Analysis of the Ventricular Repolarization in Relation to the Development of Proarrhythmic Effects Induced by Non-Cardiac Drugs in Mammalian Hearts

PhD Thesis

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1. Introduction

Prolongation of the effective refractory period (ERP) by lengthening of the cardiac action potential duration (APD) is a common mechanism in the mode of action of certain dysrhythmic drugs which was termed by Vaughan Williams as Class III antiarrhythmic action. The delayed rectifier potassium current (I_K) is a major outward current responsible for ventricular muscle action potential repolarization. The I_K in most species including man consists of both a rapid (I_Kr) and slow (I_Ks) component. Specific I_Kr blockers greatly prolong APD and several are well recognized as useful in ablating cardiac arrhythmias. Although lengthening repolarization can terminate both ventricular tachycardia and atrial fibrillation, it can, in certain situations, also evoke torsade de pointes (TdP) ventricular arrhythmias, which may degenerate into ventricular fibrillation, causing sudden death. The proarrhythmic potential of Class III antiarrhythmic drugs greatly limits their usefulness in therapy.

The APD increase induced by selective I_Kr blockade displays reverse use-dependency. The reverse use dependent prolongation of cardiac repolarization means greater increases in APD at long diastolic intervals than at short ones. Thus, when the time between successive action potentials is long, I_Kr block produces a far greater increase in APD. Long APDs due to block of I_Kr at long diastolic intervals and slow heart rates are associated with induction of early after depolarizations (EAD) believed to trigger TdP ventricular arrhythmias.

Previously, selective I_Ks block has generally been assumed to increase APD and refractoriness in a frequency-independent manner. Because of this, the search for selective I_Ks blockers has intensified as they may represent novel antiarrhythmic agents devoid of the risk of TdP arrhythmia induction. Some compounds have recently been reported to selectively block I_Ks. Available published results of describing chromanol 293B and L-735821 effects, in fact, have often contradicted each other. These contradictory findings have caused us to question whether these differences may be due to species variations in I_Ks. The effects of these compounds on QT_c and cardiac action potential configuration have not been characterized in the rabbit, which represents a species widely used to determine the effects of new antiarrhythmic agents intended for use in man. Therefore, one of the major objectives of this study was to characterize the effects of chromanol 293B and L-735,821 on whole heart QTc, papillary muscle APD, and isolated myocyte membrane current in rabbit, a species widely used for antiarrhythmic drug testing. We compared the effects of the two I_Ks blockers to those of E-4031, a recognized I_Kr blocker.

Under normal conditions block of one type of outward potassium channel is not likely to cause excessive and potentially dangerous APD lengthening, since the other types of potassium channels provide sufficient repolarization strength which was termed as "repolarization reserve". The different potassium channels may compensate each other to secure the repolarization process. If repolarization is excessively lengthened due to drug induced I_Kr block, hypokalaemia, genetic abnormality, or bradycardia, the subsequent increase in APD would favour I_Ks activation and provide a negative feedback mechanism to limit further APD lengthening. Without such a mechanism because of inheritance (e.g. LQT syndromes) or ion channel remodellings in different pathophysiological conditions (heart failure, acut myocardial infarction, diabetes mellitus, etc.), excessive APD lengthening might lead to enhanced repolarization prolongation and increase propensity for development of EAD associated with TdP induction. Accordingly, in situations where the "repolarization reserve" is impaired, even relatively weak inhibition of another potassium channel may lead to excessive APD prolongation which can result in increased risk of proarrhythmia.

In the past few years it became evident that several non-cardiac drugs can also moderately prolong repolarization by inhibition of one or more potassium currents. These noncardiac drugs such as psychotropic medication, antihistamines and antibiotics can induce TdP. The macrolide antibiotic erythromycin is a commonly prescribed antibiotic and is known to be associated with QT prolongation and TdP. In the present work a clinical case is reported in which erythromycin induced TdP ventricular tachycardia in a patient with hypokalemia. In our case, the life-threatening ventricular proarrhythmia disappeared in response to mexiletine.
Eliprodil, a newly developed NMDA (\(n\)-methyl-\(d\)-aspartate) receptor antagonist neuroprotective agent, has been at times observed to prolong the QT interval in patients, which may involve the risk of development of proarrhythmic complications. Therefore, the next purpose of the present study was to analyse the effect of eliprodil, a noncardiac drug, on the cardiac repolarization under \textit{in vitro} circumstances, under normal conditions and after the attenuation of the "repolarization reserve" by blocking the \(I_{K1}\) current with \(\text{BaCl}_2\).

