

REVIEW

Cardiac ventricular repolarization reserve: a principle for understanding drug-related proarrhythmic risk

András Varró^{1,2} and István Baczkó¹¹Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary, and²Division of Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged, Hungary**Correspondence**

András Varró, Department of Pharmacology and Pharmacotherapy, University of Szeged, Dóm tér 12, H-6720 Szeged, Hungary. E-mail: a.varro@phcol.szote.u-szeged.hu

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Cardiac repolarization abnormalities can be caused by a wide range of cardiac and non-cardiac compounds and may lead to the development of life-threatening Torsades de Pointes (TdP) ventricular arrhythmias. Drug-induced Torsades de Pointes is associated with unexpected and unexplained sudden cardiac deaths resulting in the withdrawal of several compounds in the past. To better understand the mechanism of such unexpected sudden cardiac deaths, the concept of repolarization reserve has recently emerged. According to this concept, pharmacological, congenital or acquired impairment of one type of transmembrane ion channel does not necessarily result in excessive repolarization changes because other repolarizing currents can take over and compensate. In this review, the major factors contributing to repolarization reserve are discussed in the context of their clinical significance in physiological and pathophysiological conditions including drug administration, genetic defects, heart failure, diabetes mellitus, gender, renal failure, hypokalaemia, hypothyroidism and athletes' sudden deaths. In addition, pharmacological support of repolarization reserve as a possible therapeutic option is discussed. Some methods for the quantitative estimation of repolarization reserve are also recommended. It is concluded that repolarization reserve should be considered by safety pharmacologists to better understand, predict and prevent previously unexplained drug-induced sudden cardiac deaths.

Abbreviations

APD, action potential duration; ATP, adenosine triphosphate; ATX II, Anemonia sulcata toxin; cAMP, 3'-5'-cyclic adenosine monophosphate; DAD, delayed afterdepolarization; EAD, early afterdepolarization; ERP, effective refractory period; $I_{Ca,L}$, L-type inward calcium current; I_{Kr} , rapid delayed rectifier potassium current; I_{Ks} , slow delayed rectifier potassium current; I_{Na} , inward sodium current; I_{to} , transient outward potassium current; LQT1, long QT syndrome 1; LQT2, long QT syndrome 2; LQT3, long QT syndrome 3; LQT7, long QT syndrome 7; LQTS, long QT syndromes; miRNA, micro ribonucleic acid; NCX, Na^+ - Ca^{2+} exchanger; PKA, protein kinase A; PKC, protein kinase C; TdP, Torsades de Pointes

Introduction

It is well known that certain antiarrhythmic drugs induce life-threatening Torsades de Pointes (TdP) arrhythmia in 3–5% of patients. This was not considered particularly surprising because the drugs in question were expected to affect

cardiac transmembrane ion channels and the electrophysiological properties of the heart as part of their therapeutic effect. Recently, however, there has been increasing concern about TdP arrhythmia and sudden death caused by non-cardiac drugs. The incidence of TdP induced by non-cardiac drugs is usually low (less than 1:10 000 or 1:100 000).

However, several of them including astemizole, cisapride, grepafloxacin, terfenadine, terolidine have been withdrawn from the market due to their torsadogenic properties preventing their further therapeutic application in patients not susceptible to TdP and leading to the loss of several billion dollars in development costs and increasing uncertainty for future drug development programmes. Therefore, the need for minimizing the proarrhythmic risk of novel developmental drugs is urgent and represents a significant challenge (Pugsley *et al.*, 2008; Farkas and Nattel, 2010).

In the background of these arrhythmias and sudden deaths, variable repolarization abnormalities are suspected due to unrecognized drug adverse effects, possibly in combination with other factors including genetic mutations and polymorphisms, electrophysiological remodelling, and changes in serum ion concentrations. Although unexpected and unexplained sudden deaths related to TdP have low incidence, they often occur at a young age or in individuals without previous medical history. Because our understanding of the underlying mechanisms is still poor, effective prevention remains elusive. In many cases, the degree of repolarization defect is so subtle that warning signs cannot be detected by common and widely used diagnostic tools such as ECG or routine preclinical drug safety methods. It is therefore almost impossible to identify the population at highest risk. During the last decade the concept of repolarization reserve has emerged (Roden, 1998; 2006; 2008) and has been demonstrated experimentally (Iost *et al.*, 1999; Varró *et al.*, 2000; Lengyel *et al.*, 2001; Jost *et al.*, 2005). This concept seems to be a useful approach for safety pharmacologists to better understand, predict and possibly prevent unexpected drug related, and previously unexplained, sudden cardiac deaths.

Definitions and background

The term of repolarization reserve was first coined by Roden (Roden, 1998) in an editorial in 1998:

The greater problem is predicting the development of torsade de pointes in the absence of high doses or plasma concentrations. We have developed the concept of **“repolarization reserve”** to address this issue. We postulate that there exist in the normal ventricle and conducting system mechanisms to effect orderly and rapid repolarization that runs essentially no risk of setting up re-entrant circuits or of generating EADs. Indeed, the normal function of the delayed rectifier currents I_{Kr} and I_{Ks} is a major contributor to such stable repolarization, or a large **repolarization reserve**. Identified risk factors for torsades de pointes reduce this repolarization reserve, making it more likely that the further added stress of, for, e.g. an I_{Kr} blocking drug or a subtle genetic defect, is sufficient to precipitate torsades de pointes in individual patients.

At the time, experimental evidence was not available to support this concept; instead, it represented a way of thinking about cardiac repolarization based on general principles. Even so, the impact of this editorial was immediate and

substantial. It is now an important principle in understanding unexpected proarrhythmic complications associated with genetic disorders of ion channels, certain diseases and drug effects. The concept has also served to provide a theoretical background for safety pharmacology related to drug-induced QT prolongation.

During the 10 years after the concept of repolarization reserve was first proposed, it has evolved yielding the following definition (Roden, 2008):

The concept of “repolarization reserve”, the idea is that the complexity of repolarization includes some redundancy. As a consequence, loss of 1 component (such as I_{Kr}) ordinarily will not lead to failure of repolarization (i.e. marked QT prolongation); as a corollary, individuals with subclinical lesions in other components of the system, say I_{Ks} or calcium current, may display no QT change until I_{Kr} block is superimposed.

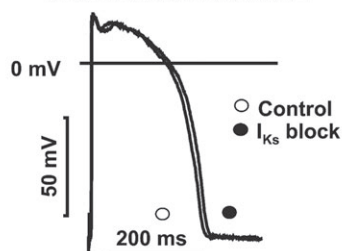
At around the same time of the original proposal, specific inhibitors (chromanol 293B, L-735,821 and HMR-1556) of the slow delayed rectifier potassium current (I_{Ks}) became available (Busch *et al.*, 1996; Salata *et al.*, 1996; Gögelein *et al.*, 2000), and both researchers and pharmaceutical industry considered I_{Ks} block to have beneficial class III antiarrhythmic effects causing less or negligible proarrhythmic complications compared with rapid delayed rectifier potassium current (I_{Kr}) inhibition (Hondeghe, 1992; Busch *et al.*, 1994; Nair and Grant, 1997). Several drug companies therefore launched projects to develop novel and specific I_{Ks} inhibitors. Surprisingly, however, in our experiments as shown on Figure 1, I_{Ks} block did not elicit any measurable effects on repolarization in either canine papillary muscle or Purkinje fibre preparations (Iost *et al.*, 1999; Varró *et al.*, 2000). These observations contradicted some published studies (Bosch *et al.*, 1998; Cordeiro *et al.*, 1998), as well as the general belief that decrease in a major and important potassium current should substantially delay cardiac repolarization. However, when action potential duration (APD) was lengthened by pharmacological means, I_{Ks} inhibition further lengthened repolarization (Figure 1C). Based on these results, we hypothesized that the slowly activating and relatively small I_{Ks} represented only a negative feedback mechanism limiting excessive repolarization lengthening. In particular, we stated that:

Although I_{Ks} may have little role in normal action potential repolarization, it probably plays a vital role when cardiac APD is abnormally lengthened by other means (e.g. by reductions in I_{Kr} or I_{K1} or increases in I_{Na} or I_{Ca}). As such, pharmacological block of I_{Ks} might be expected to have severe detrimental consequences when this protective mechanism is eliminated. For example, 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.

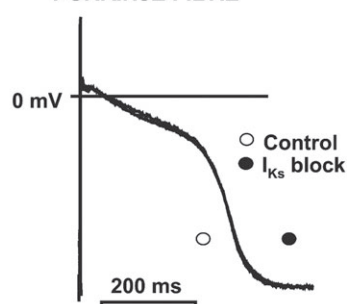
The principle of repolarization reserve was thereby directly and experimentally demonstrated (Iost *et al.*, 1999; Varró *et al.*, 2000), and in the conclusion of this paper the concept was described without the term ‘repolarization reserve’.

Normal repolarization reserve

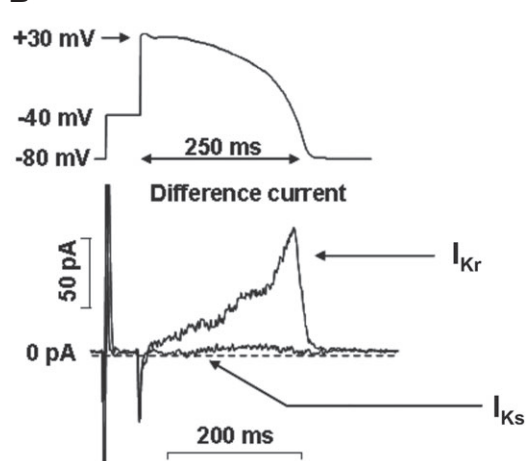
A VENTRICULAR MUSCLE



PURKINJE FIBRE

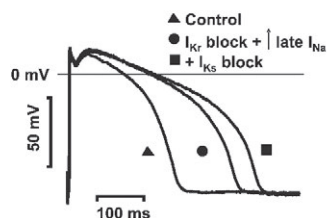
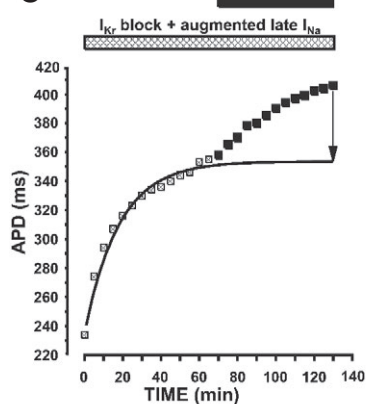


B



Impaired repolarization reserve

C



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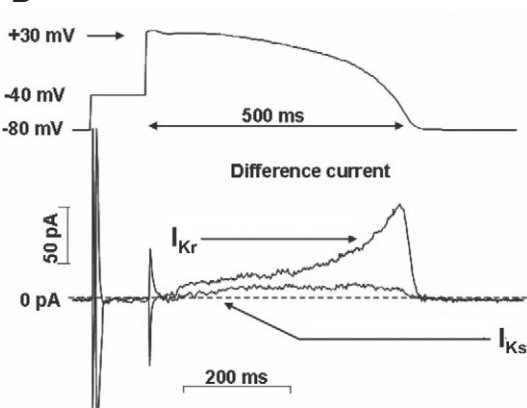


Figure 1

Experimental evidence of repolarization reserve. (A) The lack of marked prolongation of the action potential after I_{Ks} block (by L-735,821) on dog right ventricular papillary muscle and Purkinje fibres at 1 Hz stimulation frequency. (B) The magnitude of I_{Kr} and I_{Ks} after normal action potential-like voltage clamp pulses. Short voltage clamp pulses corresponding to 'normal action potential' elicit relatively large I_{Kr} (E-4031 sensitive current) but small I_{Ks} (L-735,821 sensitive current). (C) Effect of I_{Ks} block on dog ventricular action potential duration (APD) previously prolonged by application of E-4031 (I_{Kr} block) and veratrine (augmented late I_{Na}). Note that in preparations where repolarization was lengthened, inhibition of I_{Ks} resulted in significant action potential prolongation. (D) Increasing the duration of the voltage clamp pulses beyond 'normal' did not change the density of I_{Kr} but increased that of I_{Ks} . From Varró *et al.* (2000), with permission.

