

©2004, Acta Pharmacologica Sinica  
Chinese Pharmacological Society  
Shanghai Institute of Materia Medica  
Chinese Academy of Sciences  
<http://www.ChinaPhar.com>

## Two components of delayed rectifier K<sup>+</sup> current in heart: molecular basis, functional diversity, and contribution to repolarization<sup>1</sup>

Jian-hua CHENG<sup>2</sup>, Itsuo KODAMA<sup>3</sup>

*Department of Pharmacology, Faculty of Basic Medical Sciences, School of Medicine, Tongji University, Shanghai 200331, China;*

*<sup>3</sup>Department of Circulation, Research Institute of Environmental Medicine, Nagoya University, Nagoya 464-8601, Japan*

**KEY WORDS** potassium channels; long QT syndrome; beta-adrenergic receptors; action potentials; anti-arrhythmia agents

### ABSTRACT

Delayed rectifier K<sup>+</sup> current ( $I_K$ ) is the major outward current responsible for ventricular repolarization. Two components of  $I_K$  ( $I_{Kr}$  and  $I_{Ks}$ ) have been identified in many mammalian species including humans.  $I_{Kr}$  plays a pivotal role in normal ventricular repolarization. A prolongation of action potential duration (APD) under a variety of conditions would favor the activation of  $I_{Ks}$  so that to prevent excessive repolarization delay causing early afterdepolarization. The pore-forming  $\alpha$  subunits of  $I_{Kr}$  and  $I_{Ks}$  are composed of HERG (KCNH2) and KvLQT1 (KCNQ1), respectively. KvLQT1 is associated with a function-altering  $\beta$  subunit, minK to form  $I_{Ks}$ . HERG may be associated with minK (KCNE1) and/or minK-related protein (MiRP1) to form  $I_{Kr}$ , but the issue remains to be established.  $I_{Ks}$  is enhanced, whereas  $I_{Kr}$  is usually attenuated by  $\beta$ -adrenergic stimulation via cyclic adenosine 3',5'-monophosphate (cAMP)/protein kinase A-dependent pathways. There exist regional differences in the density of  $I_{Kr}$  and  $I_{Ks}$  transmurally (endo-epicardial) and along the apico-basal axis, contributing to the spatial heterogeneity of ventricular repolarization. A decrease of  $I_{Kr}$  or  $I_{Ks}$  by mutations in either HERG, KvLQT1, or KCNE family results in inherited long QT syndrome (LQTS) with high risk for Torsades de pointes (TdP)-type polymorphic ventricular tachycardia and ventricular fibrillation. As to the pharmacological treatment and prevention of ventricular tachyarrhythmias, selectively block of  $I_{Ks}$  is expected to be more beneficial than selectively block of  $I_{Kr}$  in terms of homogeneous prolongation of refractoriness at high heart rates especially in diseased hearts including myocardial ischemia.

### INTRODUCTION

Delayed rectifier K<sup>+</sup> current ( $I_K$ ) is the major outward current involved in ventricular repolarization.  $I_K$

in the heart has two major components with different biophysical properties and different drug sensitivities<sup>[1]</sup>. A rapidly activating component ( $I_{Kr}$ ) rectifies inwardly at depolarized membrane potential due to C-type inactivation, whereas a slowly activating component ( $I_{Ks}$ ) has almost linear current voltage-relationship.  $I_{Kr}$  is highly sensitive, but  $I_{Ks}$  is resistant to the blockade by methanesulfonamide class III antiarrhythmic agents<sup>[1]</sup>.

In most mammalian species,  $I_{Kr}$  plays a pivotal role in normal ventricular repolarization. A lengthening

<sup>1</sup> Project supported in part by grant from China Ministry of Education, No 2010241002.

<sup>2</sup> Correspondence to Dr Jian-hua Cheng.

Phn 86-21-5103-0733. Fax 86-21-6562-8322.

E-mail [dewang@online.sh.cn](mailto:dewang@online.sh.cn)

Received 2002-12-29

Accepted 2003-06-02

of cardiac action potential duration (APD) under a variety of conditions would favor  $I_{Ks}$  activation so that to prevent excessive APD prolongation and generation of early afterdepolarization (EAD)<sup>[2,3]</sup>. A decrease of  $I_{Kr}$  or  $I_{Ks}$  by mutations of their pore-forming  $\alpha$  subunits (HERG and KvLQT1) or regulatory  $\beta$  subunits (KCNE family) underlies the inherited long QT syndrome (LQTS) with increased risk of Torsades de Pointes (TdP)-type polymorphic ventricular tachycardia and ventricular fibrillation (VF)<sup>[4]</sup>.

The present review summarizes recent progress in our understanding on  $I_{Kr}$  and  $I_{Ks}$  in terms of molecular basis, biophysical and pharmacological properties, functional roles in ventricular repolarization of normal and diseased hearts.

## $I_K$ IN MAMMALIAN VENTRICULAR CELLS

**Functional properties** The existence of the two components of  $I_K$  ( $I_{Kr}$  and  $I_{Ks}$ ) has been recognized in many mammalian species including human. There are substantial species differences in their functional properties. Activation and deactivation kinetics of  $I_{Kr}$  and  $I_{Ks}$  of ventricular myocytes from dogs, rabbits, and human (undiseased hearts) are compared (Tab 1)<sup>[2,5-8]</sup>. Compared with guinea pig<sup>[1]</sup>,  $I_{Kr}$  deactivates more slowly and  $I_{Ks}$  deactivates more rapidly in other animal species and human. Lu *et al* compared the accumulation of  $I_{Ks}$  at high stimulation rates in guinea pig and rabbit ventricular myocytes<sup>[9]</sup>. After thirty 200-ms depolarization pulses to +30 mV applied at 3.0 Hz to mimic high frequency ventricular excitation,  $I_{Ks}$  tail in guinea pig was markedly augmented (from 2.3 pA/pF to 3.5 pA/pF), whereas the current amplitude in rabbit was little affected<sup>[9]</sup>. This can be explained by a longer time required for full deactivation of  $I_{Ks}$  during electrical diastole in guinea pig than in rabbit.

