] Heidbuchel H, Vereecke J, Carmeliet E: The electrophysiological effects of acetylcholine in single human atrial cells. *J Mol Cell Cardiol* 19:1207-1219, 1987.

[45] Nánási P, Varró A, Lathrop DA: Ionic currents in ventricular myocytes isolated from the heart of a patient with idiopathic cardiomyopathy. *Cardioscience* 3:85-89, 1992.

[46] Ten Eick RE, Robertson L: Cat papillary muscle seems to lack transient outward current. *Fed Proc* 42:730, 1983.

[47] Litovsky SH, Antzelevitch C: Transient ouward current prominent in canine ventricular epicardium but not endocardium. *Circ Res* 62:116-126, 1988.

[48] Coraboeuf E, Deroubaix E, Escande D, Coulombe A: Comparative effects of three class I antiarrhythmic drugs on plateau and pacemaker currents of sheep cardiac Purkinje fibres. *Cardiovasc Res* 22:375-384, 1988.

[49] Virág L, Varró A, Papp J Gy: Disopyramide decreases the transient outward current in rabbit isolated cardiac myocytes. *J Mol Cell Cardiol* 26:LXXXVII, 1994 (abstract).

[50] Virág L, Varró A, Papp J Gy: Effect of disopyramide on potassium currents in isolated rabbit cardiac myocytes. *J Mol Cell Cardiol* 27:A141, 1995 (abstract).

[51] Virág L, Varró A, Papp J Gy: Effect of disopyramide on potassium currents in rabbit ventricular myocytes. *Naunyn-Schmiedeberg's Arch Pharmacol* 357:268-275, 1998.

[52] Virág L, Iost N, Varró A, Papp J Gy: Comparison of several antiarrhythmic drugs on potassium currents in rabbit ventricular myocytes. *J Mol Cell Cardiol* 28:A58, 1996 (abstract).

[53] Fazekas T, Virág L, Varró A, Papp J Gy, Berlin KD, Scherlag BJ, Lazzara R: Effects of GLG-V-13, a novel class III antiarrhythmic agent, on cardiac K^+ currents in rabbit ventricular myocytes. *J Mol Cell Cardiol* 29:A118, 1997 (abstract).

[54] Virág L, Iost N, Fazekas T, Varró A, Scherlag BJ, Lazzara R, Papp J Gy: Modulation of various cardiac potassium currents by a new class III antiarrhythmic drug, GLG-V-13, in rabbit ventricular myocytes. *Naunyn-Schmiedeberg's Arch Pharmacol* 356(Suppl 1):R7, 1997 (abstract).

[55] Németh M, Virág L, Varró A, Papp J Gy: Comparison of the effects of restacorin and flecainide on various cardiac transmembrane potassium currents. *Acta Physiol Hung* 84:317-318, 1996.

[56] Imaizumi Y, Giles WR: Quinidine-induced inhibition of transient outward current in cardiac muscle. *Am J Physiol* 253:H704-H708, 1987.

[57] Wang Z, Fermini B, Nattel S: Effects of flecainide, quinidine, and 4-aminopyridine on transient outward and ultrarapid delayed rectifier currents in human atrial myocytes. *J Pharmacol Exp Ther* 272:184-196, 1995.

[58] Dukes ID, Morad M: Tedisamil inactivates transient outward K^+ current in rat ventricular myocytes. *Am J Physiol* 257:H1746-H1749, 1989.

[59] Yamashita T, Nakajima T, Hamada E, Hazama H, Omata M, Kurachi Y: Flecainide inhibits the transient outward current in atrial myocytes isolated from the rabbit heart. *J Pharmacol Exp Ther* 274:315-321, 1995.

[60] Slawsky MT, Castle NA: K^+ channel blocking actions of flecainide compared with those of propafenone and quinidine in adult rat ventricular myocytes. *J Pharmacol Exp Ther* 269:66-74, 1994.

[61] Varró A, Nánási P, Lathrop DA: Effect of sotalol on transmembrane ionic currents responsible for repolarization in cardiac ventricular myocytes from rabbit and guinea pig. *Life Sci* 49:PL7-12, 1991.

[62] Berger F, Borchard U, Hafner D: Effects of (+)- and (+/-)-sotalol on repolarizing outward currents and pacemaker current in sheep cardiac Purkinje fibres. *Naunyn-Schmiedeberg's Arch Pharmacol* 340:696-704, 1989.

[63] Gintant GA: Two components of delayed rectifier current in canine atrium and ventricle. *Circ Res* 78:26-37, 1996.

[64] Salata JJ, Jurkiewicz NK, Jow B, Folander K, Guinosso PJ, Raynor B, Swanson R, Fermini B: I_K of rabbit ventricle is composed of two currents: evidence for I_{Ks}. *Am J Physiol* 271: H2477-H2489, 1996.