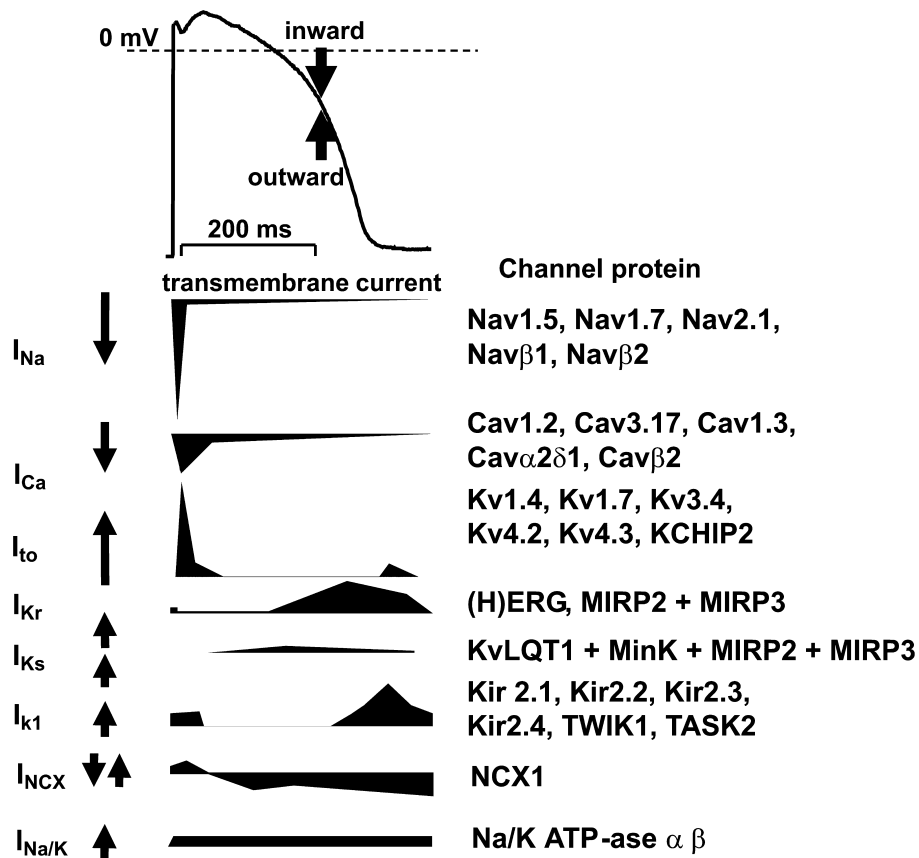


Figure 2

Ventricular action potential (top) and schematic, illustrative representation (not proportional to actual current densities) of the most important underlying transmembrane ionic currents and electrogenic pumps (bottom). Channel proteins, responsible for mediating the respective current, are indicated on the right. Upward and downward deflections and arrows denote outward and inward currents respectively.

Major currents influencing repolarization reserve

Cardiac muscle repolarization is governed by the simultaneous and balanced activities (activation and inactivation) of several inward and outward currents flowing through different ion channels or electrogenic ion pumps (Figure 2). Although this review focuses on ventricular tissue and it is well known that there are significant differences in the density of these channels and exchangers between various regions of the heart (Nerbonne and Kass, 2005; Gaborit *et al.*, 2007), the concept of repolarization reserve can also be applied to these areas.

Inward sodium current (I_{Na})

The large fast sodium current is inactivated within a few milliseconds but the sodium current does not vanish completely during the plateau phase. Over the limited range of membrane potentials where activation and inactivation overlap, a relatively small steady-state sodium current can be recorded known as the 'Na⁺ – window current' (Attwell *et al.*, 1979). In addition, a small fraction of the sodium current slowly inactivates during the plateau phase (Carmeliet, 1987).

This so-called persistent sodium current functionally opposes repolarizing potassium currents and delays repolarization. It is augmented in a number of situations, for example, by administration of veratrine and Anemonia sulcata toxin (ATX II), in long QT syndrome 3 (LQT3) syndrome and heart failure (Schwartz *et al.*, 1995), further opposing the function of outward currents. In case there are compensatory ionic movements for this current, the net result is diminished repolarization reserve; however, when the increase of this inward current is substantial, prolongation of repolarization may occur.

L-type inward calcium current ($I_{Ca,L}$)

The influence of inward calcium currents on repolarization is more complex than that of I_{Na} and is still not completely understood. The calcium current, like I_{Na} , exhibits both slowly inactivating and window components but in addition, inactivation of the current is dependent on the free cytosolic calcium concentration ($[Ca^{2+}]_i$). Because $[Ca^{2+}]_i$ changes dynamically during the action potential, and is regulated by many factors (Bers, 2001) including sarco/endoplasmic reticulum Ca^{2+} ATPase, phospholamban, ryanodine receptor, calmodulin and protein kinase A (PKA) and C (PKC), the impact of inward calcium currents on the repolarization is

difficult to predict accurately; and it represents one of the most problematic targets for mathematical modelling of cardiac action potentials. Moreover, if inward currents (both I_{Na} and I_{Ca}) are augmented, the plateau voltage is shifted towards more positive values that may result in enhanced activation of outward potassium currents that, depending on the actual balance of currents, may shorten repolarization.

Rapid delayed rectifier outward potassium current (I_{Kr})

I_{Kr} activates rapidly on membrane depolarizations (Gintant, 1996) that are more positive than -30 mV ($\tau \sim 40$ ms at $+30$ mV) but its inactivation precedes depolarization-dependent activation (Spector *et al.*, 1996). As a consequence, I_{Kr} channels are largely closed during the plateau phase and only open when membrane potential returns to around 0 mV. Recovery from inactivation is very rapid, and as membrane potential slowly changes towards negative values these channels open again and then slowly deactivate (close), resulting in the transient nature of I_{Kr} . Contrary to what one might expect from the Nernst equation, increased extracellular K^+ concentration is known to enhance, while decreased $[K^+]_o$ acutely reduces I_{Kr} (Sanguinetti and Jurkiewicz, 1992; Yang *et al.*, 1997). Interestingly, the surface density of I_{Kr} in rabbit hearts and human ether-a-go-go-related gene (HERG) channel in human cell lines have been recently found to be under direct control of extracellular K^+ concentration, with $[K^+]_o$ reduction resulting in accelerated internalization and degradation of the channel (Guo *et al.*, 2009). These mechanisms may play important roles in changes in repolarization reserve following electrolyte disturbances. Due to its peculiar gating properties, that is, its very fast inactivation and recovery from inactivation, an apparent inward rectification (also see I_{ki} later) occurs. I_{Kr} may play a positive feedback role contributing to repolarization lengthening when repolarization is already prolonged (Virág *et al.*, 2009) due to other factors such as LQT3 or bradycardia. In this setting shown on Figure 3, with longer action potentials there is less I_{Kr} (and also less I_{ki} , see later) developing at isochronal points further weakening the force of repolarization in a complex voltage and time dependent process (Virág *et al.*, 2009). Thus, repolarization becomes more vulnerable when depolarizing factors [I_{Na} , $I_{Ca,L}$, Na^+ - Ca^{2+} exchanger (NCX)] are augmented or repolarizing factors (I_{ki} , I_{Ks} , Na/K) are reduced. Even a small decrease in I_{Kr} may lead therefore to substantial prolongation of APD at slow rates or may have an additive effect with other interventions that lengthen repolarization. I_{Kr} is widely recognized as one of the most important outward currents controlling repolarization. A marked decrease or inhibition of I_{Kr} results in substantial repolarization lengthening in many species, including human (Varró *et al.*, 2000; Lengyel *et al.*, 2001; Jost *et al.*, 2005). When the decrease in I_{Kr} is only fractional, the APD is not necessarily prolonged due to possible compensatory functions by other outward currents. However, further impairment of the current may have detrimental consequences such as excessive repolarization lengthening leading to TdP arrhythmia. This is the most likely reason why relatively weak I_{Kr} inhibition by certain non-cardiac drugs provokes arrhythmias related to repolarization abnormalities. Thus, I_{Kr} is not only a robust outward current

determining the course of apparent repolarization but itself is clearly the most important contributor to repolarization reserve.

Slow delayed rectifier outward potassium current (I_{Ks})

I_{Ks} , which flows through KvLQT1 + MinK + MIRP protein constituted channels, activates slowly (500–1000 ms) during the plateau phase and deactivates rapidly (100–200 ms) at negative membrane potentials (for a recent review, see Jost *et al.*, 2007). Its relatively small amplitude between 0 mV and $+20$ mV and slow activation kinetics, mean that there is relatively little current activating during the normal course of the action potential (Varró *et al.*, 2000; Jost *et al.*, 2005) and consequently little influence on repolarization (Varró *et al.*, 2000; Jost *et al.*, 2005). However, when action potential is lengthened and plateau voltage is shifted to more positive voltage, I_{Ks} (Han *et al.*, 2001) 'creates an available reserve of channels that are ready to open on demand' (Carmeliet, 2006), thereby forming a transmembrane current that has the main function of participating in repolarization reserve (Figure 1D), and opposing excessive APD lengthening (Varró *et al.*, 2000; Jost *et al.*, 2005). I_{Ks} is activated by PKC and by sympathetic stimulation via PKA (Yazawa and Kameyama, 1990; Walsh and Kass, 1991). The latter has important consequences, because increases in the amplitude of I_{Ks} and shifts in its activation voltage towards negative potentials enhance I_{Ks} current density during elevated sympathetic tone. Moreover, sympathetic stimulation enhances $I_{Ca,L}$ that in turn shifts the plateau towards more positive voltage and lengthening repolarization. These changes further activate I_{Ks} which, as a negative feedback limits the effect of the enhancement of $I_{Ca,L}$ (Han *et al.*, 2001; Volders *et al.*, 2003). It becomes evident therefore that impairment of I_{Ks} due to loss of function mutation (LQT1) or I_{Ks} blocking drugs in the presence of elevated sympathetic tone, leaves the repolarization lengthening caused by augmented $I_{Ca,L}$ unopposed and substantial prolongation of APD can develop. Thus, I_{Ks} does not necessarily contribute to repolarization in normal settings and at low sympathetic tone. However, under certain conditions (e.g. enhanced sympathetic activation), this current provides a protective mechanism that creates a repolarization reserve counterbalancing repolarization lengthening only when necessary (Johnson *et al.*, 2010). It should be noted that I_{Ks} has exhibited transmural expression differences (Gintant, 1995) that, with the transmural and regional expression heterogeneities other currents may also contribute to enhanced heterogeneity of repolarization and, possibly, regional differences in repolarization reserve. In canine wedge preparations, Akar *et al.* (2002) have clearly demonstrated a direct link between such transmural repolarization heterogeneity and the development of TdP.