There are considerable species differences between the relative expression of  $I_{Kr}$  and  $I_{Ks}$  in ventricular myocytes. The relative current density of  $I_{Ks}$  to  $I_{Kr}$  in guinea pig is 11.4 (tail current measured at -40 mV after 7.5-s depolarization to +60 mV)<sup>[1]</sup>. In dogs, the ratio is about 5.0 (tail current measured at -40 mV after 3-s depolarization to +65 mV)<sup>[10]</sup>. In rabbits, we demonstrated that the ratio was about 3.0 at the basal and about 0.5 at the apical myocytes (tail current measured at -40 mV after 3-s depolarization to +40 mV)<sup>[3]</sup>. However, caution should be taken when these values are extrapolated to the intact heart. The relative contribution of  $I_{Ks}$  in ventricular repolarization (APD is less

**Tab 1. Comparison of the activation and deactivation time constants of  $I_{Kr}$  and  $I_{Ks}$  in mammalian ventricular myocytes<sup>[2,5-8]</sup>.**

Species	Current	Activation/ms	Deactivation/ms
Rabbit	$I_{Kr}$	35±3	641±29 6531±343
	$I_{Ks}$	888±48	57±4
Dog	$I_{Kr}$	53±5	360±26 3310±280
	$I_{Ks}$	1045±103	86±12
Human	$I_{Kr}$	31±7	600±53 6297±875
	$I_{Ks}$	903±101	122±11

Values are expressed as mean±SD. Activation time kinetics for  $I_{Kr}$  and  $I_{Ks}$  were measured as tail current at -40 mV after test pulses to +30 mV (+50 mV for human  $I_{Ks}$ ) with gradually increasing durations. Deactivation kinetics of  $I_{Kr}$  and  $I_{Ks}$  were measured as tail current at -40 mV after a long test pulse to +30 mV (+50 mV for human  $I_{Ks}$ ). The activation time constants of  $I_{Kr}$  and  $I_{Ks}$  and the deactivation time constants of  $I_{Ks}$  were approximated by a single-exponential function, while the deactivation time constants of  $I_{Kr}$  were approximated by a double-exponential function.

than several hundred milliseconds) must be much less than the above-described ratio of  $I_{Ks}/I_{Kr}$ .

In mouse and rat, ventricular repolarization depends primarily on a large transient outward  $K^+$  current ( $I_{to}$ ), and the relative contribution of  $I_{Kr}$  and  $I_{Ks}$  is small. The contribution of  $I_{to}$  to ventricular repolarization in dog, rabbit and human is much less, and  $I_{to}$  is undetectable in guinea pig. These species differences should be taken into account in clinical implications of the experimental results.

**Regional difference** There exists a prominent transmural electrophysiological gradient in the mammalian ventricles<sup>[7,11-13]</sup>. In dog ventricles,  $I_{Ks}$  density was shown to be significantly less in the midmyocardial ventricular (M) cell compared with sub-epicardial and sub-endocardial cells (0.92 pA/pF vs 1.99 pA/pF and 1.83 pA/pF, respectively), whereas  $I_{Kr}$  density was comparable among the three layers<sup>[12]</sup>. The lower  $I_{Ks}$  density is supposed to contribute to the longer APD in M cells. In the rabbit ventricle,  $I_{Ks}$  density in sub-epicardial myocytes was shown to be greater than that in sub-endocardial myocytes (1.1 pA/pF vs 0.43 pA/pF), whereas their  $I_{Kr}$  densities were similar (0.31 pA/pF vs

0.36 pA/pF)<sup>[7]</sup>. In guinea pig,  $I_{Kr}$  and  $I_{Ks}$  densities in sub-endocardial myocytes are both smaller than those in mid-myocardial and sub-epicardial myocytes<sup>[13]</sup>.

However, these observations obtained in voltage-clamp experiments in isolated ventricular myocytes can not be extrapolated directly to the intact heart. The transmural gradient of refractoriness observed in the animal hearts (including dogs) is moderate or minimal<sup>[14-16]</sup>. The apparent discrepancy between *in vivo* and *in vitro* experiments could be attributed to electrotonic cell-cell interactions, an influence of mechanical stress to the ventricular wall, or other neurohumoral factors.

A substantial electrophysiological gradient was also recognized along the apico-basal axis of ventricles in some animal species<sup>[16,17]</sup>. In the isolated Langendorff-perfused rabbit heart, the QT interval of ventricular surface electrograms was shown to be significantly longer in the apex than in the base, and the regional difference was enhanced by the application of methanesulfonamide Class III antiarrhythmic drugs<sup>[17]</sup>. In the intact canine heart *in vivo*, the effective refractory period (ERP) was shown to be longer in the apex than in the base, and application of dofetilide enhanced the gradient through a greater ERP prolongation in the apex than in the base<sup>[16]</sup>.

In rabbit ventricular myocytes, Cheng *et al* demonstrated significant differences in  $I_K$  density between the base and the apex; the total  $I_K$  density was higher in the base than in the apex (2.09 pA/pF vs 1.56 pA/pF), and the  $I_{Ks}$  density was also higher in the base than in the apex (1.43 pA/pF vs 0.40 pA/pF), whereas the  $I_{Kr}$  density was lower in the base than in the apex (0.66 pA/pF vs 1.15 pA/pF)<sup>[3]</sup>. In concordance with this observation, Brahmajothi *et al* reported that the expression of ERG transcript (mRNA) and protein in ferret ventricles was more abundant in the apex than in the base<sup>[18]</sup>. Recently, interventricular differences of  $I_K$  were demonstrated in dog hearts;  $I_{Ks}$  density was significantly higher (approximately double) in the right ventricle (RV) than in the left ventricle (LV), whereas  $I_{Kr}$  density was comparable in the two ventricles<sup>[19]</sup>.

## MOLECULAR BASIS OF $I_K$ AND LONG QT SYNDROME

**Molecular basis of  $I_{Kr}$  and  $I_{Ks}$**  The  $K^+$ -selective channel encoded by HERG (KCNH2) shows currents similar to native  $I_{Kr}$  in terms of the characteristic inward rectification and high sensitivity to  $La^{3+}$ <sup>[20,21]</sup>.

