Cellular elctrophysiological and pharmacological measurements on undiseased human myocardium

Summary of PhD Thesis

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1. BACKGROUND

Ischaemic heart disease and its complications, among them cardiac arrhythmias and ventricular fibrillation, are a common cause of death in developed countries. Although the medical technical developement facilitates the use of more invasive antiarrhythmic treatments which includes intracardiac defibrillator implantation and transcatheter radiofrequency ablation, the vast majority of patients are treated with oral or intravenous antiarrhythmic drugs.

The treatment strategy of the ventricular arrhythmias turned from class I antiarrhythmic drugs used most commonly in the last decades, towards the potassium chanel blocker class III antiarrhythmic drugs, with their action potential duration (APD) and effective refractory period (ERP) lenghthening properties, without influence on the conduction velocity. In the SWORD trial though patients treated with oral d-Sotalol (a class III antiarrhythmic drug) showed increased mortality due to occurence of life-threatening ventricular arrhythmias –torsade des pointes. Experiments made on mammalian heart preparations revealed a significant lenghthening of APD_{50} and APD_{90} after administration of sotalol to the tissue bath, facilitating the occurrence of early afterdepolarisation (EAD) and enhanced inhomogeneity of repolarisation causing fatal ventricular arrhythmias .

Since the vast majority of experimental data available at present come from studies performed on mammalian or diseased human hearts explanted during cardiac transplantation, we evaluated the cellular electrophysiological effects of antiarrhythmic drugs on undiseased human donor heart preparations. We measured the possible beneficial effects of the combination of a class I/B antiarrhythmic drug mexiletine with sotalol aiming to prevent its pharmacologically disadvantageous effect of reverse rate dependent APD prolongation.

Large effort is made worldwide to develop new antiarrhythmic agents with no or minimal proarrhythmic and extracardiac side effects. In such a chemical synthetic program of the Drug Reserch Institute new compounds were obtained with combined class I/B and III antiarrhythmic properties, structurally different from amiodarone. In this work we also described the cellular electrophysiological effects of one of these compounds, GYKI 16638, on the action potential of undiseased human ventricular muscle in comparison with sotalol and mexiletine.

The experimental data obtained from mammalian hearts of different species often give rise to controversies regarding the effects and mechanisms of action of several compounds. Such a relevant example is the case of endothelin, a group of three vasoactive peptides released from vascular and endocardial endothelium. From these, endothelin-1 (ET 1) is a

potent vasoconstrictor which was described to be present in the human pericardial fluidum. The cellular electrophysiological effects of ET 1 are controversal, depending on the origine of the cardiac cells. Administrated intrapericardially in a rather high concentration, ET 1 causes in dog a significant prolongation of APD with EAD and severe ventricular arrhythmias. On the other hand ET 1 shortens APD by inhibiting L-type calcium current (I_{Ca}) and activating potassium currents in rabbit and guinea pig cardiac preparatios. Since human data were not available, we investigated the electrophysiological effects of ET-1 on L-type I_{Ca}, I_{to}, I_{Kr} and I_{K1} in undiseased left ventricular isolated myocardial cells.

It is obvious that experimental data obtained from undiseased *human* myocardium is a sine qua non condition to fully characterise the effects and mechanisms of action of different antiarrhythmic drugs or other compounds, or to find the mammalian species which electrophysiologically resembles the human myocardium. This is true also in the case of the slow component of the delayed rectifier potassium current, since I_{Ks} show species and tissue dependent variations. Therefore, we further described this slowly activating and relatively fast deactivating current.

The small amount of experimental data obtained from human myocardium (mostly from diseased, cardiomyopatic explanted hearts,) and even less from donor human hearts, along with the sometimes controversial results obtained from experiments made on different mammalian myocardial tissue, underlines the great value of the electrophysiological and pharmacological measurements made on undiseased human myocardium. In the homograft program of the Cardiac Surgery Department of the Szeged University we prepare aorta and pulmonary valvular conduits for aorta, pulmonary valve replacements or pulmonary valve autotransplantations (Ross procedure). The hearts are obtained from beating heart donors. The homograft preparation protocol uses only the aortic and pulmonary valvulated conduits, the rest of the heart (atrial and ventricular myocardial mass) being destroyed. This raised the idea of explanting the hearts in conformity with the transplantation protocol, i.e after administration of cold myocardial protective solutution in the coronary arteries, thus preserving entirely for 6 hours the mechanic and electrophysiologic properties of the undiseased donor heart. This protocol was approved by the Ethical Review Board of the Szeged Medical University (No. 51-57/1997 OEj).