Inward rectifier potassium current (I_{ki})

I_{ki} flows through a mixture of Kir 2.1, 2.2, 2.3 showing strong inward rectification (for a recent review, see Anumonwo and Lopatin, 2010). Consequently, I_{ki} current is decreasing close to zero at membrane potentials more positive than -30 mV and reopening very rapidly when membrane potentials reach more negative values. The inward rectification is due to

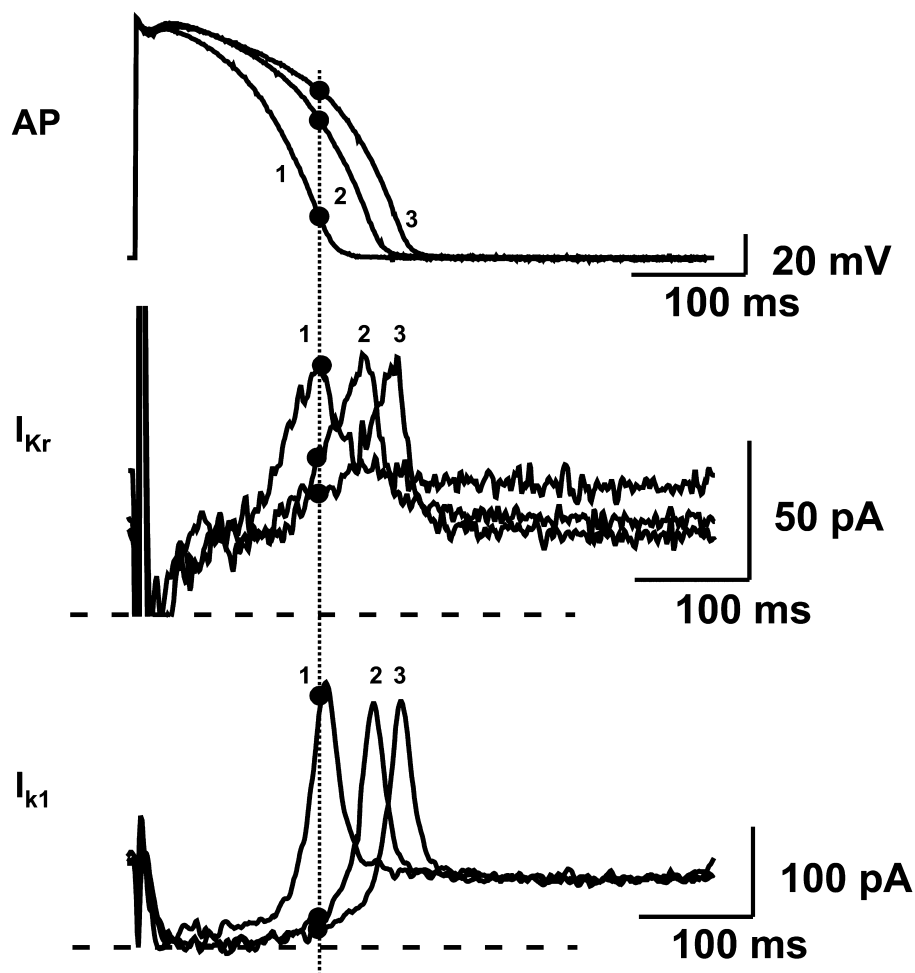


Figure 3

When action potential duration has been prolonged previously by other means in dog papillary muscle (top panel), the positive feedback mechanism by which reduction of I_{Kr} (middle panel) and I_{k1} (lower panel) further augments repolarization delay. Increased duration and thereby decreased rate of repolarization decreases the contribution of I_{Kr} and I_{k1} currents to the repolarization process during the time course of the action potential, in turn producing a further slowing of repolarization. Numbers denote three recordings with different action potential durations. Dotted line identifies isochronal points on these recordings. I_{Kr} and I_{k1} were measured from isolated ventricular myocytes subjected to action potential waveforms used as command signals in voltage-clamp conditions. AP, action potentials. Modified from Virág *et al.* (2009), with permission.

channel block by Mg^{2+} and polyamines from intracellular sites on depolarization (Matsuda *et al.*, 1987). When the positive membrane potential is changing to the negative direction, the Mg^{2+} and polyamine-induced channel block is relieved and I_{k1} increases. This process forms a negative feedback during repolarization making it a regenerative process. This phenomenon resembles that mentioned in connection with I_{Kr} , but the potential range of I_{k1} is more negative than that of I_{Kr} . I_{k1} plays a similar role to I_{Kr} in the self-limiting repolarization process after APD lengthening (Figure 3) following bradycardia (Virág *et al.*, 2009). These channels are fully open at rest (i.e. during diastole) so that I_{k1} opposes any kind of depolarization due to enhanced pacemaker activity and Ca^{2+} overload-related delayed afterdepolarizations (DAD). This feature represents a special form of repolarization reserve. Consequently, the impairment of I_{k1} may have a proarrhythmic potential by not opposing depolarization that can thereby reach the threshold for propagation of extrasys-

toles. In addition, smaller I_{k1} also increases duration of repolarization and its heterogeneity. The density of I_{k1} shows considerable variations among species being relatively weak in human ventricle (Jost *et al.*, 2008) but strong in dog, rabbit and guinea pig (Varró *et al.*, 1993). A possible result in humans is that a moderate I_{k1} defect or block is not reflected in significant repolarization lengthening. However, the inhibition of I_{k1} , the current being a part of the repolarization reserve, together with the malfunction of other ion channels, may lead to excessive lengthening of the APD. Indeed, loss of function mutations of Kir 2.1 channels in LQT7 (Andersen-Tawil syndrome), although substantially decreasing I_{k1} , do not result in marked QTc lengthening yet greatly enhance proarrhythmic risks for these patients (Zhang *et al.*, 2005). An important role for I_{k1} in repolarization reserve has also been suggested using mathematical modelling where reduction of I_{k1} in addition to I_{Kr} inhibition resulted in early afterdepolarizations (EAD) (Ishihara *et al.*, 2009). Based on this analysis, it

is likely that I_{K1} is an important contributor to repolarization reserve both in diastole and during the final repolarization phase.

Transient outward potassium current (I_{to})

I_{to} consists of the rapidly ($I_{to,f}$) and slowly recovering ($I_{to,s}$) components that are carried by channels with distinct pore-forming subunits, Kv4.3/Kv4.2 for $I_{to,f}$, and Kv1.4 for $I_{to,s}$, respectively, and show considerable species variations (recently reviewed by Niwa and Nerbonne, 2010). I_{to} has not been recorded in guinea pig. In human (Gaborit *et al.*, 2007) and dog (Fülöp *et al.*, 2006), Kv4.3 is considered to provide the α subunit, while in rabbit Kv1.4 seems to be the dominant protein (Wang *et al.*, 1999). In general, I_{to} is a large and rapidly activating ($\tau \sim 1\text{--}2$ ms) and inactivating (5–10 ms) outward current at membrane potentials more positive than -20 mV (Patel and Cambell, 2005). Therefore, I_{to} contributes to the initial phase 1 repolarization and its influence on the APD is believed to be limited. It must be emphasized that I_{to} can alter the amplitude of the plateau and thereby indirectly change the activation, inactivation and deactivation of other transmembrane currents. In addition, I_{to} (Kv4.3 current) has a second, slower phase of inactivation ($\tau \sim 15\text{--}30$ ms) which may be responsible for a reasonable amplitude during the plateau, and its activation and inactivation may overlap producing a similar type of window current during the final repolarization as described for I_{Ca} or I_{Na} . Thus, one can suspect that although changes in I_{to} current may not lead to marked changes in repolarization; however, as part of the repolarization reserve together with other currents, I_{to} may also have an impact on repolarization. Because I_{to} has been shown to vary between subendocardial, subepicardial and midmyocardial layers (Litovsky and Antzelevitch, 1988; Antzelevitch and Fish, 2001) it may contribute to spatial repolarization heterogeneity as well.

Sodium – potassium pump current ($I_{Na/K}$)

The $I_{Na/K}$ is due to the activity of the cardiac Na/K pump that hydrolyses adenosine triphosphate (ATP) and is stimulated by increased intracellular cAMP concentrations. This electrogenic ion transport mechanism is mainly responsible for maintaining resting membrane potential and pumps out three Na^+ from the cell in exchange for the inward movement of two K^+ . In the range of membrane potentials corresponding to the action potential and diastole, the pump current is outward (De Weer *et al.*, 1988) and therefore contributes to the repolarization reserve during both systole and diastole. The magnitude of this current depends primarily on intracellular $[\text{Na}^+]$ which explains why $I_{Na/K}$ is so sensitive to frequency of stimulation. In certain pathological settings, including heart failure, Na/K pump expression is altered and $I_{Na/K}$ also shows a characteristic transmural distribution (Gao *et al.*, 2005).

$\text{Na}^+/\text{Ca}^{2+}$ exchange current (I_{NCX})

In physiological conditions, the sarcolemmal NCX is one of the main mechanisms that are responsible for restoring physiological low concentrations of intracellular Ca^{2+} in diastole (Blaustein and Lederer, 1999). Because three Na^+ are transported in exchange for one Ca^{2+} per cycle, the transport

is electrogenic and critically depends on membrane potential and intracellular $[\text{Ca}^{2+}]$, both changing dynamically during the action potential. It is difficult therefore to estimate accurately the current magnitude produced by NCX, and efforts to this end are also hampered by the lack of sufficiently specific inhibitors. At the beginning of the action potential when intracellular $[\text{Ca}^{2+}]$ is low and membrane potential is positive, the NCX current is outward; on the other hand, during the later plateau phase, in early and late repolarization and also during diastole, it is inward. When calcium overload is present, NCX can carry a depolarizing current that may provoke both DAD and EAD contributing to arrhythmogenesis (Bers *et al.*, 2002). NCX also exhibits differences in transmural expression (Xiong *et al.*, 2005) and it is known that NCX is up-regulated in heart failure (Schillinger *et al.*, 2000). Therefore, this trigger mechanism is more likely to induce arrhythmias in case repolarization reserve is decreased, as it is the case in heart failure.

Alterations in calcium cycling

The function and/or density of several ion channels have been shown to be modulated by $[\text{Ca}^{2+}]_i$. Calmodulin, a ubiquitous Ca^{2+} -sensing protein regulates human cardiac sodium channels (Tan *et al.*, 2002), and I_{Na} density was found to be acutely modulated by $[\text{Ca}^{2+}]_i$ (Casini *et al.*, 2009). I_{to} is regulated by Ca^{2+} and calmodulin-dependent protein kinase II (Tessier *et al.*, 1999; Sergeant *et al.*, 2005) and I_{to} is down-regulated following rapid ventricular pacing in a dog model of tachycardiomyopathy via Ca^{2+} /calmodulin-dependent protein kinase II and calcineurin/nuclear factor of activated T-cells systems (Xiao *et al.*, 2008a). Importantly, calmodulin has been shown to be necessary for both correct channel assembly and gating of I_{Ks} (Ghosh *et al.*, 2006; Shamgar *et al.*, 2006) and Ca^{2+} was found to modulate the delayed rectifier potassium current in guinea pig (Tohse *et al.*, 1987) and to inhibit HERG (Schönherr *et al.*, 2000). As these currents play important roles in repolarization, it is reasonable to assume that disease (e.g. heart failure) or drug-induced impairment of calcium cycling will have significant effects on repolarization which might be important for a better understanding of the molecular mechanisms responsible for repolarization reserve. In this regard, the activation of calmodulin-dependent protein kinase II in heart failure and cardiac hypertrophy has been shown to exert direct or indirect effects on the expression and functional properties of I_{to} and I_{K1} (recently reviewed by Nerbonne, 2011). Also, preclinical measures of repolarization reserve, particularly mechanisms underlying beat-to-beat variability of repolarization are probably at least in part related to alterations of Ca^{2+} cycling in the chronic atrioventricular block dog model (Oros *et al.*, 2008). Furthermore, cardiac repolarization alternans is mechanistically related to changes in Ca^{2+} cycling (Pruvot *et al.*, 2004).