[65] Beuckelmann DJ, Näbauer M, Erdmann E: Alterations of K^+ currents in isolated human ventricular myocytes from patients with terminal heart failure. *Circ Res* 73:379-385, 1993.

[66] Li GR, Feng J, Yue L, Carrier M, Nattel S: Evidence for two components of delayed rectifier K^+ current in human ventricular myocytes. *Circ Res* 78:689-696, 1996.

[67] Jurkiewicz NK, Sanguinetti MC: Rate-dependent prolongation of cardiac action potentials by a methanesulfonanilide class III antiarrhythmic agent. Specific block of rapidly activating delayed rectifier K^+ current by dofetilide. *Circ Res* 72:75-83, 1993.

[68] Kotake H, Hasegawa J, Hata T, Mashiba H: Electrophysiological effect of disopyramide on rabbit sinus node cells. *J Electrocardiol* 18:377-383, 1985.

[69] Hiraoka M, Kuga K, Kawano S, Sunami A, Fan Z: New observations on the mechanisms of antiarrhythmic actions of disopyramide on cardiac membranes. *Am J Cardiol* 64:15J-19J, 1989.

[70] Fazekas T, Carlsson L, Scherlag B, Mabo P, Poty H, Palmer M, Patterson E, Berlin KD, Garrison GL, Lazzara R: Electrophysiological and inotropic characterization of a novel class III antiarrhythmic agent, GLG-V-13, in the mammalian heart. *J Cardiovasc Pharmacol* 28:182-191, 1996.

[71] Follmer CH, Colatsky TJ: Block of delayed rectifier potassium current, I_K, by flecainide and E-4031 in cat ventricular myocytes. *Circularion* 82:289-293, 1990.

[72] Wettwer E, Grundke M, Ravens U: Differential effects of the new class III antiarrhythmic agents almokalant, E-4031 and d-sotalol, and of quinidine, on delayed rectifier currents in guinea pig ventricular myocytes. *Cardiovasc Res* 26:1145-1152, 1992.

[73] Kass RS, Arena JP, Walsh B: Measurement and block of potassium channel currents in the heart: importance of channel type. *Drug Dev Res* 19:115-127, 1990.

[74] Martin CL, Chinn K: Contribution of delayed rectifier and inward rectifier to repolarization of the action potential: pharmacologic separation. *J Cardiovasc Pharmacol* 19:830-837, 1992.

[75] Shimoni Y, Clark RB, Giles WR: Role of an inwardly rectifying potassium current in rabbit ventricular action potential. *J Physiol* 448:709-727, 1992.

[76] Salata JJ, Wasserstrom JA: Effects of quinidine on action potentials and ionic currents in isolated canine ventricular myocytes. *Circ Res* 62:324-337, 1988.

[77] Duan D, Fermini B, Nattel S: Potassium channel blocking properties of propafenone in rabbit atrial myocytes. *J Pharmacol Exp Ther* 264:1113-1123, 1992.

[78] Martin DK, Nakaya Y, Wyse KR, Campbell TJ: Effects of disopyramide and flecainide on the kinetics of inward rectifier potassium channels in rabbit heart muscle. *Br J Pharmacol* 111:873-879, 1994.

[79] Rees S, Curtis MJ: Which cardiac potassium channel subtype is the preferable target for suppression of ventricular arrhythmias? *Pharmacol Ther* 69:199-217, 1996.

[80] Nakaya H, Tohse N, Takeda Y, Kanno M: Effects of MS-551, a new class III antiarrhythmic drug, on action potential and membrane currents in rabbit ventricular myocytes. *Br J Pharmacol* 109:157-163, 1993.

[81] Opthof T: IK1 blockade is unlikely to be a useful antiarrhythmic mechanism. *Cardiovasc Res* 28:420, 1994.

[82] Varró A, Virág L, Papp J Gy: The effects of chronic amiodarone treatment on the cardiac calcium and potassium channels. *J Mol Cell Cardiol* 27:A140, 1995 (abstract).

[83] Varró A, Virág L, Papp J Gy: Comparison of the chronic and acute effects of amiodarone on the calcium and potassium currents in rabbit isolated cardiac myocytes. *Br J Pharmacol* 117:1181-1186, 1996.

[84] Debbas NM, Ducailar C, Bexton RS, Demaille JG, Camm AJ, Puech P: The QT interval: a predictor of the plasma and myocardial concentrations of amiodarone. *Br Heart J* 51:316-320, 1984.

[85] Kodama I, Suzuki R, Kamiya K, Iwata H, Toyama J: Effects of long-term oral administration of amiodarone on the electrochemical performance of rabbit ventricular muscle. *Br J Pharmacol* 107:502-509, 1992.

[86] Ikeda N, Nademanee K, Kannan R, Singh BN: Electrophysiologic effects of amiodarone: Experimental and clinical observation relative to serum and tissue drug concentrations. *Am Heart J* 108:890-898, 1984.