Aims of the study:

1.Preservation of myocardium from beating heart donors not used for cardiac transplantation

2. To investigate the cellular electrophysiological effects of sotalol, mexiletine and a new antiarrhythmic compound, GYKI 16638, on undiseased human right ventricular papillary and trabecular muscle preparations.

3. To investigate the properties (amplitudes, activation-deactivation kinetics, drug sensitivities) of the slow component of the delayed rectifier potassium current on myocardial cells isolated from healthy human hearts.

4. To study the effect of endothelin-1 on calcium and potassim currents in ventricular myocytes obtained from undiseased human donor hearts.

2. METHODS

2.1 EXPLANTATION OF THE UNDISEASED DONOR HEART.

After the brain death of the patient is established sternotomy, along with a xiphopubian laparotomy is performed. An aortic root cardioplegic needle was inserted in the ascendent aorta. Inferior and superior vena cava and the right superior pulmonary vein were prepared. The donor was fully heparinised (with iv-Na heparin). After the kidney perfusion was started, the two vena cava were clamped, the right superior pulmonary vena was cut and the heart was allowed to empty for three four beats. Then the aorta was crossclamped and 1000 ml of cold (2-4 C°) St. Thomas cardioplegic solution was administrated in the aortic root , and the hearts stopped in diastole. After explantation the hearts were divided in two parts: the basal region with the aortic and pulmonary valvular conduits (for homograft preparation), and the left and right ventricular myocardial mass along with parts of the left and right atria. Both parts were transported in cold cardioplegic solution (composition in. mM: NaCl 110, KCl 16, $MgCl_2$ 16, $CaCl_2$ 12, $NaHCO_3$ 10) to the homograft and experimental laboratories. The experimental protocol complied with the declaration of World Medical Association proclaimed in Helsinki and was approved by the Ethical Review Board of the Universsity ot Szeged (No. : 51-57/1997 OEj). Right venticular trabecular and papillary muscles were prepared and used for cellular electrophysiological measurements, with the conventional microelectrode technique. Left ventricular portions with their intact coronary arteries were isolated and enzimatcally digested for obtaining isolated human myocardial cells.

2.2. CONVENTIONAL MICROELECTRODE TECHNIQUE

16 preparations were used from 16 donors, mean age 40 ± 0.3, 8 females and 8 males. The trabecular and thin (<2mm) papillary muscle preparations were excised and mounted in a

tissue chamber (50 ml volume) filled with oxygenated (95 % O_2, 5 % CO_2) modified Tyrode's solution containing in mM: NaCL 115, KCL 4, $CaCL_2$ 1,8 $MgCL_2$ 1, $NaHCO_3$ 20 and glucose 11. The experiments were performed at 37 C°, at a pH between 7,35 and 7,45. Each preparation was initially stimulated at a basic cycle lenght of 1000 ms (frequency = 1 Hz). At least 1 hour was allowed for each preparation to equilibrate while they were continuously superfused with the Tyrode's solution. Transmembrane potentials were recorded using the conventional microelectrode technique. Microelectrodes filled with 3 M KCl and having tip resistances 5-20 MΩ were connected to the input of a high impedance electrometer (Biologic VF102), which was referenced to the ground. The resting membrane potential (RP), action potential amplitude (APA) and action potential duration (APD) measured at 50 % and 90 % reporalization (APD_{50-90}) and the maximal rate of depolarisation (V_{max}), were obtained by APES HSE software developed in our Department. After measuring the action potential parameters (maximal sampling rate = 50 kHz) at the basic cycle length of 1000 ms, 9 different constant stimulation cycle lengths (300, 400, 500, 700, 1000, 1500, 2000, 3000, and 5000 ms) were applied. Action potential parameters were measured at each cycle length following „quasi steady state" adaptation (25 beats) to the new pacing cycle length. After control measurements drugs were superfused for 40-60 minutes and measurements were repeated.