Mechanisms of arrhythmias in the setting of impaired repolarization reserve

Transmembrane potassium channel expression patterns show transmural and regional differences, probably creating only

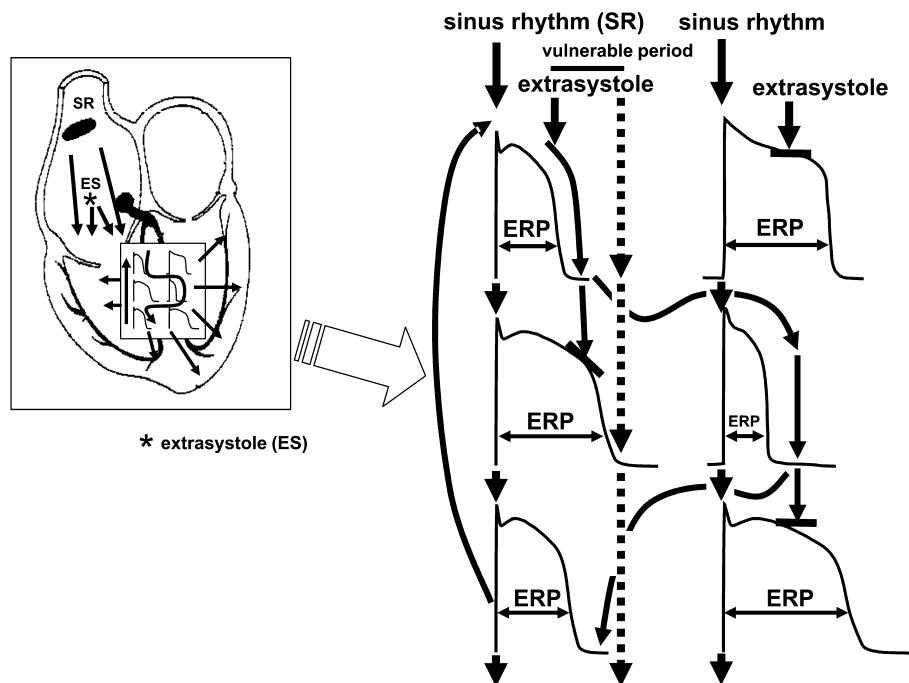


Figure 4

The simplified illustration of the mechanism of arrhythmia development due to lengthening of repolarization and decreased repolarization reserve leading to enhanced spatial repolarization heterogeneity. When the duration of repolarization and therefore the refractory period are prolonged in a heterogenous fashion, that is, the intrinsic transmural/regional repolarization heterogeneities are further augmented by repolarization reserve impairment in cells/regions in a different degree, an arrhythmia substrate is created. An extra stimulus in the vulnerable period then can travel back in a complicated path to its site of origin due to enhanced differences in refractoriness of myocardial cells leading to serious ventricular arrhythmia. Dotted arrows show an extra stimulus occurring after the vulnerable period propagating on the physiological path not leading to serious arrhythmia. ERP, effective refractory period.

relatively small repolarization heterogeneity; however, these differences can be greatly enhanced by impaired repolarization reserve.

The hypothetical mechanism of arrhythmia development due to increased repolarization heterogeneity is shown on Figure 4. In normal circumstances, conduction in the heart is fast ($1\text{--}2\text{ m}\cdot\text{s}^{-1}$) and the duration of myocardial cell action potential is long (200–300 ms). Thus, these cells are resistant to early stimulation because they are in a refractory state. The length of refractoriness is characterized by the effective refractory period. Importantly, in the normal setting the difference between the APD and consequently the effective refractory periods of adjacent cells is very small, creating only small repolarization heterogeneity. Fast conduction and relatively homogenous repolarization prevents the circular re-entry of excitation and arrhythmia will not develop. However, when the duration of repolarization and therefore the refractory period are prolonged in a heterogenous fashion, that is, the intrinsic transmural/regional repolarization heterogeneities are further augmented by repolarization reserve impairment in cells/regions in a different degree, an arrhythmia substrate is created. As a consequence, an extrasystole generated after a normal sinus stimulus can propagate in the direction of cells with shorter APD but its propagation is blocked in the direction of cells with longer APD (Figure 4). Thus, the extra stimulus can travel, usually with reduced conduction velocity, back in a complicated path towards its site of origin and

in other directions where excitability is regained, leading to the generation of TdP or even ventricular fibrillation. Ventricular fibrillation does not revert to sinus rhythm spontaneously in humans and leads to sudden cardiac death without intervention in a few minutes. It is important to note that two independent factors are needed for the arrhythmia described above, and illustrated in Figure 4, to develop. Heterogeneity of repolarization following prolongation of repolarization is itself not sufficient for arrhythmia development, establishing only the possibility of an arrhythmia ('substrate'). In order to induce arrhythmia, an extrasystole in the vulnerable period is needed ('trigger') that can travel the re-entry paths created by the heterogeneous repolarization. The timing of this trigger extrasystole is critical, because before the vulnerable period its conduction is blocked and after the vulnerable period it does not lead to tachycardia or fibrillation, only to a harmless single extrasystole. Increased repolarization heterogeneity leads to longer vulnerability periods and with more frequent extrasystoles, the chances become greater for the generation of serious arrhythmias. In an elegant study, Akar *et al.* (2002) have experimentally demonstrated the development of TdP arrhythmia in dogs based on mechanisms described above (Figure 5). In these experiments, increased transmural repolarization heterogeneity within relatively short transmural distances was observed and the development of TdP was dependent on both bradycardia and administration of the I_{K_r} blocker *d*-sotalol in canine

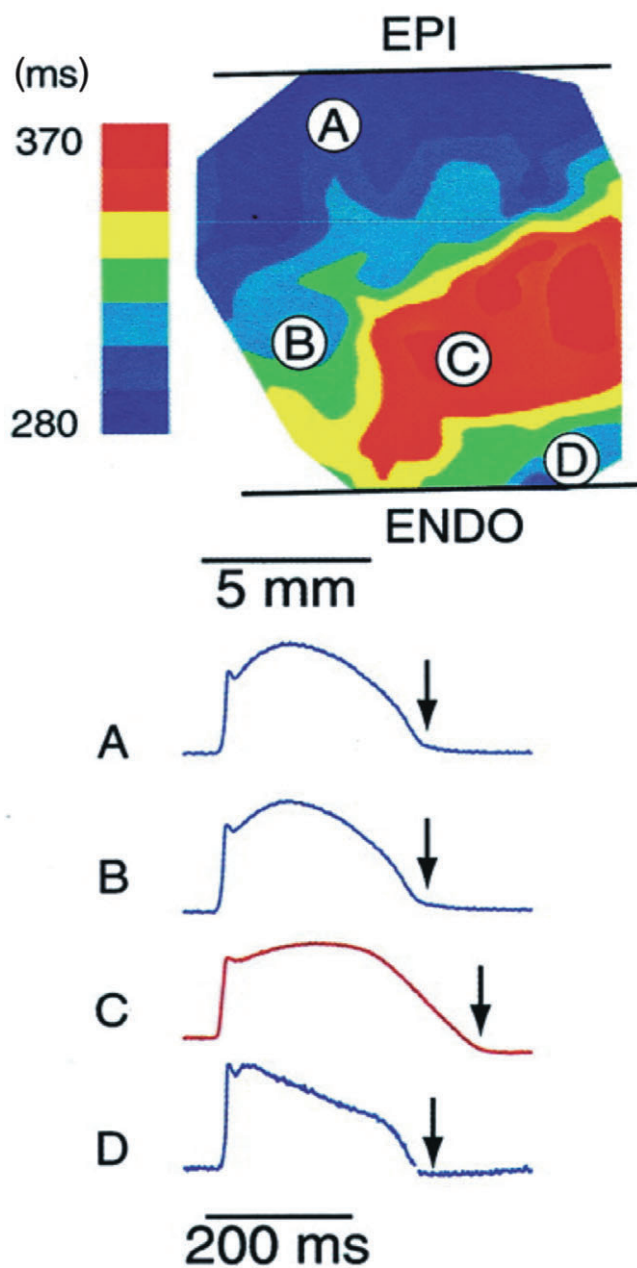


Figure 5

Experimental demonstration of spatial differences in duration of repolarization. Upper panel shows transmural heterogeneity of action potential durations using color codes in bradycardia and *d*-sotalol administration mimicking decreased repolarization reserve in the canine wedge long QT syndrome 2 model. Optical action potentials are shown from selected transmural sites (A–D) on lower panel. Arrows denote the significant differences in the action potential duration within relatively short transmural distances. EPI, epicardium; ENDO, endocardium. Adapted from Akar *et al.* (2002), with permission.

wedge preparations (Figure 5). The steep gradients in the duration of repolarization appear to be mechanistically related to the block and re-entry as shown on Figure 6. These studies also illustrate that additional hits on repolarization

during bradycardia, particularly in midmyocardial cells, where decreased I_{Ks} expression has been shown (Liu and Antzelevitch, 1995) can precipitate TdP. In this model, TdP arrhythmia was induced by applying an extra stimulus that travelled a re-entry path around a region with delayed repolarization (Figure 6), thus, decreased repolarization reserve facilitates arrhythmias at both the substrate and trigger level by increasing repolarization heterogeneity and by decreasing repolarization force during diastole which consequently helps subthreshold depolarizations to reach threshold and to produce propagating extrasystoles.

In addition, when repolarizing force is weakened (I_{K1} , I_{Kr} , I_{Ks}), it would further enhance both normal and abnormal triggered activity by counterbalancing the depolarizing influence of EAD or DAD or I_f to a lesser degree.

Clinical significance of reduced repolarization reserve

It is known that decreased repolarization reserve occurs in pathological settings and in certain special circumstances. Therefore, it should be possible to recognize diminished repolarization reserve. However, such situations are usually complex, and attenuated repolarization reserve can be recognized only retrospectively, sometimes only after a fatal outcome. Some patients with a QTc interval within the normal range may respond to drug treatment with excessive lengthening of repolarization and arrhythmias, occasionally leading to sudden cardiac death. In other patients these drugs do not even prolong cardiac APD or only lengthen it moderately. In accordance with these observations, Kääh *et al.* (2003) reported that patients who experienced TdP arrhythmia with QT prolonging drugs developed more QTc lengthening after i.v. sotalol, an I_{Kr} blocking drug, than those of the control group consisting of patients without history of TdP. An interesting observation of this study showed that in both groups the baseline QTc was normal and did not differ between the two groups (Figure 7). It can be assumed that the striking differences in response to the administered drug were due to differences between the two groups in the repolarization reserve.

Drugs and dietary constituents

Drugs that lengthen ventricular repolarization, measured on the ECG as QTc prolongation, are well known to be susceptible to potentially fatal arrhythmia, such as TdP and ventricular fibrillation (Kannankeril and Roden, 2007). Although drug-induced lethal arrhythmia related to QT prolongation is rare, its existence is well established. In the case of class III antiarrhythmics, the therapeutic mechanism includes manifest prolongation of repolarization by inhibition of one or more types of cardiac potassium channels. The proarrhythmic risk with these drugs is therefore obvious and as such it can be expected and monitored. To deal with the problem posed by non-cardiac drugs that are usually prescribed by non-cardiologists represents a significantly more difficult task. These drugs often do not cause manifest, or at least easily recognizable, cardiac repolarization lengthening because repolarization reserve compensates for relatively