[87] Northover BJ: Alteration to the electrical activity of atrial muscle isolated from rat heart, produced by exposure in vitro to amiodarone. *Br J Pharmacol* 82:191-197, 1984.

[88] Yabek S, Kato R, Singh BN: Effects of amiodarone and its metabolite, desethylamiodarone, on the electrophysiologic properties of isolated cardiac muscle. *J Cardiovasc Pharmacol* 8:197-207, 1986.

[89] Kato R, Venkatesh N, Kamiya K, Yabek S, Kannan R, Singh BN: Electrophysiologic effects of desethylamiodarone, an active metabolite of amiodarone: Comparison with amiodarone during chronic administration in rabbits. *Am Heart J* 115:351-359, 1988.

[90] Gallagher JD, Bianchi J, Gessman LJ: A comparison of the electrophysiologic effects of acute and chronic amiodarone administration on canine Purkinje fibers. *J Cardiovasc Pharmacol* 13:723-729, 1989.

[91] Valenzuela C, Bennett PB: Voltage- and use-dependent modulation of calcium channel current in guinea pig ventricular cells by amiodarone and des-oxo-amiodarone. *J Cordiovasc Pharmacol* 17:894-902, 1991.

[92] Carmeliet E: Block of I_{Kr} by amiodarone in rabbit ventricular myocytes. *J Mol Cell Cardiol* 26: LVI (abstract), 1994.

[93] Sato R, Koumi S-I, Singer DH, Hisatome I, Jia H, Eager S, Wasserstrom JW: Amiodarone blocks the inward rectifier potassium channel in isolated guinea pig ventricular cells. *j Pharmacol Exp Ther* 269:1213-1219, 1994.

[94] Toyama J, Kamiya K, Cheng J, Kodama I, Yamada K: Acute and chronic effects of amiodarone on action potential and ionic currents in single rabbit ventricular myocytes. *Eur Heart J* 15:192 (abstract), 1994.

[95] Talajic M, Nattel S, Davies M, McCans J: Attenuation of class 3 and sinus node effects of amiodarone by experimental hypothyroidism. *J Cardiovasc Pharmacol* 13:447-450, 1987.

[96] Virág L, Furukawa T, Hiraoka M: Modulation of the effect of glibenclamide on K_{ATP} channels by ATP and ADP. *Mol Cell Biochem* 119:209-215, 1993.

[97] Ripoll C, Lederer WJ, Nichols CG: On the mechanism of inhibition of K_{ATP} channels by glibenclamide in rat ventricular myocytes. *J Cardiovasc Electrophysiol* 4:38-47, 1993.

[98] Venkatesh N, Lamp ST, Weiss JN: Sulfonylureas, ATP-sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia, and metabolic inhibition in mammalian ventricle. *Circ Res* 69:623-637, 1991.

[99] Findaly I: Inhibition of ATP-sensitive K^+ channels in cardiac muscle by the sulfonylurea drug glibenclamide. *J Pharmacol Exp Ther* 261:540-545, 1992.

[100] Virág L, Furukawa T, Hiraoka M: Lactate fails to activate ATP-sensitive potassium current in membrane patches excised from guinea pig ventricular myocytes. *J Physiol* 489P:74P, 1995 (abstract).

[101] Findaly I: Sulfonylurea drugs no longer inhibit ATP sensitive K^+ channels during metabolic stress in cardiac muscle. *J Pharmacol Exp Ther* 266:456-467, 1993.

[102] Undrovinas AI, Burnashev N, Eroshenko D, Fleidervish I, Starmer CF, et al.: Quinidine blocks adenosine 5'-triphosphate-sensitive potassium channels in heart. *Am J Physiol* 259:H1609-H1612, 1990.

[103] Wu B, Sato T, Kiyosue T, Arita M: Blockade of 2,4-dinitrophenol induced ATP sensitive potassium current in guinea-pig ventricular myocytes by class I antiarrhythmic drugs. *Cardiovasc Res* 26:1095-1101, 1992.

6. Acknowledgements

This work was supported by a grant from the Hungarian National Scientific Research Foundation (OTKA F6328) and a Foreign Special Scholarship from Japanese Government (Mombusho).

I am most grateful to Professor Julius Gy Papp and Professor László Szekeres for their continuous support and for providing possibility to me to work at the Department of Pharmacology, Albert Szent-Györgyi Medical University.

I am grateful to Professor Masayasu Hiraoka for introducing me to the fundamentals of the patch-clamp technique and for helping me in all respects during my stay in Japan.

I am especially thankful to Dr. András Varró, my tutor, who always helped and guided me during my studies.

I thank Mrs. Tari Magdolna Csomor, Éva Szabadi, Ervin Lukács and Gyula Horváth for their technical assistance.