\textbf{1.1. Major experimental goals}

(1) To carry out experimental studies to establish whether \textit{in vitro} conditions mexiletine is able to decrease the erythromycin-induced cardiac electrophysiological changes leading to a tendency to torsade de pointes ventricular tachycardia.

(2) To investigate and to compare the electrophysiological properties of the rapid and slow components of the delayed rectifier potassium current (\(I_{Kr}\) and \(I_{Kr}\)) in Langendorff-perfused hearts, in multicellular right ventricular papillary muscle preparations and in ventricular myocytes isolated from rabbit hearts.

(3) To evaluate the role of \(I_{Ks}\) current when cardiac repolarization is abnormally lengthened by pharmacological means.

(4) To analyse the effect of eliprodil, a noncardiac drug, on the cardiac repolarization under \textit{in vitro} circumstances, under normal conditions and after the attenuation of the "repolarization reserve".

\textbf{2. Case report: erythromycin induced torsade de pointes ventricular tachycardia}

A 59-year-old woman was referred to our clinic because of palpitation, ventricular extrasystoles and a syncopal attack. She was regularly taking clopamid (10 mg/day) and potassium chloride (1000 mg/day) because of pedal edema. In addition to these drugs, she had taken erythromycin (500 mg three times a day) and acetylcysteine (600 mg/day) for respiratory infection three days before the syncopal attack. There was no family history of long QT syndrome, sudden death, frequent syncope or seizure. Her physical examination was unremarkable other than a moderate bilateral lower extremity edema, minimal crackles at lung bases, quiet systolic murmur at the heart apex and extrasystoles. The blood pressure was in the normal range. Chest x-ray showed a moderate pulmonary congestion. Transthoracic echocardiography demonstrated an enlarged left atrium (50 mm), normal ventricular diameters and normal wall motion. The left ventricular ejection fraction was 54%.

The admission 12-lead electrocardiogram (ECG) showed a sinus rhythm at mean heart rate of 85 beats/min, prolonged ventricular repolarization (\(QT = 560 \text{ ms}, QT_c = 663\text{ ms}\), corrected by Bazett formula), particular bigeminy and \textit{torsade de pointes} ventricular tachycardias preceded by "short-long-short" RR interval sequence. The proarrhythmic ventricular tachycardia causing syncopal attacks was abolished by the discontinuation of erythromycin treatment, parenteral potassium chloride (3000 mg) and magnesium sulphate (1000 mg) supplementation and oral mexiletine therapy (200 mg three times a day). Since then she has been free of ventricular tachycardia or ventricular ectopics. Holter monitoring for 24 hours before discharge revealed no premature beats or tachyarrhythmias. The QT interval at that time was normal (360 ms).
3. Methods of the experiments

3.1. Preparation of the rabbit and canine hearts

New Zealand rabbits were sacrificed by cervical dislocation after an intravenous injection of heparin. The chest was opened, the heart quickly removed and immediately immersed in oxygenated modified Locke's solution. Adult mongrel dogs were used in canine experiments. Following sodium pentobarbital induced anaesthesia each heart was rapidly removed through a right thoracotomy and immediately rinsed in oxygenated modified Locke's solution.

3.2. ECG measurements in Langendorff-perfused rabbit hearts

The rabbit heart was mounted on a Langendorff column and perfused with oxygenated modified Locke’s solution. After appropriate preparation, the heart was immersed in a tissue chamber filled with perfusion solution. Volume-conducted electrocardiograms (ECGs) were obtained as previously described by Zabel et al. ECG leads were acquired by an ECG signal processing system and data were analyzed off-line. After an equilibration period, baseline ECGs were obtained and a 40 min perfusion period was initiated with either investigated drug. ECG recordings were monitored continuously and compared to baseline measurements at the end of this period. QT intervals were always measured on lead II.