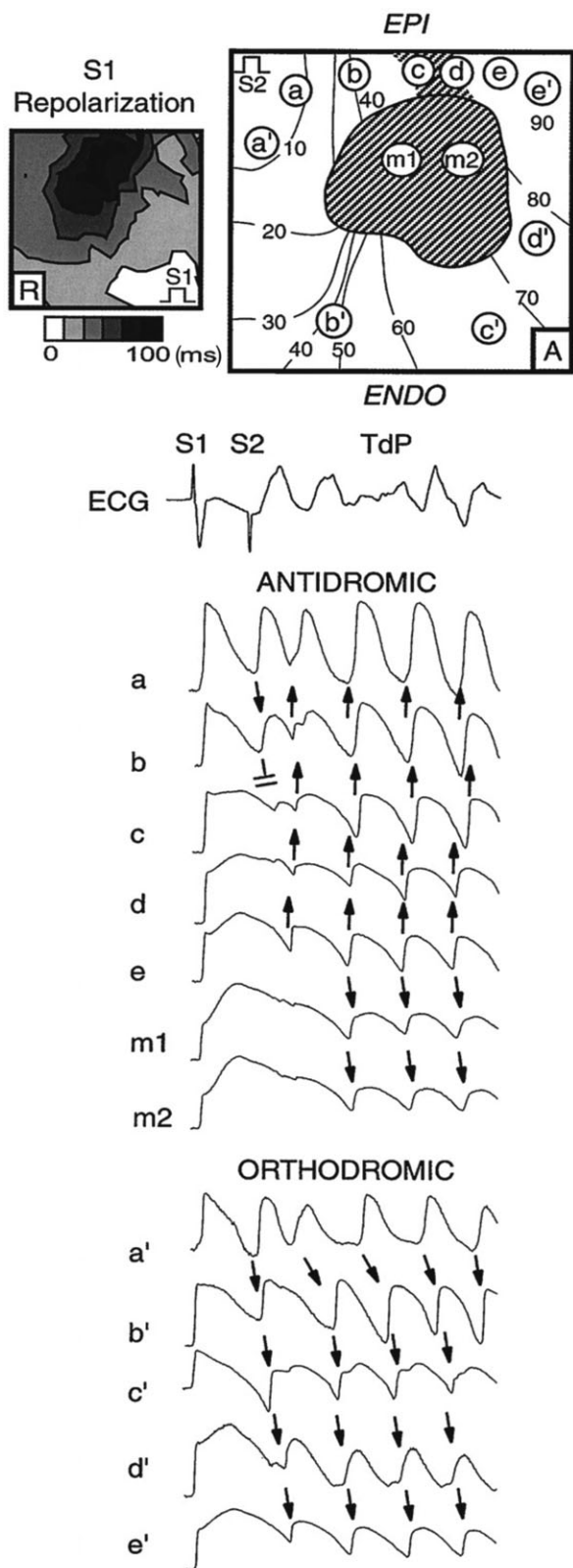


Figure 6

Experimental demonstration of the development of Torsades de Pointes provoked by a premature stimulus (S2) in the canine wedge model during bradycardia and selective I_{Kr} block by *d*-sotalol. Spatial action potential duration heterogeneity created a region with delayed repolarization (c to d and m1, m2) around which the premature stimulus could propagate only in the orthodromic direction (a' to e') but not in the antidromic direction (a to e and m1, m2). EPI, epicardium; ENDO, endocardium; TdP, Torsades de Pointes. Adapted from Akar *et al.* (2002), with permission.

tional factors such as genetic mutation or disease-induced electrophysiological remodelling alone, do not present as clinical symptoms. Moreover, dietary ingredients and supplements may have similar effects. For example, grapefruit juice has been reported to elicit moderate and marginally noticeable QT prolongation (Figure 8) in normal subjects (Zitron *et al.*, 2005) which was attributed to the HERG channel inhibitory property of some of its flavonoid ingredients (Scholz *et al.*, 2005; Zitron *et al.*, 2005). Pink grapefruit juice ingestion has also been found to increase QT variability index in a small set of patients with dilated and hypertensive cardiomyopathies (Piccirillo *et al.*, 2008). In addition, it is well known that grapefruit juice strongly inhibits P-450 which plays an important role of the metabolism of several drugs including those that block cardiac potassium channels and impair repolarization, a mechanism that further contributes to decreased repolarization reserve and enhanced proarrhythmic risk. Importantly, many old drugs, food ingredients, additives and preservatives have never been properly studied in terms of potassium channel pharmacology. Therefore, our current knowledge in this respect may represent only the tip of the iceberg. One must also take into consideration the possible individual variation in the strength of repolarization reserve; thus, evaluation of the effect of drugs on cardiac repolarization by conventional statistical approaches for entire populations can be misleading. One or two consistent positive cases in the study population deserve particular attention even without statistical significance in the incidence of drug-related events between study groups.

Genetic components

A recent study from Boulet *et al.* (2006) described a loss of function mutation in the *KCNQ1* gene encoding the pore-forming subunit of I_{Ks} channel in a 40-year-old woman. This subject had experienced TdP arrhythmia and syncope with a QTc interval of 430 ms (well within the normal range), indicating that a genetic loss of I_{Ks} function alone does not necessarily prolong repolarization. However, in case other unfavourable conditions set in (e.g. hypokalaemia, drug effects, possible down-regulation of potassium channels), the impairment of repolarization reserve by this mutation can diminish the necessary protection, making this patient more susceptible to arrhythmia than those with normal I_{Ks} channels.

This case highlights the importance of 'silent', 'subclinical' or 'concealed' long QT syndrome (Varró and Papp, 2006) where repolarization processes are not apparently affected as measured by conventional diagnostic tools, but where the

weak potassium channel inhibition. Alternatively, relatively weak potassium channel inhibition by drugs can be a source of decreased repolarization reserve which manifests as repolarization prolongation and arrhythmia in cases where addi-

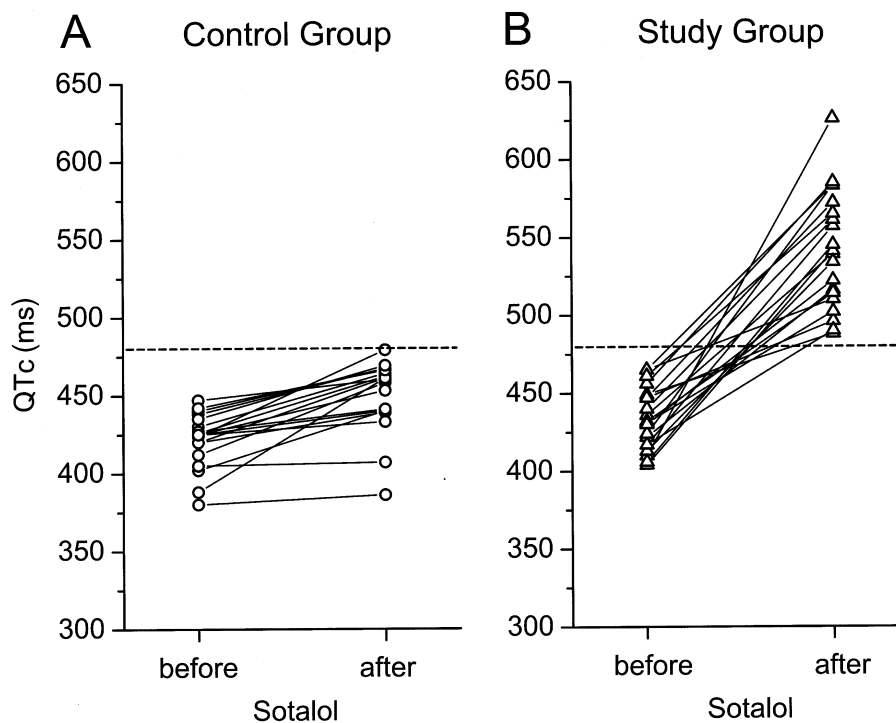


Figure 7

Individual QTc intervals in control individuals and in patients with suspected acquired long QT syndrome (study group) before and after intravenous administration of sotalol. The dotted line indicates a cut-off value of 480 ms that distinguished best between the study population and the control group. From Kääh *et al.* (2003), with permission.

repolarization reserve is impaired. These individuals may be more susceptible to drug-induced arrhythmias than those with normal genetic backgrounds.

Another very interesting case illustrating decreased repolarization reserve due to congenital factors involved a 31-year-old female patient with known asymptomatic LQT syndrome (Hermans *et al.*, 2003). The patient was also diabetic and was admitted to hospital with polydipsia where she developed convulsive syncope due to TdP arrhythmic episodes and exhibited an excessively long QTc interval of 580 ms on the ECG. She drank large amounts of grapefruit juice and tonic water because of her polydipsia before admission. Following discontinuation of these drinks, the patient's QTc interval decreased to 450 ms and the TdP episodes disappeared. In this patient, ventricular repolarization reserve was markedly impaired by congenital LQT syndrome and autonomic neuropathy due to diabetes mellitus; however, she had asymptomatic LQT. Significant QTc prolongation with concomitant TdP development most likely occurred as a result of multiple additional hits on ventricular repolarization: ingestion of HERG blocking flavonoids in grapefruit juice (naringin, naringenin, hesperetin, morin, etc.) and quinine in tonic water.

Ion channel polymorphisms represent a particularly interesting chapter in investigations on repolarization reserve, because they can cause mild ion channel dysfunction, however, might have relatively high incidence in the general population and were also reported to be associated with drug-induced arrhythmias (Sesti *et al.*, 2000; Roden,

2004; Darbar and Roden, 2006; McBride *et al.*, 2009). These polymorphisms highlight individual differences in repolarization reserve and therefore in drug-induced proarrhythmia susceptibility in the population.

Chronic heart failure

Heart failure is still a devastating disease with a 5 year life expectancy worse than cancer, with approximately half of heart failure patients dying due to sudden cardiac death caused by lethal arrhythmias and not due to end-stage pump failure (Packer, 1985; Kjekshus, 1990). It has been shown that both electrophysiological and structural remodelling occurs during the progression of heart failure. This includes the substantial degree of reduction of the repolarization reserve, due to down-regulation of different types of potassium channels. The results obtained so far regarding the nature of potassium channel down-regulation are conflicting, depending on the form of the disease (hypertrophic vs. dilated), experimental model (pacing vs. valve destruction) and the species studied (rat, rabbit, dog). In general, it has been reported that I_{K1} , I_{Ks} , I_{to} and I_{Kr} channels were down-regulated (Kääh *et al.*, 1998; Näbauer and Kääh, 1998; Li *et al.*, 2002). The persistent or slowly inactivating sodium current is also increased in heart failure (Valdivia *et al.*, 2005). This latter change in the presence of reduced repolarization reserve results in APD lengthening and increased repolarization heterogeneity, a substrate for arrhythmias. The up-regulation of NCX, pacemaker current (I_f) and malfunction of the ryanodine receptor proteins (Stillitano *et al.*, 2008; Tateishi *et al.*, 2009) enhance