HERG is also sensitive to methanesulfonamide drugs. However, there are some differences between native  $I_{Kr}$  and the first HERG clone studied (HERG1): the HERG current expressed in *Xenopus* oocytes or mammalian cell lines has 4-10 times slower activation and deactivation kinetics than native  $I_{Kr}$  in guinea pig ventricular myocytes<sup>[1,20]</sup>. Additional channel subunits, minK (KCNE1) or MinK-related peptide 1 (MiRP1 or KCNE2) have been identified, which may act as a function-altering  $\beta$  subunit and associate with the pore-forming  $\alpha$  subunit, HERG<sup>[22-24]</sup>. The mixed complexes were shown to form channels quite similar to native  $I_{Kr}$  in terms of gating kinetics, unitary conductance, regulation by potassium, and distinctive biphasic inhibition by methanesulfonamide drugs<sup>[23]</sup>.

Weerapura *et al* compared the biophysical and pharmacological properties of HERG channels expressed in Chinese hamster ovary (CHO) cells with and without MiRP1<sup>[25]</sup>. The results have revealed that MiRP1 coexpression significantly accelerates inward  $I_{HERG}$  deactivation, but does not affect more physiologically relevant outward  $I_{HERG}$  deactivation. Native  $I_{Kr}$  is activated at more positive potential range than  $I_{HERG}$ , but MiRP1 coexpression resulted in a hyperpolarizing shift of  $I_{HERG}$  activation. Moreover, the methanesulfonamide sensitivity of  $I_{HERG}$  is indistinguishable from that of native  $I_{Kr}$ , and the sensitivity is unaffected by MiRP1 coexpression. These observations seem to be unfavorable for the functional significance of MiRP1. The subunits underlying  $I_{Kr}$  and their interaction are, therefore, still under controversy, and remain to be settled.

Two N-terminal splice variants of HERG have been described<sup>[26,27]</sup>. An ERG isoform (MERG1b), which is expressed specifically in the heart, has a very short and divergent N-terminal domain, and has more rapid deactivation kinetics than HERG<sup>[26]</sup>. When MERG1b is coassembled with another isoform (MERG1a) having a long N-terminal domain in *Xenopus* oocytes, the channel showed a further acceleration of deactivation kinetics that is almost identical to native  $I_{Kr}$ <sup>[26]</sup>. An alternatively processed isoform of MERG (MERG B) expressed selectively in the heart has a unique 36-amino acid N-terminal domain, and its current characteristics closely resemble cardiac  $I_{Kr}$ <sup>[27]</sup>. HERG B, the human homologue of MERG B, has also been isolated with analogous current properties<sup>[27]</sup>. A C-terminal splice variant of HERG (HERGUSO) cloned by Kupersmidt *et al* is non-functional when expressed by itself, but modifies and reduces HERG1 current (the original clone

obtained from hippocampal cDNA library) when they are coexpressed<sup>[28]</sup>.

These observations may provide intriguing insights into the molecular basis for the interspecies and regional differences of  $I_{Kr}$ . It is possible that the native  $I_{Kr}$  could result from a mixture of N-terminal or C-terminal splice variants with HERG1 to form homomultimers or heteromultimers. Different expression of N-terminal truncated isoform would alter the kinetics of the endogenous  $I_{Kr}$ , while different expression ratio of HERGUSO vs HERG1 would alter the  $I_{Kr}$  density.

A voltage-dependent  $K^+$  channel underlying  $I_{Ks}$  is composed of a pore-forming  $\alpha$  subunit, KvLQT1 (KCNQ1), and a function-altering  $\beta$  subunit, minK (KCNE1)<sup>[29,30]</sup>. An N-terminal truncated isoform of the KvLQT1 gene product (isoform 2) has been identified in the human adult ventricle (with an amount of 28 % of total KvLQT1 expression)<sup>[31]</sup>. The isoform 2 exerts a pronounced dominant negative effect on the original isoform of KvLQT1 (isoform 1) when they are co-expressed in *Xenopus* oocytes. Péréon *et al* demonstrated that the overall expression of KvLQT1 (isoform 1, isoform 2) and minK genes in the ventricle was similar among the epicardial, midmyocardial and endocardial tissues from explanted human hearts, but the gene expression of isoform 2 was most abundant in the midmyocardial tissue<sup>[32]</sup>. This observation is in a good agreement with the least  $I_{Ks}$  density in the midmyocardial layer, and may provide a molecular basis for the longest APD in this region<sup>[12,33]</sup>.

**Dysfunction of  $I_K$  in inherited cardiac arrhythmias** Long QT syndrome (LQTS) is a cardiac disorder characterized by prolonged ventricular repolarization and a high risk for the polymorphic ventricular tachycardia known as “Torsades de Pointes (TdP)”, which often leads to sudden cardiac death as the first manifestation of the disease. Mutations in genes encoding ion channels are the main cause of the heritable (congenital) LQTS. The inheritance pattern is most frequently autosomal-dominant: alterations of a single allele are sufficient to produce the arrhythmogenic phenotype (Romano-Ward syndrome). Genetic linkage analysis has identified 6 forms of the autosomal-dominant congenital LQTS<sup>[4]</sup>. Either  $Na^+$  channel gene SCN5A or one of the four genes underlying  $I_{Kr}$  and  $I_{Ks}$  can be affected (the existence of other LQT genes is suggested, but their identity is not yet known). Chromosome 11-linked LQT1 is associated with a mutation in KvLQT1 (KCNQ1). Chromosome 7-linked LQT2 is

associated with a mutation in HERG (KCNH2). Chromosome 21-linked LQT5 and LQT6 are caused by mutations in minK (KCNE1) and MiRP1 (KCNE2), respectively. Mutations in KvLQT1 (KCNQ1) and minK (KCNE1) have also been identified in the autosomal-recessive congenital LQTS associated with deafness (Jervell-Lange-Neilsen syndrome)<sup>[4]</sup>. The LQT-associated mutations in the  $K^+$  channels decrease  $K^+$  outward current through  $I_{Kr}$  or  $I_{Ks}$  by loss-of-function or dominant-negative mechanisms<sup>[34]</sup>. This has been studied extensively in heterologous expression systems.