3.3. Conventional microelectrode measurements

Canine Purkinje strands obtained from both ventricle and rabbit or canine isolated right ventricular papillary muscle preparations were mounted individually in a tissue chamber continuously superfused with modified Locke's solution while stimulated at 1000 ms cycle length using rectangular constant current pulses 2 ms in duration. Transmembrane potentials were recorded using conventional microelectrodes connected to the input of a high impedance electrometer and continuously monitored on a dual beam storage oscilloscope. The maximum diastolic potential, action potential amplitude and action potential duration (APD) were automatically measured. In each experiment, baseline action potential characteristics were first determined during continuous pacing at 1 Hz, and then while pacing cycle length was sequentially varied between 300 and 5000 ms. Twenty-five action potential were evoked at each cycle length and the cycle length was then changed so that "quasi" steady-state frequency response relations could be rapidly generated. Each preparation was then superfused for 40 to 60 min with either investigated drug before repeating the pacing protocol.

3.4. Patch-clamp measurements

Single ventricular myocytes were obtained by enzymatic dissociation of isolated rabbit and canine hearts. After the isolation procedure, one drop of cell suspension was placed in a transparent recording chamber mounted on the stage of an inverted microscope. Myocytes were used that were rod shaped with clear striations. HEPES buffered Tyrode solution served as the normal superfusate in all experiments. Patch-clamp micropipettes were fabricated from borosilicate glass capillaries using a micropipette puller. These electrodes had resistances between 1.5 and 2.5 MΩ when filled with pipette solution. The pH of this solution was adjusted to 7.2 by addition of KOH. Nisoldipine (1 µM) in the external solution eliminated inward Ca\(^{2+}\) current (I\(_{Ca}\)) while the sodium current (I\(_{Na}\)) was inactivated during experiments by applying a holding potential of -40 mV. At this holding potential, transient outward current (I\(_{to}\)) was also largely inactivated. An amplifier was used to record membrane current in the whole-cell configuration of the patch-clamp technique. Membrane currents were digitized using an analog-to-digital converter under software control. Analyses were performed using pClamp 6.0 software. All patch-clamp data were collected at 37 °C.
4. Results

4.1. Effect of erythromycin on the action potential in isolated canine Purkinje-fiber.

The isolated, free-running Purkinje fibers (n = 9) were individually mounted in a tissue chamber containing saline. After equilibration period, erythromycin was applied in a concentration of 200 mg/l, while the electrical stimulation cycle length was progressively increased from 1000 ms to 5000 ms. In three experiments, the EAD was facilitated by addition of the K⁺-channel blocker cesium chloride (CsCl; 2 mM). Mexiletin was added to the organ bath in a concentration of 10 µM. Under control conditions, when cycle length was changed from 1000 ms to 5000 ms, although the APD increased considerably, EADs were never observed. CsCl (2 mM, n=3) lengthened APD further without eliciting EADs. Application of 200 mg/l erythromycin for 25-30 minutes at 1 Hz basic stimulation frequency did not significantly influence the maximal diastolic potential, the action potential amplitude and slightly decreased the maximal rate of depolarization (V_{max}). The APD, however, was markedly increased from 289.4 ± 20.6 ms and 387.9 ms to 360.1 ± 28.0 ms and 567.4 ± 47.7 ms, respectively (p < 0.005). Erythromycin evoked EADs after 30 minutes in all experiments when cycle length was gradually increased from 1000 ms. The average cycle length at which erythromycin-induced EADs developed was 2344 ± 310 ms (n = 9). In the continuous presence of 200 mg/l erythromycin, the addition of 10 µM/l mexiletine to the tissue bath, markedly shortened APD and, in most Purkinje fiber preparations (7/9), the abolition of EADs was observed.

4.2. Comparison of the effects of I_{Ks} and I_{Kr} block in rabbit hearts

4.2.1. The choice of drug concentrations

The concentrations of L-735,821 (100 nM) and chromanol 293B (10 µM) were comparable to those used by others and shown to block I_{Ks} in other species. We used 100 nM L-735,821 to assure completely block I_{Ks} during assessment of I_{Kr}, and 1-5 µM E-4031 to fully block I_{Kr} during assessment of I_{Ks}. E-4031 concentrations (100 nM) were also similar to those used previously by us and others.