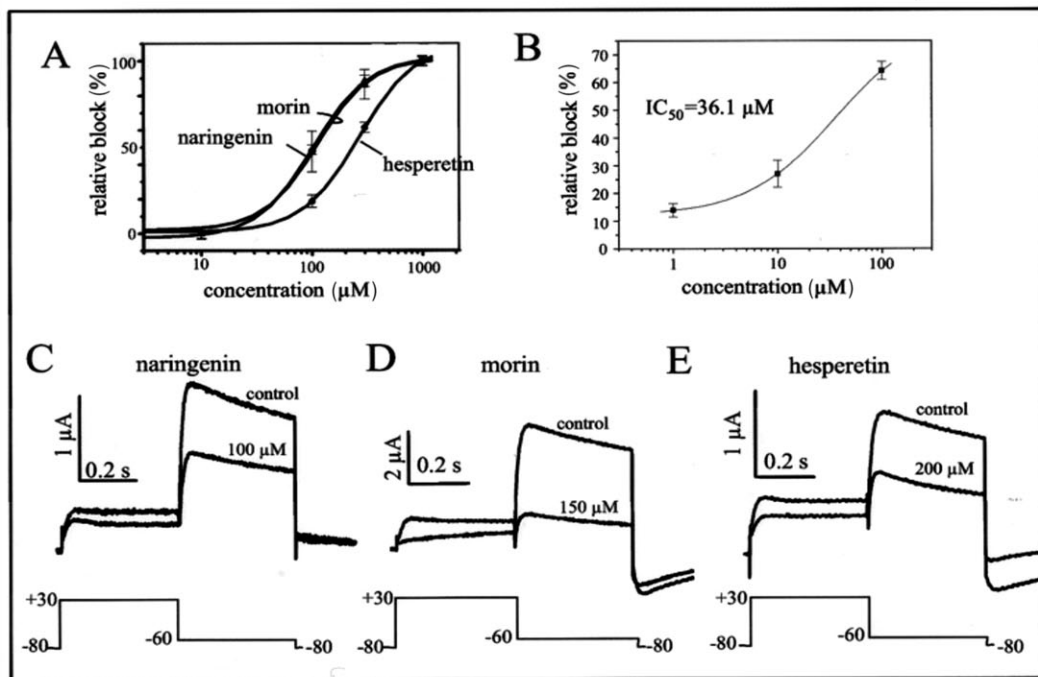
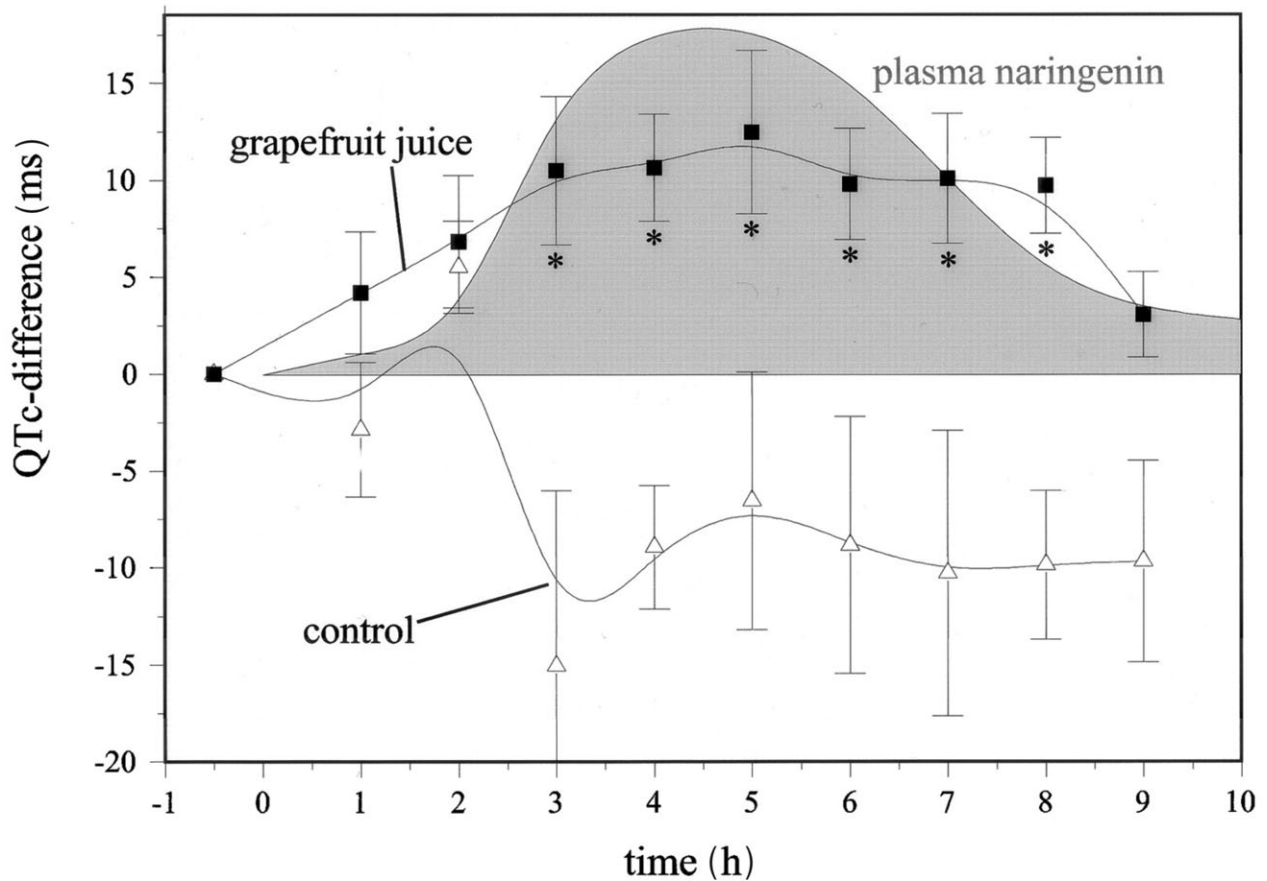


Figure 8

Prolongation of repolarization following grapefruit juice ingestion in healthy volunteers (upper panel) and inhibition of the human ether-a-go-go-related gene current by grapefruit juice flavonoids naringenin, morin and hesperetin in *Xenopus* oocytes (lower panel). From Zitron *et al.* (2005), with permission.

ectopic activity because transient depolarizations are not opposed properly during diastole due to impaired repolarization reserve which later also contributes to the resting potential. These also increase arrhythmia risks by trigger mechanisms.

Diabetes mellitus

Repolarization abnormalities, including mild prolongation of the QT interval have been observed in patients with diabetes mellitus (Giunti *et al.*, 2007); thus, both type 1 and type 2 diabetes mellitus are associated with increased risk for sudden cardiac death (Gill *et al.*, 2008), which is not attributable to general pathophysiological changes, such as atherosclerosis, hyperlipidaemia or hypertension, commonly observed in diabetes. The mechanisms responsible for repolarization abnormalities in long term chronic diabetes are not well explored. The reduction of I_{to} , I_{Ks} and I_{Kr} in different diabetic animal models (Shimoni *et al.*, 1998; Lengyel *et al.*, 2007b) of short-term type 1 diabetes suggests that decreased density of these currents may contribute to reduced repolarization reserve in diabetes mellitus. In accordance with these experimental data, increased beat-to-beat QT variability was also recently reported (Lengyel *et al.*, 2008) in patients with type 1 diabetes mellitus.

In addition to the repolarization abnormalities caused by endocrine diseases, compounds used for the treatment of these pathologies can also adversely influence cardiac ventricular repolarization resulting in QTc prolongation and impairment of repolarization reserve (Gonzalez *et al.*, 2010).

Gender

It is well known that women are at a significantly greater risk than men for developing TdP arrhythmia in response to drugs that prolong cardiac repolarization. This is likely to be related to the fact that women have longer QTc and reduced repolarization reserve compared with men (James *et al.*, 2007).

The basis for the gender differences in risk of TdP and QTc is not clear. This topic has been under intensive investigation currently (Rodriguez *et al.*, 2001; James and Hancox, 2003; Kurokawa *et al.*, 2008; Ridley *et al.*, 2008) and gender-related differences in cardiac electrophysiology were recently reviewed in detail by Jonsson *et al.* (2010). It can be assumed that gender differences are related to the influence of sex hormones on cardiac repolarization and the underlying transmembrane currents. This notion is supported by findings showing at birth QTc intervals are very similar in males and females (Stramba-Badiale *et al.*, 1995) and during puberty, the QTc intervals are shorten in men (Rautaharju *et al.*, 1992).

Evidence suggests that oestrogen reduces I_{Kr} and prolongs repolarization (Kurokawa *et al.*, 2008) while testosterone increases I_{Kr} (Ridley *et al.*, 2008). Sex hormones can also alter the interactions of drugs with these potassium channels (James and Hancox, 2003; Kurokawa *et al.*, 2008). A smaller I_{to} and a larger $I_{Ca,L}$ were measured in female cardiomyocytes (Verkerk *et al.*, 2005); however, these results were obtained using cells isolated from failing hearts.

Interestingly, when studying I_{Kr} block-induced QTc prolongation in women at different stages of the menstrual cycle,

drug-induced QTc prolongation was greatest during menses and at the ovulatory stage of the cycle, compared with the luteal phase (Figure 10), despite the fact that in pre-drug conditions the QTc did not differ in the cycle (Rodriguez *et al.*, 2001). This observation suggests that progesterone may be protective by opposing repolarization lengthening caused by I_{Kr} inhibition. In a recent study, it was shown that testosterone activated HERG channels via androgen receptors (Ridley *et al.*, 2008). This finding further emphasizes the possibility of gender-based adverse consequences of drug-receptor interactions.

A very recent review has concluded that testosterone mediates the increased repolarization reserve in men by decreasing $I_{Ca,L}$ and enhancing I_K and therefore testosterone plays a significant role in the reduced susceptibility to drug-induced ventricular arrhythmias in males (Jonsson *et al.*, 2010).

Renal failure

It is known that patients with chronic renal failure are at increased risk for ventricular arrhythmia and sudden death (Johansson *et al.*, 2004). In chronic renal failure, QT dispersion (Lórinicz *et al.*, 1999) and the QT variability index are increased compared with normal subjects. These results suggest the presence of impairment of repolarization reserve. Chronic renal failure patients are prone to electrolyte derangements both due to the disease and medications used for its treatment. Importantly, rapid electrolyte shifts may also alter repolarization (Severi *et al.*, 2008) and significantly contribute to the high, arrhythmia-related cardiac mortality rates seen in dialysis patients (Herzog *et al.*, 2008). However, it must be also noted that chronic renal failure patients are usually co-morbid patients, that is, they have additional diseases such as diabetes, coronary artery sclerosis, hypertension, cardiac hypertrophy and are on multiple medications (for a recent review, see Schmid *et al.*, 2010). All these factors may affect repolarization, making it difficult to determine the extent renal dysfunction contributes to alterations in repolarization reserve.

Body temperature abnormalities

Fever can indirectly interfere with repolarization reserve which may have important clinical implications (Burashnikov *et al.*, 2008). Elevation of body temperature by 1–3°C enhances both inward and outward currents. Therefore, pharmacological inhibition, loss of function mutations or down-regulation of potassium currents may shift the balance of currents towards depolarization and thereby unexpected repolarization lengthening may occur when body temperature increases. On the other hand, hypothermia has also been shown to profoundly affect cardiac repolarization by prolonging APD in guinea pig hearts (Duker *et al.*, 1987) and papillary muscle (Bjørnstad *et al.*, 1993) and by promoting monophasic APD heterogeneities in open chest dogs (Kuo *et al.*, 1983). In a recent study using canine left ventricular wedge preparations Piktel *et al.* (2011) found that therapeutic hypothermia (cooling to 32°C) increased dispersion of repolarization to a lesser degree and was associated with decreased arrhythmia susceptibility compared with severe hypothermia (cooling to 26°C) and subsequent rewarming.

Hypokalaemia

Hypokalaemia is a common factor that can decrease repolarization reserve. Low extracellular potassium increases I_{Ks} and I_{K1} according to the Nernst equation but paradoxically reduces I_{Kr} and prolongs QT interval (Sanguinetti and Jurkiewicz, 1992; Yang *et al.*, 1997). Furthermore, lowering of extracellular potassium was reported to increase drug-induced I_{Kr} block (Yang and Roden, 1996), although this latter observation has recently been questioned (Limberis *et al.*, 2006).

Hypothyroidism

Hypothyroidism decreases (i.e. down-regulates) a number of different potassium currents (Bosch *et al.*, 1999; Le Bouter *et al.*, 2003) and pacemaker channels (Le Bouter *et al.*, 2003), resulting in cardiac repolarization lengthening (Galetta *et al.*, 2008) and bradycardia (Galetta *et al.*, 2008). Even in its sub-clinical form, it decreases repolarization reserve as reflected by an increased QTc dispersion and prolonged QTc (Galetta *et al.*, 2008). The reduction of repolarization reserve induced by hypothyroidism is also associated with an increased incidence of TdP arrhythmias (Fredlund and Olsson, 1983).

Increased sympathetic tone

As mentioned earlier, increased sympathetic tone enhances I_{Ks} , I_{Ca} and possibly I_{Kr} (Volders *et al.*, 2003; Lengyel *et al.*, 2004). In this situation, pharmacological inhibition, loss of function mutation and down-regulation of I_{Ks} and possibly I_{Kr} all tend to leave the enhanced depolarizing effect of augmented I_{Ca} unopposed, producing repolarization lengthening. In this regard, increased instability of repolarization was demonstrated *in vivo* in dogs following intense β -adrenergic receptor stimulation followed by ventricular extrasystoles and TdP (Gallacher *et al.*, 2007). A very recent *in vitro* study by Johnson *et al.* (2010) has shown that β -adrenergic stimulation of I_{Ks} rescued dog left ventricular myocytes from excessive increase in beat-to-beat variability of their APD following I_{Kr} blockade and augmented late I_{Na} (impaired repolarization reserve). Also, β -adrenergic stimulation elicited largely increased repolarization variability in these cells when I_{Ks} was pharmacologically inhibited. As both I_{Ks} and I_{Kr} exhibit transmural and apico-basal heterogeneity (Antzelevitch, 2007), dispersion of repolarization can also be substantially increased in these circumstances providing further substrate for arrhythmia.