2.3. PATCH CLAMP TECHNIQUE

2.3.1. *Myocardial cell isolation*

Part of the left ventricular myocardium along with its adjacent coronary artery was prepared for myocardial cell isolation from 33 patients with a mean age of 42,5±8,95 years, 20 female and 13 male. The cells were used for:

a. Demonstration of existence and characterisation of the slow component of the delayed rectifier potassium current in undiseased human myocardium.

b. Study of the electrophysiological effects of endothelin-1 on Ca and K currents in undiseased human ventricular cells.

The left venticular wall together with its arterial branch was mounted on a modified Langendorff apparatus, where it was perfused through the left anterior descending coronary artery according to the following sequence: (1) modified Tyrode solution (containing, in mmol/l: NaCl, 135; KCl, 4,7; KH_2PO_4, 1,2; $MgSO_4$ 1,2; HEPES,10; $NaHCO_3$, 4,4; glucose 10; pH=7,2) for 10 min; (2) Ca^{2+} -free modified Tyrode solution for 10 min; (3) Ca^{2+} -free modified Tyrode solution containing collagenase(660 mg/l, type I, Sigma), elastase (45mg/l, type III, Sigma), taurine (50 mmol/l) and bovine albumin (2 g/l, fraction V, fatty acid free, Sigma) for 45 min; (4) after this step of enzymatic digeston the solution was supplemented

with protease (120 mg/l, type XIV, Sigma) for a further 40-60 min. Portions of the left ventricular wall, which was clearly digested by the enzymes, were cut into small pieces and either stored in KB medium, or equilibrated for 15 min in modified Tyrode solution containing 1,25 mmol/l $CaCl_2$ and 50 mmol/l taurine. Single myocytes were obtained from the tissue chunks after gentle agitation.

2.3.2. *Voltage clamp*

Experiments were perfomed on calcium-tolerant cells, which were rod shaped and maintained clear cross-striations following exposure to oxygenated Tyrode solution containing (in mmol/l): NaCl, 140; KCl, 5,4; $CaCl_2$, 2,5; $MgCl_2$, 1,2; Na_2HPO_4, 0,35; HEPES, 5; glucose, 10 at pH 7,4. This solution was supplemented with either 3 mmol/l 4-aminopyridine or 0,25 mmol/l $CdCl_2$, when measuring calcium or potassium currents, respectively. Suction pipettes, fabricated from borosilicate glass (Clark), had tip resistances of 2-3 MΩ after filling with pipette solution. When measuring potassium currents, the solution composition was (in mmol/l): K-aspartate, 100; KCl, 20; Mg-ATP, 5; HEPES, 10; K_4BAPTA, 5; glucose, 5. When measuring calcium current the pipette solution contained (in mmol/l): KCl, 110; KOH, 40; K-ATP, 3; HEPES, 10; EGTA, 10; tetraethylammonium chloride (TEACI), 20; glucose, 5; GTP, 0,25. The pH of both solutions was adjusted to 7,2 using KOH. Membrane currents were recorded at 37° C using an Axopatch-1D amplifier (Axon Instruments) in the whole-cell configuration of the pach-clamp technique (10). After establishing a high-resistance (1-10 GΩ) seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by applying 1,5-V electrical pulses for 1-5 ms.

Results are expressed as mean ±SEM values. Student's t-test for paired data was used to determine statistical significance. Changes were considered significant when p was less then 0,05.

3. RESULTS

3.1. The cellular electrophysiological properties of GYKI 16638.

The effect of 5 μM GYKI 16638 on the basic action potential parameters was studied at 1 Hz stimulatinon frequency and compared with that of 30 μM sotalol and 10 μM mexiletine.GYKI 16638 lengthened APD_{90} but not APD_{50}, without significantly changing other action potential parameters. Sotalol (30 μM) lengthened both APD_{50} and APD_{90}, while mexiletine (10 μM) significantly shortened repolarization measured at both APD_{50} and APD_{90}.

The frequency dependent effect of GYKI 16638, sotalol and mexiletine was investigated on the APD_{90} and V_{max} in the range of stimulation cycle length between 300 and 5000 ms.