4.2.2. Comparison of the effects of I_{Ks} and I_{Kr} block on QTc interval in isolated Langendorff-perfused rabbit hearts

Neither I_{Ks} blocker, chromanol 293B (10 µM) nor L-735,821 (100 nM) significantly lengthened QTc or increased RR interval in isolated, Langendorff-perfused rabbit hearts after 40 min of exposure. The I_{Kr} blocker E-4031 (100 nM), significantly increased QTc under identical conditions. This E-4031 induced increase in QTc was associated with a significant increase in RR interval (420.5 ± 17.5 ms at baseline vs. 463.5 ± 17.8 ms after E-4031, n = 8, p < 0.05).

4.2.3. Effects of I_{Ks} and I_{Kr} block on ventricular action potential duration in isolated rabbit papillary muscle

Concentrations of chromanol 293B (10 µM) and L-735,821 (100 nM) reported to block I_{Ks} in other species failed to significantly affect rabbit papillary muscle APD while pacing at a constant pacing cycle length of 1000 ms. On the other hand, E-4031 (100 nM) markedly and significantly increased rabbit papillary muscle APD under identical conditions. A similar difference in the effects of chromanol 293B (10 µM) and L-735,821 (100 nM) compared to E-4031 (100 nM) on APD was observed in rabbit ventricular muscle over a wide range of pacing cycle lengths. Chromanol 293B and L-735,821 produced only small changes in APD over this entire range of pacing rates while E-4031 markedly lengthened rabbit papillary muscle APD in a reverse frequency-dependent fashion so that the increase in APD was greater at long cycle lengths than at short ones.
4.2.4. Effect of $I_{Ks}$ block in the presence of forskolin

Because $I_{Ks}$ is modulated by changes in intracellular cAMP, we also examined the effect of $I_{Ks}$ block on APD in the presence of 1 µM forskolin to activate adenylcyclase and thereby increase intracellular cAMP. Forskolin (1 µM) alone shortened APD in rabbit papillary muscle paced at cycle length of 1000 ms from $217.4 \pm 18.7$ ms to $194.0 \pm 15.7$ ms ($n=5$, $p<0.05$). Addition of 10 µM chromanol 293B in the continuous presence of forskolin had little effect on APD ($194.0 \pm 15.7$ ms versus $190.8 \pm 12.9$ ms, $n=5$). Similar result was obtained with L-735,821 (100 nM). These results show that selective $I_{Ks}$ block does not alter APD substantially even in the presence of elevated intracellular cAMP.

4.2.5. Effect of L-735,821 on $I_{Ks}$ compared to that of E-4031 on $I_{Kr}$ in isolated rabbit ventricular myocytes

L-735,821 (100 nM) completely abolished $I_{Ks}$. In comparison, a greater concentration of E-4031 (1 µM) than used in examining its effects on rabbit QTc and APD was required to fully block $I_{Kr}$ tail currents.

4.2.6. $I_{Ks}$ and $I_{Kr}$ activation and deactivation kinetics in rabbit ventricular myocytes

$I_{Ks}$ kinetics were assessed in rabbit ventricular myocytes using an envelope of tails protocol in the presence of 5 µM E-4031 to eliminate $I_{Kr}$. Under these conditions, $I_{Ks}$ activation was slow ($\tau = 888.1 \pm 48.2$ ms, $n = 21$, at +30 mV) and $I_{Ks}$ deactivation was fast ($\tau = 157.1 \pm 4.7$ ms, $n = 22$, at –40 mV).

In the presence of 100 nM L-735,821 to block $I_{Ks}$, the activation time constant ($\tau$) for $I_{Kr}$ was $35.5 \pm 3.1$ ms ($n = 26$) and deactivation was slow and best fit as the sum of two exponentials; $\tau_1 = 641.5 \pm 25.0$ ms and $\tau_2 = 6531 \pm 343$ ms with amplitudes of $A_1 = 32.8 \pm 1.7$ pA and $A_2 = 42.4 \pm 2.1$ pA, respectively ($n = 35$).

The E-4031 sensitive current ($I_{Kr}$) amplitude at the end of the 150 ms long test pulse was $34.1 \pm 4.2$ pA ($n = 14$), or about 30% of the tail current amplitude measured after the voltage test pulse returned to -40 mV ($85.8 \pm 9.2$ pA, $n = 14$). The L-735,821 sensitive current ($I_{Ks}$) during the test pulse to +30 mV was larger than its tail current on return to -40 mV. The magnitude of $I_{Ks}$ during the test pulse was $13.27 \pm 1.2$ pA at +30 mV vs. $6.6 \pm 0.8$ pA at -40 mV, ($n = 15$), approximately an order of magnitude less than the $I_{Kr}$ tail current.