The penetrance of genetic effects of LQT1 is relatively lower than other forms of LQT. Priori *et al* reported that only about 25 % of patients with genetic defects for  $I_{Ks}$  channels actually had abnormally long QT intervals<sup>[35]</sup>. This observation suggests that  $I_{Ks}$  may play a less important role in human ventricular repolarization than  $I_{Kr}$ . However, the individuals with KvLQT1 (KCNQ1) mutations but with no or only marginal QT prolongation still have a high susceptibility to arrhythmias induced by drugs or adrenergic factors (physical and emotional stress) compared with other channel mutations<sup>[36]</sup>.

## $\beta$ -ADRENERGIC MODULATION OF $I_K$

The onset of polymorphic ventricular tachycardia (TdP) in patients with LQTS is often triggered by adrenergic stress, suggesting the physiological importance of  $\beta$ -adrenergic regulation of ion channels responsible for ventricular repolarization. The  $K^+$  current through  $I_{Ks}$  is enhanced by  $\beta$ -adrenergic stimulation, that causes an elevation of intracellular cAMP and an activation of protein kinase A (PKA)<sup>[3,37,38]</sup>. An elevation of cytosolic cAMP is shown to increase the  $I_{Ks}$  current amplitude, slow down the deactivation kinetics and shift the activation curve to more negative potential range<sup>[38]</sup>.

The cAMP regulation of recombinant KvLQT1 (KCNQ1)/minK (KCNE1) complex requires PKA anchoring by A-kinase anchoring proteins (AKAP). The PKA-mediated response of native cardiac  $I_{Ks}$  may, therefore, depend on the coexpression of AKAP in the cell membrane together with the channel subunits (KvLQT1 and minK)<sup>[39]</sup>. Marx *et al* have recently demonstrated that the  $\beta$ -adrenoceptor modulation of hKCNQ1 is mediated by both PKA and protein phosphatase 1 via the targeting protein, yotiao<sup>[40]</sup>. Thus, the precise molecular mechanisms of signal transduction for the  $\beta$ -adrenergic modulation of  $I_{Ks}$  are still under investigation.

Previous studies reported that  $\beta$ -adrenergic stimulation by isoproterenol had no substantial effects on  $I_{Kr}$  in guinea pig and rabbit ventricular myocytes<sup>[3,37]</sup>. However, HERG contains a cyclic nucleotide binding domain in the C-terminal<sup>[41]</sup>, and several recent studies have provided evidences of cAMP/PKA-dependent modulation of  $I_{Kr}$ . In guinea pig ventricular myocytes,  $I_{Kr}$  is shown to be inhibited by  $\beta_1$ -adrenoceptor activation through cAMP/PKA pathway<sup>[42]</sup>. In HERG channels, Cui *et al* showed that an activation of cAMP-dependent PKA results in HERG phosphorylation accompanied by a rapid reduction of the current amplitude, acceleration of deactivation, and depolarizing shift of the activation curve<sup>[43]</sup>. cAMP may also directly bind to the HERG protein with a hyperpolarizing shift of the activation curve, and this stimulatory effect of cAMP on HERG is enhanced by coexpression of accessory proteins (MiRP1 or minK)<sup>[43]</sup>.

#### CONTRIBUTION OF $I_K$ TO REPOLARIZATION AND ITS MODULATION BY $I_K$ BLOCKERS

**Contribution of  $I_{Kr}$  and  $I_{Ks}$  to action potential repolarization** The relative contributions of  $I_{Kr}$  and  $I_{Ks}$  to repolarization have been studied in cardiac myocytes of different species by using a rectangular test pulse (about 200 ms) or an artificial action-potential like test pulse. In guinea pig ventricular myocytes, the two components have similar amplitude at membrane potentials corresponding to the plateau phase of action potential (-20 mV to +20 mV), suggesting their roughly equal contribution to repolarization<sup>[1]</sup>. In isolated guinea pig hearts, pharmacological block of either  $I_{Kr}$  or  $I_{Ks}$  resulted in a similar moderate prolongation of ventricular repolarization, whereas concomitant block of both  $I_{Kr}$  and  $I_{Ks}$  resulted in a much greater prolongation of repolarization<sup>[44]</sup>. In the rabbit ventricle, the tail current amplitude of  $I_{Kr}$  measured after a 200-ms depolarization to +40 mV is 3-fold as large as  $I_{Ks}$  in the myocytes from apex, but their amplitudes are similar in the myocytes from base. The overall contribution of  $I_{Kr}$  to ventricular repolarization in rabbits is, therefore, much greater than that in guinea pigs<sup>[3]</sup>.

In dog ventricular myocytes, the relative amplitude of  $I_{Kr}$  was shown to be larger than  $I_{Ks}$  when they were measured with test pulses corresponding to normal APD (a 200-ms rectangular pulse to +30 mV or a 250-ms action potential like pulse), suggesting a greater contribution of  $I_{Kr}$ <sup>[2]</sup>. However, when the currents were

measured at a longer test pulse (500 ms),  $I_{Ks}$  had a larger amplitude than  $I_{Kr}$ . The greater contribution of  $I_{Ks}$  to the repolarization of long action potentials in dog ventricular muscle was confirmed in experiments using selective  $I_{Ks}$  blockers (L-735,821 and chromanol 293B)<sup>[2]</sup>.

In dog and rabbit (and perhaps in human) hearts under the normal condition,  $I_{Kr}$  may provide the major source of outward current responsible for ventricular repolarization, whereas  $I_{Ks}$  may play a minimal role. However, when the repolarization is delayed by certain factors, the prolonged APD would favor  $I_{Ks}$  activation to limit a further APD prolongation. This may act as a repolarization reserve to prevent excessive APD prolongation, which will lead to EAD and TdP-type polymorphic ventricular tachycardia.

**Modulation of repolarization by selective  $I_{Kr}$  and  $I_{Ks}$  blockers**  $I_{Kr}$  is the primary target of most class III antiarrhythmic drugs currently available. They are supposed to exert antiarrhythmic actions by preventing or terminating the reentrant excitation through a prolongation of APD and the refractory period. These drugs possess a common unfavorable feature; the drug-induced APD prolongation is enhanced at lower stimulation frequencies, but diminished at higher frequencies. This "reverse" frequency-dependence limits their antiarrhythmic potential at higher heart rates, and favors a proarrhythmic propensity through an excessive repolarization delay at lower heart rates<sup>[45,46]</sup>.