Sotalol (30 μM) increased APD considerably more at long cycle lengths than at short ones, i.e. it induced strong reverse rate-dependent APD prolongation. In contrast, mexiletine (10 μM) shortened APD. This APD shortening was minimal and statistically not significant at high stimulation frequencies i.e. at short cycle lengths, while it proved to be significant at longer cycle lengths. The magnitude of the APD lengthening effect of GYKI 16638 (5 μM) was marked but less than that of sotalol. This APD prolongation, however, unlike that induced by sotalol, was apparently independent of the rate of stimulation.

The frequency dependent effect of GYKI 16638, sotalol and mexiletine on the V_{max} was also studied. Sotalol (30 μM), as expected from previous animal studies, did not change significantly V_{max} at any stimulation cycle lengths, while both 10 μM mexiletine and 5 μM GYKI 16638 exerted rate dependent V_{max} depression i.e. the higher stimulation rate was associated with more V_{max} block.

The characteristics of this frequency dependent V_{max} block was very similar with mexiletine and GYKI 16638 i.e. both drugs depressed V_{max} only at cycle lengths shorter than 1000 ms. This rate-dependent V_{max} block was the consequence of the slowing of the recovery of sodium channels from inactivation, which was studied by applying second stimuli with increasing coupling intervals during the diastole from a basic cycle length of 1000 ms. In control condition i.e. before drug administration and also after sotalol superfusion, V_{max} value returned, within 30-40 ms during early diastole, almost to its value characteristic at the basic cycle length. In marked contrast, after mexiletine and GYKI 16638 administration the recovery of V_{max} was considerably delayed and could be characterised with a recovery time constant of 310.4±71.5 ms (amplitude 96.9±12.7 V/s, n=6) and 298.6±70.2 ms (amplitude 74.9±13.7 V/s, n=8), respectively.

3.2. The slow component of the delayed rectifier potassium current

Results were obtained from 31 cells from 15 hearts (average 2.1 cells/heart, range 1-4). We specifically chose to use only cells with high-quality seals and low, stable series resistance (Rs), opting for a smaller number of excellent recordings from each preparation rather than more recordings of variable quality. In additional 27 cells we could either not observe I_{Ks} or the I_{Ks} amplitudes were so small that it was difficult to distinguish the current from the noise.

The applied pulse frequency was 0,1. Hz wich allowed the E-4031 insensitive tail current to be fully deactivated. The current was activated with a double exponential time course (γfast=22,4 ms, γslow=2388 ms) suggesting that it represented both I_{Kr} and I_{Ks} currents. Complete block of I_{Kr} by 5 μM E-4031 markedly reduced the amplitude of the tail currents and changed its activation to a single exponential (γ=1763 ms) with a slow time course.

Additional superfusion of the cell with 30 μM chromanol 293B, a blocker of I_{Ks}, entirely abolished this residual tail current suggesting that this current indeed represented I_{Ks}.

All of the other experiments on I_{Ks}, therefore, were perfomed in the presence of 1 or 5 μM E-4031 to completely block I_{Kr} , thereby facilitaing the separation of I_{Ks} from I_{Kr}.

The activation kinetics of the I_{Ks} tail current measured at -40 mV was apparently not voltage dependent in the test range of 10-60 mV. In the presence of the selective blocker of I_{Kr} (1 μM E-4031) and I_{Ks} (100 nM L-735,821), as expected, no tail current was recorded at -40 mV after a step potential to +30 mV for various durations. Cells were then exposed to 0,5 mM $BaCl_2$ that elicited a slowly developing tail current that resembled I_{Ks}, as it was also found in two other cells. However, since both I_{Ks} and I_{Kr} were completely blocked by 100 nM L-735,821 and 1 μM E-4031 this current could not be due to the activation of I_K.

3.3 The electrophysiological effects of endothelin-1

3.3.1 *Effect of ET-1 on calcium current*

Families of I_{Ca-L} were recorded before and 5 min after ET-1 treatment. Superfusion with 8 nmol/l ET-1 significantly decreased I_{Ca-L} at all membrane potentials studied.

At +5 mV, I_{Ca-L} was reduced by ET-1 from 10.2±0.6 to 6.8±0.8 pA/pF (66.7±8.9 % of control, $P<0,05$ n=5). This reduction of peak current was accompanied by a lengthening of inactivation. Inactivation of I_{Ca-L} was fitted as a sum of two exponentials, with estimated time constants of 12.8±1.1 and 97.4±11 ms at +5 mV in Tyrode solution. Both time constants were increased by ET-1 to 17.2±1.2 and 122±9 ms, respectively.