4.3. Effect of $I_{Ks}$ block in canine hearts

4.3.1. The effects of L-735,821 and chromanol 293B on action potential repolarization in dog ventricular muscle

Chromanol 293B and L-735,821 produced small changes in APD amounting to less than a 7% increase over baseline measurements, and these unremarkable effects of $I_{Ks}$ demonstrated little frequency dependence in right ventricular papillary muscles. Selective $I_{Ks}$ block in dog has little effect on normal cardiac APD in ventricular muscle fibres.

4.3.2. The effects of L-735,821 and chromanol 293B on pharmacologically lengthened action potentials

The effects of both L-735,821 and chromanol 293B were tested in dog ventricular papillary muscle action potentials, lengthened pharmacologically by exposure to 1 µM E-4031 (to block $I_{Kr}$) and 1 µg/ml veratrine (a recognized sodium channel agonist). L-735,821 markedly lengthened APD under these conditions from $383.5 \pm 25.2$ to $442.1 \pm 32.3$ ms ($p < 0.01$, $n = 7$). This effect was in
sharp contrast to the negligible effect of L-735,821 on normal APD. Comparable effects on APD were obtained with chromanol 293B in the continuous presence of E-4031 and veratrine (APD was 366.1 ± 13.1 ms before chromanol 293B versus 429.5 ± 23.5 ms after its addition, p < 0.01, n = 8).

4.4. Effect of eliprodil on ventricular repolarization

4.4.1. Effect of eliprodil on ventricular action potential duration in isolated canine ventricular papillary muscle under normal condition

Eliprodil (1 µM) lengthened APD moderately (<10%) from 235.3±5.9 ms to 257.3±9.0 ms (1Hz, n = 9, p < 0.05) without causing significant change in the resting membrane potential, the action potential amplitude, and the V<sub>max</sub>. Under normal condition, the drug produced a moderate reverse rate-dependent APD prolongation (7.4 ± 1.5%, 8.9 ± 2.1% and 9.9 ± 1.8% at cycle lengths of 300, 1000 and 5000 ms, respectively; n = 9).

4.4.2. Effect of eliprodil on ventricular action potential duration in isolated canine ventricular papillary muscle after IK<sub>1</sub> inhibition

Partial block of IK<sub>1</sub> by 10 µM BaCl<sub>2</sub> lengthened APD in a reverse frequency dependent manner (7.0 ± 1.3%, 14.2 ± 1.6% and 28.1 ± 2.1% at cycle lengths of 300, 1000 and 5000 ms, respectively; n = 8). In the presence of BaCl<sub>2</sub>, 1 µM eliprodil induced a marked further lengthening relative to the APD values measured after the administration of BaCl<sub>2</sub> (12.5 ± 1.0%, 17.6 ± 1.5% and 20.5 ± 0.9% at cycle lengths of 300, 1000 and 5000 ms, respectively; n = 8), ie. the APD lengthening effect of eliprodil was significantly augmented in preparations where the "repolarization reserve" was attenuated by previous application and presence of BaCl<sub>2</sub>.

4.4.3. Effect of eliprodil on QT<sub>c</sub> interval in isolated Langendorff-perfused rabbit hearts in the absence and presence of IK<sub>1</sub> block

In the normal Langendorff-perfused rabbit heart, eliprodil (1 µM) produced a significant QT<sub>c</sub> prolongation (12.7 ± 1.8%, n = 9). After the attenuation of the "repolarization reserve" by the IK<sub>1</sub> blocker BaCl<sub>2</sub> (10 µM), this eliprodil evoked QT<sub>c</sub> prolongation was greatly enhanced (28.5 ± 7.9%, n = 6). In 2 out of 6 Langendorff preparations the QT<sub>c</sub> lengthening degenerated into torsade de pointes (TdP) ventricular tachycardia.