In guinea pig ventricular myocytes,  $I_{Ks}$  deactivates slowly, and it will accumulate at fast stimulation rates<sup>[9]</sup>. Based on this behavior, selective block of  $I_{Ks}$  has been expected to cause a greater APD prolongation at higher stimulation frequencies. However, this is not always the case because of the species difference of  $I_{Ks}$  deactivation kinetics and frequency-dependent changes of other ionic currents during the repolarization phase.

The APD prolongation by selective  $I_{Ks}$  blockers (chromanol 293B, L-735,821) has been studied in multicellular tissue preparations or intact hearts. In dog papillary muscles and Purkinje fibers, chromanol 293B (10  $\mu$ mol/L) or L-735,821 (100 nmol/L) caused only a slight (<7%) but uniform APD prolongation over a wide range of pacing cycle length (300-5000 ms), whereas *d*-sotalol (30  $\mu$ mol/L) or E-4031 (1  $\mu$ mol/L) caused a prominent APD prolongation (by 20%-80%) with typical "reverse" frequency-dependence<sup>[2]</sup>. In support of this observation, *in vivo* application of these  $I_{Ks}$  blockers to anesthetized dogs resulted in minimal QTc prolongation<sup>[2]</sup>. Similar results were reported by Lengyel *et al* in

experiments using rabbits; chromanol 293B (10  $\mu\text{mol/L}$ ) and *L*-735 821 (100  $\text{nmol/L}$ ) caused a minimal prolongation of APD (<7 %) in papillary muscles in a frequency-independent manner, but no significant QT prolongation in Langendorff-perfused hearts<sup>[8]</sup>.

Selective block of  $I_{Ks}$  causes much more prominent prolongation of cardiac APD under  $\beta$ -adrenergic stimulation<sup>[38,47]</sup>. In dog Purkinje fibers and guinea pig papillary muscles pretreated with isoproterenol (100  $\text{nmol/L}$ ), additional application of chromanol 293B (10 or 50  $\mu\text{mol/L}$ ) resulted in a marked APD prolongation (around 25 %) often associated with early afterdepolarization (EAD) or delayed afterdepolarization (DAD)<sup>[38,47]</sup>. In the presence of  $\beta$ -adrenergic stimulation, which is known to enhance L-type  $\text{Ca}^{2+}$  current ( $I_{CaL}$ ), the role of  $I_{Ks}$  in the regulation of repolarization may be much greater than that in the resting state. In arterially perfused wedge preparations of the dog left ventricle, selective block of  $I_{Ks}$  by chromanol 293B was shown to cause a pronounced transmural dispersion of repolarization sufficient to induce TdP only when the drug treatment was accompanied by  $\beta$ -adrenergic stimulation<sup>[48]</sup>.

In anesthetized dogs with recent myocardial infarction or acute myocardial ischemia, intravenous application of *L*-768 673 was quite effective in the prevention of ventricular tachycardia and fibrillation despite of a modest prolongation of ventricular refractory period (3 %-10 %) and QTc interval (4 %-6 %) by the drug treatment<sup>[49]</sup>. It was also demonstrated in dogs with recent myocardial infarction that concomitant application of *L*-768 673 and timolol at low doses had a potent protective action against malignant ventricular tachyarrhythmias, although a prolongation of QTc and paced QT intervals by the treatment was minimal (4.5 %-5.5 %)<sup>[50]</sup>. These observations suggest that selective block of  $I_{Ks}$  may be a potentially useful intervention to prevent ischemic ventricular tachyarrhythmias.

## MODULATION OF $I_K$ UNDER PATHOLOGICAL CONDITIONS

$I_{Kr}$  and  $I_{Ks}$  in cardiomyocytes are modulated under a variety of pathological conditions including ventricular hypertrophy, myocardial infarction, and congestive heart failure. In a rabbit model of left ventricular hypertrophy produced by renal artery clipping, Xu *et al* reported a significant reduction of  $I_{Ks}$  density in both subepicardial and subendocardial ventricular myocytes

by similar extents (about 40 %) with no significant changes in  $I_{Kr}$  density<sup>[7]</sup>. Application of dofetilide (a specific  $I_{Kr}$  blocker) to the hypertrophied myocytes resulted in a greater prolongation of APD (by 31 %-53 %) than control myocytes (18 %-32 %)<sup>[7]</sup>. In a dog model of myocardial infarction, ventricular myocytes in a border zone 5 d after the coronary occlusion showed significantly less densities of both  $I_{Kr}$  and  $I_{Ks}$ , and the electrophysiological changes were accompanied by reductions of transcripts (mRNA) of dERG and dmink (by 52 % and 76 %, respectively)<sup>[51]</sup>.

In a rabbit model of pacing-induced heart failure, Tsuji *et al* observed significant reductions of both  $I_{Kr}$  and  $I_{Ks}$  densities in ventricular myocytes (by about 50 %) in association with a significant prolongation of APD (by 15 %-18 %) at physiological cycle lengths (333 ms and 1000 ms)<sup>[52]</sup>. In a dog model of pacing-induced heart failure, Li *et al* demonstrated a significant reduction of  $I_{Ks}$  density (by about 30 %) in atrial myocytes<sup>[53]</sup>. In atrial muscle sampled from patients with persistent atrial fibrillation, transcripts (mRNA) of HERG (KCNH2) and KvLQT1 (KCNQ1) were shown to be decreased significantly, whereas that of mink (KCNE1) was increased<sup>[54]</sup>.

The dog with chronic atrioventricular block (AVB) has been described as an animal model of acquired QT prolongation and TdP<sup>[55]</sup>. In the model, the bradycardia-induced volume overload causes biventricular hypertrophy and heterogeneous prolongation of the ventricular APD<sup>[56-58]</sup>. Significant reduction of  $I_{Ks}$  in both ventricles (by about 50 %) and that of  $I_{Kr}$  only in the right ventricle (by 45 %) were demonstrated<sup>[58]</sup>. TdP was easily induced in the dog model by class III antiarrhythmic drugs (*d*-sotalol and almokalant) and programmed stimulation, but documented spontaneous TdP episodes and the incidence of sudden cardiac death were relatively rare. Recently, Tsuji *et al* reported a chronic AVB model in the rabbit<sup>[59]</sup>. The rabbit model showed a prominent (52 %-120 %) QT prolongation and a high incidence (71 %) of spontaneous TdP and sudden cardiac death. Ventricular myocytes isolated from the AVB rabbits were characterized by significant APD prolongation (by 20 %-60 %) and reductions of both  $I_{Kr}$  and  $I_{Ks}$  densities (by 50 %-55 %).