Lenghthening of the faster component was statistically significant ($P<0,05$ n=5), while that of the slower component was not. The voltage dependence of steady-state activation and inactivation was not altered by ET-1.

The effect of ET-1 on peak I_{Ca-L} developed rapidly (within 2 min) and was not reversible within a 7 min period of superfusion with ET-1-free Tyrode solution.

ET-1 did not affect the voltage dependence of activation of I_{Ca-L}: half-activation voltages and slope factors, obtained by fitting the activation curves to a two-state Boltzmann model, were 4.7±0.7 versus -4.9±0.7 mV, and 3.7±0.3 versus 4.1±0.8 mV in absence and presence of ET-1, respectively, (n=5, N.S.,).

Steady-state inactivation curves, obtained in the absence and presence of ET-1, were almost identical (midpoint potentials and slope factors were -19.3±1.2 versus -21.6±0.7 mV, and 3.45±0.27 versus 3.83±0.63 mV, respectively, n=5, N.S.).

3. 3. 2. *Effect of ET-1 on potassium currents*

I_{Kr} was activated using deporalizing voltage pulses of 1000 ms duration clamped from a holding potential of -40 mV to test potentials ranging between -20 and +50 mV. The decaying tail current recorded at -40 mV after the end of the test pulse was assessed as I_{Kr}. The current was fully abolished by 5 µmol/l E-4031, the selective blocker of I_{Kr}. ET-1 significantly decreased the amplitude of the tail current at each membrane potential studied.

Following depolarization to +40 mV , ET-1 decreased I_{Kr} tails from 0.31±0.02 to 0,06±0.02 pA/pF (20.3 % of control, $P<0.05$, n=4).

Transient outward K^+ current (I_{to})was measured at a rate of 0.2 Hz using deporalizing voltage pulses of 400 ms duration clamped from a holding potential of -80 mV to test potentials ranging between -10 and +60 mV.

ET-1 failed to change the peak amplitude of I_{to} (9.3±4.6 and 9.0±4.4 pA/pF at +50 mV before and after ET-1, respectively, n=4, N.S.). The steady-state current-voltage relationship of the membrane was studied between -130 and 0 mV at the end of the 400-ms deporalization. At these potentials the current is most likely I_{K1}. No change of this current (I_{K1}) was noted after superfusion with 8 mmol/l ET-1. Applying hyperpolarization to -100 mV, the I_{K1} current densities were 3.6±1.4 and 3.7±1.4 and 3.7±1.4 pA/pF in control conditions and in the presence of ET-1, respectively (n=4, N.S.)

3. 3. 3. *Effect of ET-1 on action potetial characteristics*

In multicellular left ventricular muscle preparations (n=3) paced at 1.2 Hz, ET-1 caused a moderate change in action potential morphology. Action potential duration, measured at 50 % of repolarization, was slightly increased (from 146±6 to 160±3 ms) by 8 nmol/l ET-1. This effect was reverted by isoproterenol. Application of isoprotenol (50 nmol/l) in the presence of ET-1 shortened APD_{50} from 160±3 to 144±11 ms, a value close to the control level .

The corresponding changes in APD_{90} were less pronounced (APD_{90} values of 213±7, 221±3 and 209±7 ms were obtained in control, after application of ET-1, and in the presence of ET-1 plus isoproterenol, respectively). ET-1 had no effect on the resting potential (-81.8±3.3 versus 107.8±4.6 mV) and maximum rate of deporalization (215±33 versus 204±21 V/s), measured before and after superfusion with ET-1, respectively.

4. DISCUSSION

The homograft bank of the Department of Cardiac Surgery uses mainly beating heart donors for prepaing aortic and pulmonary human valvular conduits which are used for aortic valve replacement with aortic homografts or pulmonary autografts (Ross procedure). Since the myocardial mass of the explanted heart is otherwise destroyed after valvular conduit preparation, the purpose of this work was to preserve the undiseased myocardium and use parts of it for electrophysiological and pharmacological studies. This experimental protocol complied with the declaration of the World Medical Association proclaimed in Helsinki and was approved by the Ethical Review Board of the University of Szeged. (No.: 51-57/1997 Oej)

To achieve this, the hearts were explanted according to the transplantation protocol (cold cardioplegic solution administrated into the aortic root after decompression of the heart and crossclamping of the aorta). The hearts were transported in cold (+4 °C) cardioplegic solution and both the isolated myocytes and the trabecular and papillary muscles were used for experiments within 5-12 hours after aortic crossclamping. With this method the mechanic and electrophysiologic properties of the myocardium were entirely preserved.