4.4.4. The effect of eliprodil on the transmembrane potassium currents in canine ventricular myocytes

Eliprodil (1 µM) does not considerably influence IK<sub>K1</sub> or I<sub>to</sub> in canine ventricular myocytes (IK<sub>K1</sub> current values at -60 mV: 365.7±29.2 pA as control and 321.5±30.5 pA in the presence of 1 µM eliprodil, n=5; I<sub>to</sub> current values at 50 mV: 6017.2±963.0 pA as control and 5617.5±1025.0 pA in the presence of 1 µM eliprodil, n=5). The drug does not significantly affect IK<sub>s</sub> in canine ventricular myocytes (IK<sub>s</sub> tail current was 261.6±53.0 pA under control conditions and 202.4±40.0 pA after application of 1 µM eliprodil, at 50 mV of potential of activation, n=6). 1 µM eliprodil, however, abolished IK<sub>K1</sub> tail current completely.
5. Discussion

5.1. Suppression of erythromycin-induced early afterdepolarizations and torsade de pointes ventricular tachycardia by mexiletine

A recent electrophysiological analysis of erythromycin at a cellular and ion channel level revealed that, in clinically relevant concentrations (10-200 mg/l), the macrolide antibiotic selectively blocks the I_Kr. Following single iv. injection of 1 g erythromycin, an average serum level of 30 mg/l may be measured. This exceeds the threshold concentration of 10-20 mg/l which induces a significant APD prolongation in the Purkinje and M cells. In the presence of predisposing pathogenic factors (bradycardia, hypokalemia, congenital LQTS, etc.), even smaller oral doses of erythromycin may induce proarrhythmia. A relatively frequent side effect of erythromycin treatment is diarrhoea, due to the prokinetic effect of the drug. This can give rise to clinically considerable myocardial potassium loss. In our case report, however, the hypokalemia was induced by concomitant diuretic therapy.

Our experiments demonstrated that mexiletin, an inhibitor of slowly inactivating window sodium current, may prevent I_Kr-blocking drug induced TdP ventricular tachycardia by abolishing APD prolongation and EADs. *In vitro*, mexiletin is capable of limiting the APD prolongation and EAD-inducing effects not only of erythromycin, but also of other I_Kr-blocking drugs. In accordance with this, mexiletin is also suitable for *in vivo* elimination of the TdP induced by antiarrhythmic agents with Class IA and III actions. Shortening of the plateau phase of the action potentials is accompanied by decreases in the transsarcolemmal calcium inflow and in the intracellular calcium loading of the heart cells. This is clearly a beneficial effect, if it is taken into consideration that cytosolic calcium overload is a fundamental pathogenetic factor of EAD formation.

5.2. Comparison of the electrophysiological properties of the I_Kr and I_Ks in isolated rabbit heart preparations

Because the surface electrocardiogram remains the best clinical means of assessing antiarrhythmic drug therapy and monitoring development of proarrhythmic side effects, we determined the effects of two potentially beneficial antiarrhythmic agents, chromanol 293B and L-735,821, on QTc in isolated, Langendorff-perfused rabbit hearts. This experimental preparation allowed us to also determine the effect of the exactly the same drug concentrations on rabbit ventricular papillary muscle action potential configuration as well as the underlying membrane currents in isolated rabbit ventricular myocytes. We compared the effects of these two reportedly selective I_Ks blockers to those of a recognized selective I_Kr blocker, E-4031. This comparison allowed direct assessment of the degree of QTc and action potential lengthening produced by complete, selective block of I_Ks versus I_Kr block in rabbit ventricular tissue. We found that chromanol 293B and L-735,821 did not substantially increase QTc in Langendorff-perfused rabbit hearts, nor did they increase isolated rabbit ventricular muscle APD. L-735,821, however, did completely block I_Ks in isolated rabbit ventricular myocytes. In contrast, a concentration of E-4031 an order of magnitude less than that which totally blocked I_Kr, markedly increased QTc and rabbit ventricular muscle APD. Thus, if the basis of the ventricular antiarrhythmic effectiveness of I_Kr block by agents like E-4031 is cardiac APD prolongation reflected as an increase in QTc, selective I_Ks block is unlikely to prove to be of antiarrhythmic benefit.

Based on the earlier results in guinea pig ventricular myocytes, where I_Ks activates and deactivates slowly, selective I_Ks block was expected to increase APD without inducing reverse use-dependent lengthening as associated with I_Kr block. In rabbit, I_Ks is usually recorded as a large membrane current relative to other species. We found in patch clamp experiments that in rabbit ventricular myocytes I_Ks activated slowly and deactivated rapidly in relation to the time of normal electrical diastole. We also found that I_Kr activated rapidly as expected, but deactivated slowly. Former report that I_Ks deactivates rapidly in human myocytes while I_Kr in human myocytes
deactivates slowly strongly suggests that rabbit would serve as better preclinical models for examining the effects of new antiarrhythmic agents than guinea pig.