The reduction of  $I_{Kr}$  and/or  $I_{Ks}$  under these pathological conditions, that is most likely the result of a down regulation of the channel subunits (HERG, KvLQT1, mink, and MiRP1), may contribute to the arrhythmogenic substrate in the diseased hearts through spatially

inhomogeneous prolongation of APD and the refractory period.

## CONCLUSION

$I_{Kr}$  is the main outward  $K^+$  current contributing to the ventricular repolarization in most mammalian species.  $I_{Ks}$  may have relatively small contribution in normal action potential repolarization, but it may act as an important "repolarization reserve" when APD is abnormally lengthened by pharmacological treatments or under a variety of pathological conditions (eg, hypokalemia, bradycardia, and genetic disorders of ion channels). Selective block of  $I_{Ks}$  produces minimal to moderate, but relatively frequency-independent prolongation of ventricular repolarization and refractoriness, that would be therapeutically relevant to reduce proarrhythmic propensity in ischemic hearts.  $I_{Ks}$  blockers are, therefore, expected to be more beneficial than  $I_{Kr}$  blockers (eg, *d*-sotalol, dofetilide, and E-4031) as class III antiarrhythmic agents.

Many drugs block  $K^+$  channels unintentionally, and  $I_{Kr}$  is a common target. Apart from antiarrhythmic drugs, a growing number of non-cardiovascular drugs (eg, antihistamines, antipsychotics, and antibiotics) are included in this category<sup>[60]</sup>. This is probably due to a unique structural feature of the HERG (KCNH2) inner vestibule that renders it rather non-selective binding of small organic molecules<sup>[61]</sup>. Drug-induced long QT syndrome occurs more frequently in women than in men. The basis for this gender differences remains to be clarified. It is not known why relatively a small percentage of recipients are more prone to drug-induced LQTS. The repolarization process of cardiac cells has a substantial "reserve" built-in by the redundancy of  $K^+$  channels. Certain mutations or polymorphisms of  $K^+$  channels, which are otherwise innocent, could reduce such a repolarization reserve and increase the susceptibility for the acquired LQTS and TdP<sup>[62]</sup>. Remodeling of ion channels in diseased hearts (eg, down regulation of  $I_{Kr}$  and  $I_{Ks}$  in hypertrophied or failing ventricles) can also reduce the repolarization reserve and contribute to their proarrhythmic propensity. Further experimental and clinical studies will be required to unravel these issues.

## REFERENCES

- Sanguinetti MC, Jurkiewicz NK. Two components of cardiac delayed rectifier  $K^+$  current: differential sensitivity to

- block by class III antiarrhythmic agents. *J Gen Physiol* 1990; 96: 195-215.
- Varro A, Balati B, Iost N, Takacs J, Virag L, Lathrop DA, *et al*. The role of the delayed rectifier component  $I_{Ks}$  in dog ventricular muscle and Purkinje fiber repolarization. *J Physiol* 2000; 523: 67-81.
- Cheng J, Kamiya K, Liu W, Tsuji Y, Toyama J, Kodama I. Heterogeneous distribution of the two components of delayed rectifier  $K^+$  current: a potential mechanism of the proarrhythmic effects of methanesulfonanilide class III agents. *Cardiovasc Res* 1999; 43: 135-47.
- Roden DM, Balsler JR, George Jr AL, Anderson ME. Cardiac ion channels. *Annu Rev Physiol* 2002; 64: 431-75.
- Virag L, Iost N, Opincariu M, Szolnok J, Szecsi J, Bogats G, *et al*. The slow component of the delayed rectifier potassium current in undiseased human ventricular myocytes. *Cardiovasc Res* 2001; 49: 790-7.
- Iost N, Virag L, Opincariu M, Szecsi J, Varro A, Papp JG. Delayed rectifier potassium current in undiseased human ventricular myocytes. *Cardiovasc Res* 1998; 40: 508-15.
- Xu X, Rials SJ, Wu Y, Salata JJ, Liu T, Bharucha DB, *et al*. Left ventricular hypertrophy decreases slowly but not rapidly activating delayed rectifier potassium currents of epicardial and endocardial myocytes in rabbits. *Circulation* 2001; 103: 1585-90.
- Lengyel C, Iost N, Virag L, Varro A, Lathrop DA, Papp JG. Pharmacological block of the slow component of the outward delayed rectifier current ( $I_{Ks}$ ) fails to lengthen rabbit ventricular muscle QTc and action potential duration. *Br J Pharmacol* 2001; 132: 101-10.
- Lu Z, Kamiya K, Opthof T, Yasui K, Kodama I. Density and kinetics of  $I_{Kr}$  and  $I_{Ks}$  in guinea pig and rabbit ventricular myocytes explain different efficacy of  $I_{Ks}$  blockade at high heart rate in guinea pig and rabbit: implications for arrhythmogenesis in humans. *Circulation* 2001; 104: 951-6.
- Gintant GA. Two components of delayed rectifier current in canine atrium and ventricle. Does  $I_{Ks}$  play a role in the reverse rate dependence of class III agents? *Circ Res* 1996; 78: 26-37.
- Furukawa T, Kimura S, Furukawa N, Bassett AL, Myerburg RJ. Potassium rectifier currents differ in myocytes of endocardial and epicardial origin. *Circ Res* 1992; 70: 91-103.
- Liu DW, Antzelevitch C. Characteristics of the delayed rectifier current ( $I_{Kr}$  and  $I_{Ks}$ ) in canine ventricular epicardial, midmyocardial, and endocardial myocytes. A weaker  $I_{Ks}$  contributes to the longer action potential of the M cell. *Circ Res* 1995; 76: 351-65.
- Bryant SM, Wan X, Shipsey SJ, Hart G. Regional differences in the delayed rectifier current ( $I_{Kr}$  and  $I_{Ks}$ ) contribute to the differences in action potential duration in basal left ventricular myocytes in guinea-pig. *Cardiovasc Res* 1998; 40: 322-31.
- Anyukhovskiy EP, Sosunov EA, Rosen MR. Regional differences in electrophysiological properties of epicardium, midmyocardium, and endocardium: *in vitro* and *in vivo* correlations. *Circulation* 1996; 94: 1981-8.
- Bauer A, Becker R, Freigang KD, Senges JC, Voss F, Hansen A, *et al*. Rate- and site-dependent effects of propafenone,