In addition, elevated sympathetic tone enhances I_f and spontaneous Ca^{2+} release from sarcoplasmic reticulum favouring the triggering of arrhythmias. In accordance with this, in LQT1 (I_{Ks} loss of function mutation) and LQT2 (I_{Kr} loss of function mutation), but not in LQT3 (I_{Na} gain function mutation), sympathetic tone is well recognized as a precipitating factor for arrhythmias.

Competitive athletes

Top athletes represent a special population where repolarization reserve may play an important role (Varró and Baczkó, 2010). Competitive sports activities and training induces physiological adaptation in the cardiovascular system leading to the development of athlete's heart that is characterized by reversible cardiac hypertrophy, where down-regulation of

certain potassium channels can be expected (Hart, 2003). In top competitive athletes, significant bradycardia develops that is another feature of athlete's heart and is attributed to increased vagal activity. Vagally mediated bradycardia has been identified as a key factor in the induction of TdP arrhythmias in an *in vivo* rabbit proarrhythmia model (Farkas *et al.*, 2008).

In competitive athletes, mild changes in repolarization reserve alone probably do not represent a significant risk for arrhythmia development, but the effects of otherwise mild factors such as different medications, bradycardia, certain ingredients of dietary supplements, early and undiagnosed cardiomyopathy and doping agents (steroids or amphetamines) on repolarizing currents can superimpose and result in repolarization abnormalities that can occasionally lead to sudden cardiac death (Maron, 2007). Indeed, in spite of the fact that the preconditioning effect of regular exercise has been shown to protect against arrhythmias (Szekeres *et al.*, 1993) and tissue damage (Das and Das, 2008), top athletes have two to four times higher mortality due to sudden cardiac death compared with age and sex matched controls not involved in sports activities (Corrado *et al.*, 2007).

Repolarization reserve: a dynamically changing factor

Recent research indicates that repolarization reserve is not a static feature of cardiac tissue but a dynamically changing one. After 24 h incubation with dofetilide, a potent I_{Kr} blocker, continuously paced dog ventricular myocytes elicited shorter APD and blunted dofetilide response (Xiao *et al.*, 2008b). Patch-clamp measurements revealed enhancement of I_{Ks} in cultured myocytes incubated with dofetilide compared with I_{Ks} in control cells. Accordingly, the KvLQT1 and MinK protein levels were increased. Interestingly, the mRNA for both KvLQT1 and MinK did not change, suggesting regulatory events at the posttranslational level. In the same experiments, the expression of microRNA 133a and 133b (miR-133a and miR-133b) were diminished in cells previously incubated with dofetilide. In another earlier study, it was shown that muscle specific miR-133 depressed KvLQT1 protein expression without changing KvLQT1 mRNA (Luo *et al.*, 2007). Based on these results, it was proposed that sustained reduction of I_{Kr} leads to compensatory up-regulation of I_{Ks} consistent with a feedback control of ion channel expression. This newly recognized modulation of repolarization reserve might happen via changes in certain miRNA levels working at the translational level. In fact, enhanced miR-133 expression was associated with a decreased density of ERG protein and I_{Kr} in diabetic rabbit hearts (Xiao *et al.*, 2009). These results are not without controversy and further studies are needed to confirm them; even so, they suggest that repolarization reserve might be regulated and altered by disease and chronic drug treatment and this should be taken into account in developing future therapies.

Measurement of repolarization reserve

Despite its potential importance, the estimation of cardiac repolarization reserve of individual patients is not a common

procedure in clinical practice. One may argue that it would be more appropriate to test individuals for their status of repolarization reserve and apply drugs accordingly, rather than restrict drug development if the compound is suspected to cause HERG inhibition or QTc lengthening. It is very likely that patients with normal repolarization reserve would not respond to drugs with mild-to-moderate K⁺ channel inhibitory properties with arrhythmias and sudden cardiac death. However, patients with diminished repolarization reserve may do so. Therefore, reliably predictive non-invasive tests in clinical settings and easy to use animal models are sorely needed. In this regard, it is clear that the pharmaceutical industry needs novel and reliable models that are able to go beyond the assessment of QT interval alterations caused by developmental drugs, thus enabling the industry to predict the proarrhythmic side effects of compounds under development (Pugsley *et al.*, 2009).

Due to the complex nature of repolarization reserve, its accurate measurement in a quantitative manner is difficult. In experimental settings, repolarization reserve based on theoretical considerations can be estimated by drugs which increase depolarization force, as would be produced by veratridine or the toxin ATX II. In these cases, the degree of repolarization prolongation produced would be expected to be opposed by the sum of repolarizing potassium currents. If limited APD prolongation is observed, then a strong repolarization reserve is predicted. Conversely, substantial APD lengthening argues for weak repolarization reserve. For pragmatic reasons, it is more appropriate to test HERG/I_{Kr} channel inhibition, and analyse the evoked repolarization lengthening. This is based on the common experience that most drugs reported to cause TdP arrhythmias inhibit I_{Kr} and the repolarization reserve in these cases would include repolarizing currents largely other than I_{Kr}. Drug testing for safety pharmacology purposes, should also be performed using preparations in which repolarization reserve had been previously attenuated by ion channel remodelling (Volders *et al.*, 1999) or by pharmacological means (Figure 9) (Lengyel *et al.*, 2004; 2007a). Special techniques based on action potential configurations have also been described to assess proarrhythmic drug effects based on repolarization instability (Hondeghem and Carlsson, 2001) at the preclinical level.

Differences in the QT intervals of consecutive heart beats, measured as the variability index (Thomsen *et al.*, 2004) or short-term beat-to-beat QT variability (Berger, 2003), have been suggested as a measure of proarrhythmic risk related to changes in repolarization reserve. The collected results are encouraging. These studies (Thomsen *et al.*, 2004; Lengyel *et al.*, 2007a) suggest that the development of TdP arrhythmia in dogs and rabbits correlated better with short-term QT variability than with QTc changes in general (Figure 10). In this context, the short-term temporal beat-to-beat variability of the QT interval has been found to reflect latent repolarization disorders and increased susceptibility to sudden cardiac death in patients with nonischaemic heart failure with no marked prolongation of the QT interval (Hinterseer *et al.*, 2010). Also, short-term variability of the QT interval was found to be increased in humans with congenital LQT syndromes (Hinterseer *et al.*, 2009).

Very recently, a novel parameter, the so-called 'absolute' variability has also been suggested to show good correlation

between repolarization instability and development of TdP arrhythmias in an *in vivo* rabbit proarrhythmia model (Farkas *et al.*, 2010). Further studies are needed to validate the utility of this parameter using compounds that had been withdrawn from the market due to their torsadogenic side effects.

At the clinical level, as data shown on Figure 8 suggests, simple QTc measurements from ECG recordings are not sufficient to measure repolarization reserve. Applying other ECG parameters such as QTc dispersion, Tpeak to Tend and T-wave morphology analysis, also give uncertain results. Provocative pharmacological tests have been suggested (Kilborn *et al.*, 2000) but these may raise ethical concerns. In this study, intravenous application of a short acting I_{Kr} blocking class III antiarrhythmic drug, ibutilide, lengthened QTc and the magnitude of this effect had a good Gaussian distribution (Figure 11). Furthermore, the long QTc edge of the curve (indicated by squares), and the subjects far out of the range (indicated by a circle), may identify patients with decreased repolarization reserve and increased risk for proarrhythmia following drug treatment (Kilborn *et al.*, 2000).

Lengyel *et al.* (2004) showed that eliprodil, a new investigational neuroprotective drug, did not change cardiac repolarization significantly in normal rabbit hearts, but lengthened it substantially accompanied by TdP episodes in preparations where repolarization reserve was previously reduced by inhibition of I_{K1} (Figure 12).

Possible pharmacological enhancement of repolarization reserve

As mentioned previously, decreasing repolarization reserve by drugs can have detrimental consequences by increasing the risk of proarrhythmia. As such, decreasing repolarization reserve represents an important adverse side effect of drug treatment. In addition, it must be emphasized that drugs which block multiple potassium channels without decreasing inward currents such as I_{Na} or I_{Ca} are expected to have higher proarrhythmic risk than selective potassium channel inhibitors because they tend to further depress repolarization reserve (Kodama *et al.*, 1999; Biliczki *et al.*, 2002). Therapeutically, it would be useful to enhance repolarization reserve and thereby attenuate the proarrhythmic risk caused by repolarization abnormalities.

Inhibition of inward sodium and calcium currents may represent pharmacological interventions that may have indirect beneficial effects on repolarization reserve. Amiodarone, a drug that inhibits both I_{Na} and I_{Ca}, is one of the best examples illustrating beneficial actions on repolarization. Amiodarone lengthens repolarization substantially but its proarrhythmic effect is much less than that of class III antiarrhythmics which lengthen APD by inhibiting outward potassium currents only. One may speculate that for these purposes, relatively weak I_{Na} and I_{Ca} block would be sufficient, because persistent (or window) inward currents seem more sensitive to drug-induced inhibition than the corresponding peak currents (Kodama *et al.*, 1999). In addition, if peak inward currents are substantially affected, impairment of impulse conduction both at the ventricular and atrioventricular levels would be expected with concomitant side

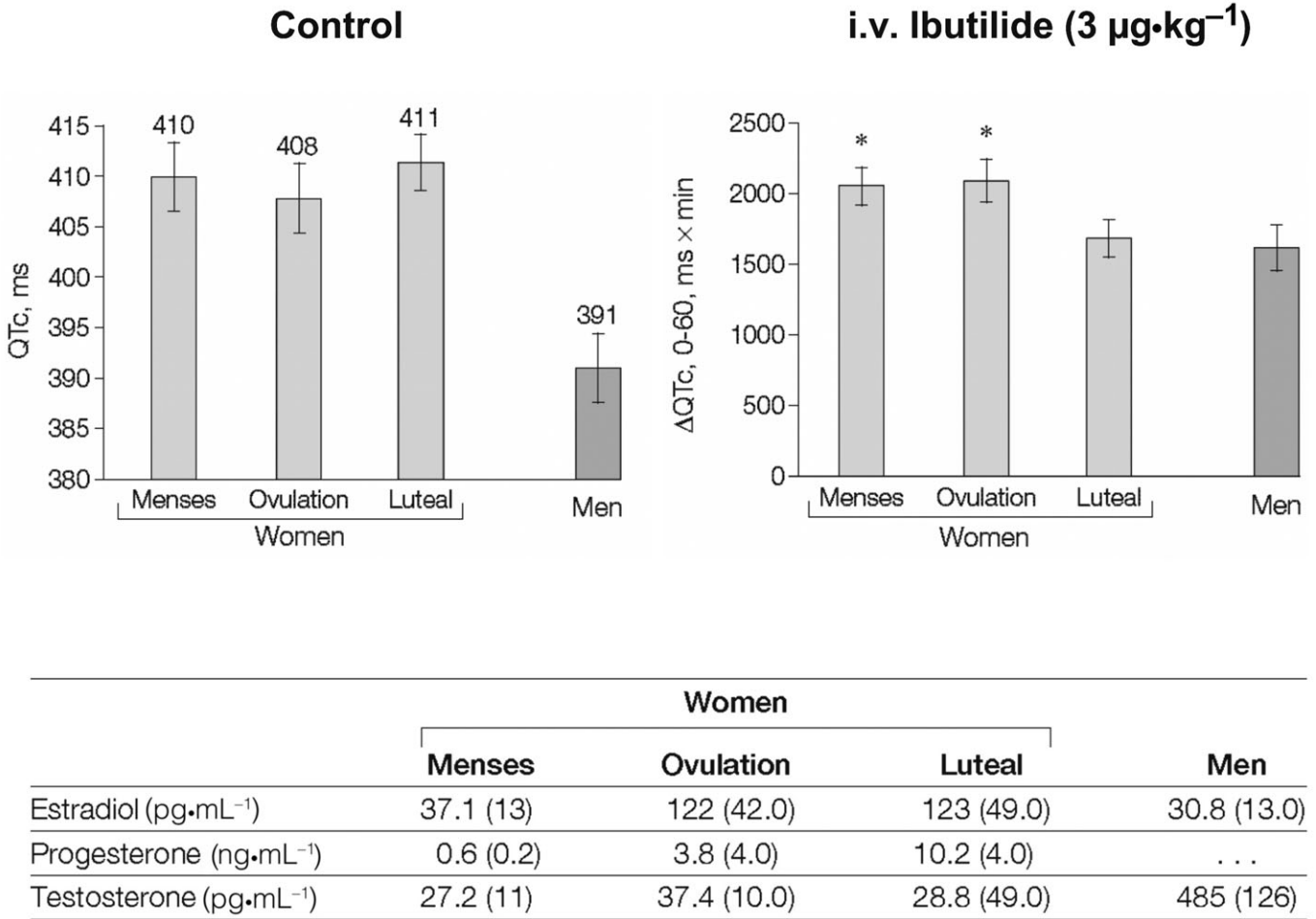


Figure 9

Possible influence of hormonal changes during the menstrual cycle on repolarization reserve. Left panel shows baseline QTc intervals in women during the different phases of the menstrual cycle and in men. Right panel shows mean change in QTc interval area under the curve during the first hour following ibutilide (I_{kr} blocker) infusion. Lower panel table shows sex hormone levels in study subjects, with low levels of estradiol and progesterone during menses, a peak of estradiol during ovulation, and the highest values of progesterone during the luteal phase. Men had the lowest estradiol serum levels, and their testosterone level was 15 times higher than in women. Table data are presented as mean (SD). These data suggest that progesterone may oppose I_{kr} block-induced repolarization lengthening in women. From Rodriguez *et al.* (2001), with permission.