The purpose of the first series of experiments was to examine the cellular electrophysiological effects of GYKI 16638, in comparison with sotalol and mexiletine, in isolated undiseased human ventricular muscle. The most important result of this study is that GYKI 16638, unlike sotalol, lengthened repolarization in a frequency independent manner, while like mexiletine, it decreased V_{max} only at heart rates faster than the normal range.The two drugs, sotalol and mexiletine, which were used for comparison, were investigated previously on ventricular action potential in various mammalian heart preparations, including in some experiments on ventricular trabecular muscle obtained from explanted endstage heart failure human hearts , but the effects of these drugs on the action potential characteristics have never been established directly in undiseased human ventricular muscle. Sotalol, which has been classified as Class III antiarrhythmic drug, has been reported to lengthen APD in a reverse rate dependent manner without affecting V_{max}. In this study we confirmed these earlier observations made in animal preparations and in vivo clinical electrophysiological measurements . Mexiletine, a drug which has been classified as a Class IB antiarrithmic drug , according to earlier investigations performed also in animal preparations and in vivo clinical electrophysiological measurements, shortened or did not change APD significantly, but induced V_{max} block with fast recorvery kinetics.

The effect of GYKI 16638 on the action potential and transmembrane ionic currents was studied in rabbit cardiac preparations. These results showed that GYKI 16638 lengthened APD and blocked V_{max} in rabbit cardiac papillary muscle and depressed the rapidly activating delayed rectifier potassium (I_{Kr}) and inward rectifier potassium (I_{K1}) currents in rabbit ventricular myocytes. In addition, in dogs, rabbits and rats the compound exerted in vivo antiarrhytmic effect comparable to that sotalol.

The purpose of the chemical synthesis of the GYKI compounds was to develop an antiarrhythmic drug, which would be devoid of the proarrhythmic potential of the selective Class III and Class I drugs, and does not exhibit serious extracardiac side effects.To achieve this latter goal, the chemical structure of GYKI does not resemble that of amiodarone, but shows similarities with the structure of both sotalol and mexiletine. Combining Class IB and Class III antiarrhythmic effects was reported to attenuate reverse rate-dependent prolongation of APD and abolished EAD formation commonly seen after administration of pure Class III drugs (4). It was also reported that erythromycine induced torsade des pointes arrhythmia was succesfully abolished with mexiletine in a patient. Although some clinical reports suggested strong antiarrhythmic effect of the combination of Class IB and Class III drugs, no large scale clinical trial has been initiated to establish the real therapeutic value of such combination.

Amiodarone, however, is a drug, which has strong antiarrhythmic efficacy in patients, while showing promising results regarding mortality in multicenter clinical trials. In addition, amiodarone treatment is associated with low incidence of proarrhytmic complication. Amiodarone, besides other effects, possesses strong rate-independent Class III and Class IB properties. These favourable effects of amiodarone can be, at least partly, attributed to its peculiar cellular electrophysiologic properties: strong rate dependent V_{max} block with fast offset kinetics. Also, chronic amiodarone treatment lengthened repolarization rate-independently and decreased I_{Kr} density in rabbit ventricular muscle. It is important to note that both acute and chronic amiodarone administration abolished EAD formation in dog Purkinje fibers and M cells alike. This latter effect can be attributed to the depression of the slowly inactivating, or window sodium current, which would limit the undesirable consequence of I_{Kr} block, resulting in rate independent APD prolongation and minimal increase of dispersion of repolarization. Based on its cellular electrophysiological effects, a similar mechanism can be postulated with GYKI 16638.

The present study has, however, some possible limitations. We have applied only one drug concentration. Taking into consideration the diffusion barrier and the time course of the

experiments, the selected drug concentrations were somewhat higher, but still close to the therapeutic plasma concentrations measured in patients with sotalol and mexiletine and established with GYKI 16638 (1-2μM) in pharmacokinetic investigations. Also, we applied only right ventricular endocardial preparations, which may not well represent the whole heart. These limitations are explained by the difficulty in obtaining human ventricular preparations for experimental purpose.