5.3. Evaluation of the role of I_{KS} current when cardiac repolarization is abnormally lengthened by pharmacological means

Our results indicate that both chromanol 293B and L-735,821, purportedly selective I_{KS} blockers did not substantially lengthen APD in dog right ventricular papillary muscle preparations. These drugs produced small changes in APD amounting to less than a 7% increase over baseline measurements, and these unremarkable effects of I_{KS} demonstrated little frequency dependence in right ventricular papillary muscles. Selective I_{KS} block in dog has little effect on normal cardiac APD in ventricular muscle fibres. This observation is in good agreement with our above-mentioned findings in rabbit ventricle.

However, in papillary muscle preparations where APD was extremely prolonged by the I_{Kr} blocker E-4031 and the I_{Na} activating veratrine, both chromanol 293B and L-735,821 increased repolarization considerably. Our finding suggests that I_{KS}, unlike I_{Kr}, plays little role during normal action potential repolarization. Such a conclusion is well supported by the negligible effect of I_{KS} block on isolated ventricular muscle APD. If repolarization is excessively lengthened due to drug induced I_{Kr} block, hypokalaemia, genetic abnormality, or bradycardia, the subsequent increase in APD would favour I_{KS} activation and provide a negative feedback mechanism to limit further APD lengthening. Without such a mechanism, excessive APD lengthening might lead to enhanced regional repolarization dispersion and increase propensity for development of EAD associated with TdP induction.

5.4. Analysis of the effect of eliprodil, a non-cardiac drug, on the cardiac repolarization under normal conditions and after the attenuation of the "repolarization reserve".

It has become apparent that not only antiarrhythmic drugs but a variety of non-antiarrhythmic agents may provoke TdP tachycardia. The number of non-cardiac drugs reported to induce QT interval prolongation with or without TdP continues to increase. A number of clinically available or still investigational non-cardiovascular agents have been implicated. Therefore, there is a great importance of the preclinical detection of torsadogenic propensity of the newly developed agents to decrease the proarrrhythmic risk. The most important finding of this part of the present work was that eliprodil which blocks I_{Kr} current without considerably interfering with I_{K1}, I_{KS} and I_{To}, caused moderate APD and QTc lengthening when it was applied alone, but when the "repolarization reserve" was attenuated by BaCl_2, it evoked augmented prolongation of repolarization, occasionally resulting in torsade de pointes ventricular tachycardia.

The present experiments may have important therapeutical and practical implications. Some non-cardiac drugs exhibit weak inhibition of one or more potassium, most frequently the I_{Kr} (HERG/MiRP) channel. Since this effect does not markedly influence repolarization in normal situation, their effect on QT is often unmasked. Therefore the potential proarrhythmic danger can be easily underestimated in individuals who have decreased "repolarization reserve" in spite of their baseline QTc falls within the normal range. Accordingly, eliprodil or any drug which is known to inhibit potassium current and exert only moderate or not even consistent repolarization lengthening, should be administered under repeated or continuous ECG control, and if QTc prolongation longer than expected is noticed, the therapy with such a drug should be discontinued. Also, the concept of attenuated "repolarization reserve" should be considered during safety pharmacology studies, since the rabbit and guinea pig possessing fast heart rate or even the dog, all of which probably have relatively strong repolarization reserve, can not be expected to respond with significant QT lengthening when drugs partially block only one type of cardiac potassium channels. Instead of studying drug effects on the cardiac repolarization and proarrrhythmic risk in the normal heart, it would certainly be more useful to develop and apply screening tests where repolarization reserve is attenuated.
6. Summary: conclusions and potential significance

1. Erythromycin is a selective IKr-blocking, APD-prolonging antibiotic drug, which may induce in patients QT interval prolongation and in particular cases TdP ventricular tachycardia. Under in vitro circumstances, at therapeutic concentration (200 mg/l), erythromycin was able to lengthen APD and induce EADs in isolated Purkinje fibers. After the addition of mexiletin (10 µM), a marked shortening of APD and the disappearance of EADs (7/9) were observed. Our experiments demonstrated that mexiletin, an inhibitor of slowly inactivating sodium current, may prevent IKr-blocking drug induced TdP ventricular tachycardia by abolishing APD prolongation and EADs.