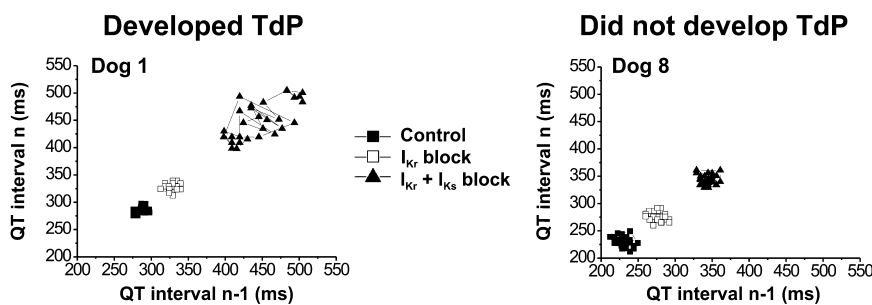


Figure 10

The development of Torsades de Pointes (TdP) arrhythmia correlated better with the increase in short-term QT variability than with QTc changes in dogs. Two representative individual Poincaré plots show increased short-term variability of QT following combined I_{kr} (by dofetilide) and I_{ks} (by HMR-1556) block in a dog that later developed TdP (left panel) compared with a dog where TdP did not occur (right panel). From Lengyel *et al.* (2007a), with permission.

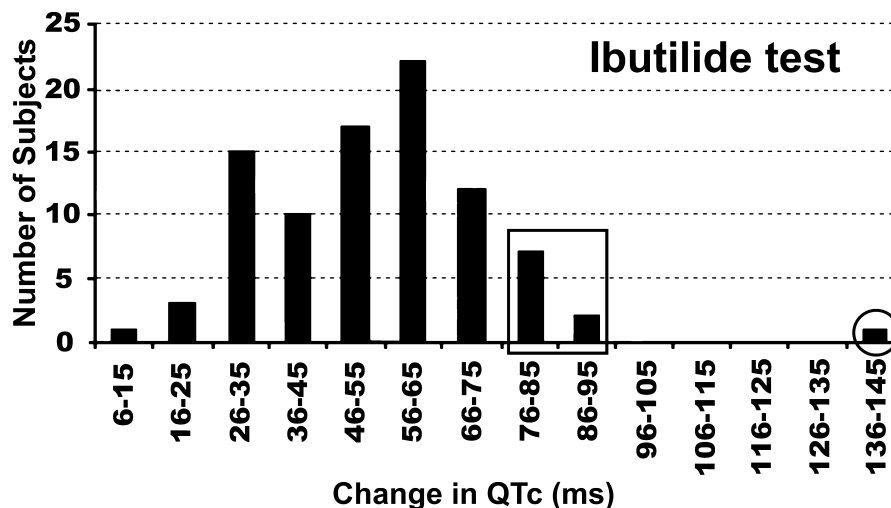


Figure 11

Possible clinical manifestation of reduced repolarization reserve. Distribution of QTc prolongation in patients following ibutilide (I_{Kr} blocker) administration. Patients who had QTc prolongation far out of range may be individuals with decreased repolarization reserve and increased proarrhythmic risk (indicated by circle). From Kilborn *et al.* (2000), with permission.

effects of proarrhythmia and decreased force of contraction. These effects may have detrimental consequences, especially in diseased hearts.

Another option is to enhance repolarization reserve by increasing outward potassium currents. The best candidate in this case is I_{Ks} , because stimulating this current should not abbreviate repolarization markedly when APD is in the normal range; however, as a negative feedback mechanism, enhanced I_{Ks} would limit excessive proarrhythmic repolarization lengthening. In this regard, the benzodiazepine derivative L-364,373 enhanced I_{Ks} in guinea pig and rabbit myocytes (Salata *et al.*, 1998; Xu *et al.*, 2002); however, this effect was not confirmed in dog (Magyar *et al.*, 2006). Further studies are needed on the effect and potency of L-364,373 before firm conclusions can be drawn. In a recent study, the pharmacological activation of I_{Ks} by the benzodiazepine R-L3 resulted in enhanced repolarizing capacity and counteracted pharmacologically reduced I_{Kr} in isolated guinea pig cardiomyocytes (Nissen *et al.*, 2009).

Recent data indicates that I_{Kr} can be augmented by NS-3623 and NS-1643, novel compounds that shorten ventricular action potentials in different preparations (Hansen *et al.*, 2006a,b; 2007; Diness *et al.*, 2008). NS-3623 was also shown to elicit post-repolarization refractoriness as an additional antiarrhythmic mechanism (Hansen *et al.*, 2007).

Repolarization reserve could also be enhanced by opening ATP-sensitive potassium channels (K_{ATP}). There are a number of compounds that activate K_{ATP} (Lathrop *et al.*, 1990; Carlsson *et al.*, 1992; Jahangir and Terzic, 2005), but these are not sufficiently selective for the cardiac sarcolemmal K_{ATP} channels. Therefore, unwanted effects (e.g. hypotension and altered glucose homeostasis) resulting from activation of K_{ATP} channels expressed in other tissues can be expected following their application. However, pinacidil and nicorandil have been shown to suppress abnormal automaticity, triggered

activity and TdP in a guinea-pig LQT1 model (Yang *et al.*, 2004). It should be noted that excessive cardiac sarcolemmal K_{ATP} activation would shorten action potential in the myocardium promoting the development of re-entry arrhythmias (Janse and Wit, 1989).

Conclusions

Repolarization reserve is a relatively new concept that is helpful in explaining the development of arrhythmias based on repolarization abnormalities and drug-induced proarrhythmic adverse effects. According to this concept, inhibition or functional impairment of one transmembrane ion channel does not necessarily result in excessive repolarization changes because other currents can compensate. Impaired repolarization reserve, which is common in certain pathological settings and following drug treatment, is not easily detected by conventional *in vivo* or *in vitro* electrophysiological measurements. In some circumstances, however, reduction in repolarization reserve leads to marked repolarization lengthening and is proarrhythmic. Better predictive methods are needed to identify the conditions in which preventable arrhythmias due to impaired repolarization reserve occur. Another important future goal is to avoid discrediting otherwise useful novel compounds solely on their minor I_{Kr} (HERG) blocking properties. Instead, the repolarization reserve status of individual patients could be assessed when they have a specific need for a drug with known effects on cardiac repolarization and thus unwanted drug-induced arrhythmias could be limited in these individuals as, for example, is the case with patients with a specific requirement for penicillin therapy who have presumed penicillin allergy.

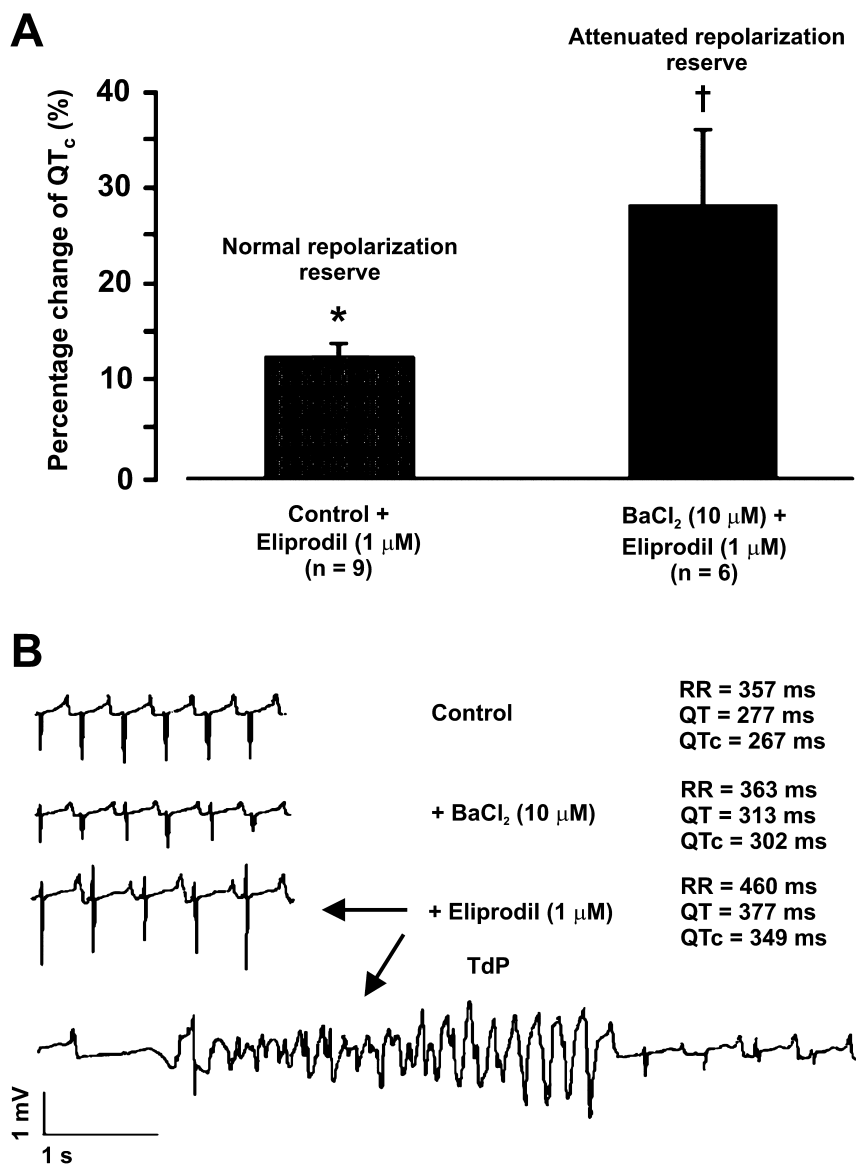


Figure 12

Repolarization (QT_c) lengthening effect of eliprodil, a new investigational neuroprotective compound, in Langendorff-perfused rabbit hearts with normal and attenuated repolarization reserve (upper panel). Eliprodil never induced arrhythmias in normal hearts but often caused Torsades de Pointes (TdP) arrhythmias in preparations where repolarization reserve was reduced via I_{K1} block by BaCl₂ (lower panel). From Lengyel *et al.* (2004), with permission.

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Conflict of interest

The authors declare that they have no conflicts of interest related to this work. Both authors have made written contributions to this manuscript, and have approved the final version.

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