The experiments made on isolated myocytes revealed the existence of I_{Ks} in undiseased human myocardium and like in the dog ventricle, it shows slow activation and relatively rapid deactivation kinetics. Earlier reports regarding existence of I_{Ks} in human ventricle were controversial. In some studies no evidence was found for I_{Ks} activity. In a previous study we could identity only I_{Kr} in human myocytes, but with changing of the pipette solution (more free K^+, presence of HPO_4^{2-} and ADP) in the presence of I_{Kr} blocking agents, we recorded a tail current identified as I_{Ks}. Even so the current was present only in 31 out of 58 cells, in 27 cells it was either absent or too small to identify. This could be explained by the origin of the cells from diffenrent layers of the ventricular wall, thereby reflecting regional heterogeneity of the myocytes rather than technical reasons. Earlier experiments performed in the presence of I_{Kr} block and Ba Cl_2 an Cd Cl_2 , revealed tail currents similar to I_{Ks}. In our experiments tail currents appeared also when both I_{Ks} and I_{Kr} were blocked, in the presence of Ba Cl_2. This can be explained by the interaction of Ba^{2+} with I_{K1} currents. I_{Ks} in cloned human KvLQT1+MinK channels expressed in COS cells revealed similar properties with I_{Ks} in undiseased human myocardium, only with slower deactivation. The characteristics of I_{Ks} measured in our study show similarities with those reported in dog and rabbit hearts.

The results obtained from the experiments carried out on isolated human ventricular cells in the presence of ET-1 showed that endothelin suppresses both $I_{Ca\text{-}L}$ and I_{Kr}, without affecting I_{to} and I_{K1}. The $I_{Ca\text{-}L}$ inhibition of ET-1 in human myocytes is similar to that obtained in studies on atrial and ventricular cells from rabbit and guinea pigs , and markedly differs from those obtained in rat atrial cells. The effects of ET-1 on potassium currents differ largely among species. ET-1 inhibitis I_K in rat myocytes, activates it in guinea pig cells and leaves it unaltered in canine heart cells. This latter results differ from those obtained in human ventricular cells, where I_{Kr} is markedly suppressed by ET-1. I_{to} and I_{K1} remains unaltered, as in canine ventricular cells (unpublished results). The effects of ET-1 on APD are also controversial and show great differences among species. While in rabbit and guinea pig atrial cells endothelin shortened APD, in human ventricular cells, 8 mmol/l, ET-1 moderately

lenghtened APD (similar results were obtained in canine and rat ventricular cells). The ET-1 induced lenghthening of APD is consistent with the inhibition of I_{Kr} and $I_{Ca\text{-}L}$.

6. CONCLUSIONS

6.1. The explantation of hearts from beating heart donors for aortic and pulmonary homografts preparation according to the transplantation protocol, offers a great opportunity for electrophysiological and pharmacological studies on undiseased human myocardium which is otherwise destroyed after valvular conduit isolation.

6.2. The study of the electrophysiological effects of sotalol, mexiletine and GYKI 16638 on human trabecular and papillary muscle preparations showed that GYKI 16638 has combined Class IB an III antiarrhythmic properties, similar to the electrophysiological effects seen after chronic amiodarone treatment. GYKI 16638 can therefore be a useful new antiarrhythmic drug candidate for further studies in animal experiments and also in patients.

6.3. The voltage clamp experiments on isolated human ventricular cells provided further evidence for the existence of I_{Ks} in human myocardium, with characteristics similar to I_{Ks} recorded in dog and rabbit myocytes.

6.4. ET-1 slightly prolongs action potential duration by reducing the activity of I_{Kr} and $I_{Ca\text{-}L}$, without affecting I_{to} and I_{K1}. These results help to elucidate the effects of ET-1 on human myocardium, which cannot be deduced from mammalian heart experiments, because of the marked interspecies differences.

1. LIST OF IN EXTENSO PUBLICATIONS RELATED TO THE THESIS

1. N. Iost, L. Virág, M. Opincariu, J. Szécsi, A. Varró, J. Gy. Papp: Delayed rectifier potassium current in undiseased human ventricular myocytes.
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2. ABSTRACTS

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