- dofetilide, and the new  $I_{Ks}$ -blocking agent chromanol 293b on individual muscle layers of the intact canine heart. *Circulation* 1999; 100: 2184-90.
- 16 Bauer A, Becker R, Karle C, Schreiner KD, Senges JC, Voss F, *et al*. Effects of the  $I_{Kr}$ -blocking agent dofetilide and of the  $I_{Ks}$ -blocking agent chromanol 293b on regional disparity of left ventricular repolarization in the intact canine heart. *J Cardiovasc Pharmacol* 2002; 39: 460-7.
  - 17 Iwata H, Kodama I, Suzuki R, Kamiya K, Toyama J. Effects of long-term oral administration of amiodarone on the ventricular repolarization of rabbit hearts. *Jpn Circ J* 1996; 60: 662-72.
  - 18 Brahmajothi MV, Morales MJ, Reimer KA, Strauss HC. Regional localization of ERG, the channel protein responsible for the rapid component of the delayed rectifier,  $K^+$  current in the ferret heart. *Circ Res* 1997; 81: 128-35.
  - 19 Volders PG, Sipido KR, Vos MA, Spatjens RL, Leunissen JD, Carmeliet E, *et al*. Downregulation of delayed rectifier  $K^+$  currents in dogs with chronic complete atrioventricular block and acquired torsades de pointes. *Circulation* 1999; 100: 2455-61.
  - 20 Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the  $I_{Kr}$  potassium channel. *Cell* 1995; 81: 299-307.
  - 21 Trudeau MC, Warmke JW, Ganetzky B, Robertson GA. HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* 1995; 269: 92-5.
  - 22 McDonald TV, Yu Z, Ming Z, Palma E, Meyers MB, Wang KW, *et al*. A minK-HERG complex regulates the cardiac potassium current  $I_{Kr}$ . *Nature* 1997; 388: 289-92.
  - 23 Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, *et al*. MiRP1 forms  $I_{Kr}$  potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 1999; 97: 175-87.
  - 24 Ohyama H, Kajita H, Omori K, Takumi T, Hiramoto N, Iwasaka T, *et al*. Inhibition of cardiac delayed rectifier  $K^+$  currents by an antisense oligodeoxynucleotide against IsK (minK) and over-expression of IsK mutant D77N in neonatal mouse hearts. *Pflügers Arch* 2001; 442: 329-35.
  - 25 Weerapura M, Nattel S, Chartier D, Caballero R, Hebert TE. A comparison of currents carried by HERG, with and without coexpression of MiRP1, and the native rapid delayed rectifier current. Is MiRP1 the missing link? *J Physiol* 2002; 540: 15-27.
  - 26 London B, Trudeau MC, Newton KP, Beyer AK, Copeland NG, Gilbert DJ, *et al*. Two isoforms of the mouse ether-a-go-go-related gene coassemble to form channels with properties similar to the rapidly activating component of the cardiac delayed rectifier  $K^+$  current. *Circ Res* 1997; 81: 870-8.
  - 27 Lees-Miller JP, Kondo C, Wang L, Duff HJ. Electrophysiological characterization of an alternatively processed ERG  $K^+$  channel in mouse and human hearts. *Circ Res* 1997; 81: 719-26.
  - 28 Kupersmidt S, Snyders DJ, Raes A, Roden DM. A  $K^+$  channel splice variant common in human heart lacks a C-terminal domain required for expression of rapidly activating delayed rectifier current. *J Biol Chem* 1998; 273: 27231-5.
  - 29 Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, *et al*. Coassembly of KvLQT1 and minK (IsK) proteins to form cardiac IKs potassium channel. *Nature* 1996; 384: 81-3.
  - 30 Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. KvLQT1 and IsK (minK) proteins associate to form the  $I_{Ks}$  cardiac potassium current. *Nature* 1996; 384: 78-80.
  - 31 Demolombe S, Baró I, Péréon Y, Bliker J, Mohammad-Panah R, Pollard H, *et al*. A dominant negative isoform of the long QT syndrome 1 gene product. *J Biol Chem* 1998; 273: 6837-43.
  - 32 Péréon Y, Demolombe S, Baró I, Drouin E, Charpentier F, Escande D. Differential expression of KvLQT1 isoforms across the human ventricular wall. *Am J Physiol Heart Circ Physiol* 2000; 278: H1908-H15.
  - 33 Drouin E, Charpentier F, Gauthier C, Laurent K, Le Marec H. Electrophysiologic characteristics of cells spanning the left ventricular wall of human heart: evidence for presence of M cells. *J Am Coll Cardiol* 1995; 26: 185-92.
  - 34 Sanguinetti MC. Dysfunction of delayed rectifier potassium channels in an inherited cardiac arrhythmia. *Ann N Y Acad Sci* 1999; 868: 406-13.
  - 35 Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. *Circulation* 1999; 99: 529-33.
  - 36 Swan H, Saarinen K, Kontula K, Toivonen L, Viitasalo M. Evaluation of QT interval duration and dispersion and proposed clinical criteria in diagnosis of long QT syndrome in patients with a genetically uniform type of LQT1. *J Am Coll Cardiol* 1998; 32: 486-91.
  - 37 Sanguinetti MC, Jurkiewicz NK, Scott A, Siegl PK. Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent E-4031 in guinea pig myocytes. Mechanism of action. *Circ Res* 1991; 68: 77-84.
  - 38 Han W, Wang Z, Nattel S. Slow delayed rectifier current and repolarization in canine cardiac Purkinje cells. *Am J Physiol Heart Circ Physiol* 2001; 280: H1075-80.
  - 39 Potet F, Scott JD, Mohammad-Panah R, Escande D, Baro I. AKAP proteins anchor cAMP-dependent protein kinase to KvLQT1/IsK channel complex. *Am J Physiol Heart Circ Physiol* 2001; 280: H2038-45.
  - 40 Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, *et al*. Requirement of a macromolecular signaling complex for  $\beta$ -adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science* 2002; 295: 496-9.
  - 41 Warmke JW, Ganetzky B. A family of potassium channel genes related to eag in drosophila and mammals. *Proc Natl Acad Sci USA* 1994; 91: 3438-42.
  - 42 Karle CA, Zitron E, Zhang W, Kathofer S, Schoels W, Kiehn J. Rapid component  $I_{Kr}$  of the guinea-pig cardiac delayed rectifier  $K^+$  current is inhibited by  $\beta_1$ -adrenoreceptor activation, via cAMP/protein kinase A-dependent pathways. *Cardiovasc Res* 2002; 53: 355-62.
  - 43 Cui J, Melman Y, Palma E, Fishman GI, McDonald TV. Cyclic AMP regulates the HERG  $K^+$  channel by dual pathways. *Curr Biol* 2000; 10: 671-4.
  - 44 Geelen P, Drolet B, Lessard E, Gilbert P, O'Hara GE, Turgeon