2. In rabbit ventricular myocytes, chromanol 293B (10 µM) and L-735,821 (100 nM) markedly or totally blocked IKs, and E-4031 (1 µM) completely inhibited IKr. The same concentration of chromanol 293B and L-735,821 had no significant effect on QTc interval in Langendorff-perfused rabbit hearts, whilst E-4031, even at lower concentration (100 nM), significantly increased QTc interval (~36%). Similarly both chromanol 293B (10 µM) and L-735,821 (100 nM) produced little increase in papillary muscle APD (less than 7 %) while pacing at cycle lengths between 300 and 5000 ms. In contrast, E-4031 (100 nM) markedly increased APD (30-60 %) in a reverse frequency-dependent manner. In rabbit ventricular myocytes, IKs tail currents activated slowly and deactivated rapidly, while IKr tail currents activated rapidly and deactivated slowly. IKr was estimated to contribute substantially more to total current density during normal ventricular muscle action potentials than does IKs. The kinetics of IKs and IKr, activation and deactivation in rabbit ventricular myocytes are similar to those reported in dog and man. These new findings suggest that rabbit is a good species for preclinical evaluation of new drugs believed to affect cardiac action potential repolarization. In addition, these results indicate that block of IKs is not likely to provide antiarrhythmic benefit by lengthening normal ventricular muscle QTc, APD, and refractoriness over a wide range of frequencies.

3. Similarly to the outcome of the rabbit experiments, our results indicate that neither chromanol 293B (10 µM) nor L-735,821 (100 nM) did substantially increase APD in dog papillary muscle. However, these compounds lengthened repolarization markedly, when APD was pharmacologically prolonged by E-4031 (1 µM) and veratrine (1µg/ml). We conclude that IKs plays little role in normal dog ventricular muscle action potential repolarization. In pathological situation, when APD is abnormally increased, the role of IKs in final repolarization increases to provide an important safety mechanism that reduces arrhythmia risk.

4. Eliprodil (1 µM), a non-cardiac drug with neuroprotective properties, significantly decreased the amplitude of IKr, but IKs, Ito and IK1 were not considerably affected by the drug when measured in dog ventricular myocytes by applying the patch clamp technique. In canine right ventricular papillary muscle by applying the conventional microelectrode technique, under normal conditions, eliprodil produced a moderate reverse rate-dependent prolongation of the action potential duration. This effect was augmented in preparations where IK1 was previously blocked by BaCl2 (10 µM). In the normal Langendorff-perfused rabbit heart, eliprodil produced a significant QTc prolongation (~13%). After the attenuation of the "repolarization reserve" by the IK1 blocker BaCl2, the eliprodil evoked QTc prolongation was greatly enhanced (~29%) In 2 out of 6 Langendorff preparations this QTc lengthening degenerated into TdP ventricular tachycardia. The results indicate that eliprodil, under normal conditions, only moderately lengthens cardiac repolarization by inhibition of IKr. However, after the attenuation of the normal "repolarization reserve", this drug can induce marked QT interval prolongation, which may result in proarrhythmic action.
The thesis is based on the following publications

Full length papers


IV. Lengyel, Cs., Iost, N., Virág, L., Varró, A., Lathorp D. A., Papp, J., Gy.: A késői egyenirányító kálium áram lassú komponensének ($I_{KS}$) blokkolása, a gyors komponens ($I_{Kr}$) gátlásával ellentétben, nem nyújtja meg a kamrai repolarizációt nyúlszív préparátumokon [The block of the slow component of the delayed rectifier potassium current ($I_{KS}$), unlike the rapid component ($I_{Ks}$), fails to lengthen the ventricular repolarization]. Card. Hung., 2001, 30, 193-202.


Published abstracts


VIII. Iost, N., Lengyel, Cs., Virág, L., Varró, A., Papp, J. Gy.: Does $I_{KS}$ play an important role in rabbit cardiac repolarization? PACE, 2000, 23, 661.


List of further publications


List of further published abstracts


100. Várkonyi, T. T., Takács, R., Róka, R., Légrády, P., Lázár, M., Madácsy, L., Lengyel, Cs.,


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