- J. Concomitant block of the rapid ( $I_{Kr}$ ) and slow ( $I_{Ks}$ ) components of the delayed rectifier potassium current is associated with additional drug effects on lengthening of cardiac repolarization. *J Cardiovasc Pharmacol Ther* 1999; 4: 143-150.
- 45 Roden DM. Ionic mechanisms for prolongation of refractoriness and their proarrhythmic and antiarrhythmic correlates. *Am J Cardiol* 1996; 72:12-6.
- 46 Nair LA, Grant AO. Emerging class III antiarrhythmic agents: mechanism of action and proarrhythmic potential. *Cardiovasc Drugs Ther* 1997; 11: 149-67.
- 47 Schreieck J, Wang Y, Gjini V, Korth M, Zrenner B, Schomig A, *et al*. Differential effect of beta-adrenergic stimulation on the frequency-dependent electrophysiologic actions of the new class III antiarrhythmics dofetilide, ambasilide, and chromanol 293B. *J Cardiovasc Electrophysiol* 1997; 8: 1420-30.
- 48 Burashnikov A, Antzelevitch C. Block of  $I_{Ks}$  does not induce early afterdepolarization activity but promotes  $\beta$ -adrenergic agonist-induced delayed afterdepolarization activity. *J Cardiovasc Electrophysiol* 2000; 11: 458-65.
- 49 Lynch JJ Jr, Houle MS, Stump GL, Wallace AA, Gilberto DB, *et al*. Antiarrhythmic efficacy of selective blockade of the cardiac slowly activating delayed rectifier current,  $I_{Ks}$ , in canine models of malignant ischemic ventricular arrhythmia. *Circulation* 1999; 100: 1917-22.
- 50 Lynch JJ Jr, Salata JJ, Wallace AA, Stump GL, Gilberto DB, Jahansouz H, *et al*. Antiarrhythmic efficacy of combined  $I_{Ks}$  and  $\beta$ -adrenergic receptor blockade. *J Pharmacol Exp Ther* 2002; 302: 283-9.
- 51 Jiang M, Cabo C, Yao J, Boyden PA, Tseng G. Delayed rectifier  $K^+$  currents have reduced amplitudes and altered kinetics in myocytes from infarcted canine ventricle. *Cardiovasc Res* 2000; 48: 34-43.
- 52 Tsuji Y, Opthof T, Kamiya K, Yasui K, Liu W, Lu Z, *et al*. Pacing-induced heart failure causes a reduction of delayed rectifier potassium currents along with decreases in calcium and transient outward currents in rabbit ventricle. *Cardiovasc Res* 2000; 48: 300-9.
- 53 Li D, Melnyk P, Feng J, Wang Z, Petrecca K, Shrier A, *et al*. Effects of experimental heart failure on atrial cellular and ionic electrophysiology. *Circulation* 2000; 101: 2631-8.
- 54 Lai LP, Su MJ, Lin JL, Lin FY, Tsai CH, Chen YS, *et al*. Changes in the mRNA levels of delayed rectifier potassium channels in human atrial fibrillation. *Cardiology* 1999; 92: 248-55.
- 55 Vos MA, Verduyn SC, Gorgels AP, Lipse GC, Wellens HJ. Reproducible induction of early afterdepolarization and torsades de pointes arrhythmias by d-sotalol and pacing in dogs with chronic atrioventricular block. *Circulation* 1995; 91: 864-72.
- 56 Vos MA, de Groot SH, Verduyn SC, van der Zande J, Leunissen HD, Cleutjens JP, *et al*. Enhanced susceptibility for acquired torsades de pointes arrhythmias in the dog with chronic, complete AV block is related to cardiac hypertrophy and electrical remodeling. *Circulation* 1998; 98: 1125-35.
- 57 Volders PG, Sipido KR, Vos MA, Kulcsar A, Verduyn SC, Wellens HJ. Cellular basis of biventricular hypertrophy and arrhythmogenesis in dogs with chronic atrioventricular block and acquired torsades de pointes. *Circulation* 1998; 98: 1136-47.
- 58 Volders PG, Sipido KR, Carmeliet E, Spatjens RL, Wellens HJ, Vos MA. Repolarizing  $K^+$  currents  $I_{TO1}$  and  $I_{Ks}$  are larger in right than left canine ventricular midmyocardium. *Circulation* 1999; 99: 206-10.
- 59 Tsuji Y, Opthof T, Yasui K, Inden Y, Takemura H, Niwa N, *et al*. Ionic mechanisms of acquired QT prolongation and torsades de pointes in rabbits with chronic complete atrioventricular block. *Circulation* 2002; 106: 2012-8.
- 60 De Ponti F, Poluzzi E, Montanaro N. Organizing evidence on QT prolongation and occurrence of torsades de pointes with non-antiarrhythmic drugs: a call for consensus. *Eur J Clin Pharmacol* 2001; 57: 185-209.
- 61 Mitcheson JS, Chen J, Lin M, Culberson C, Sanguinetti MC. A structural basis for drug-induced long QT syndrome. *Proc Natl Acad Sci USA* 2000; 97: 12329-33.
- 62 Roden DM. Pharmacogenetics and drug-induced arrhythmias. *Cardiovasc Res* 2001; 50: 224-31.