PHYSIOLOGY IN MEDICINE

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Molecular Biology of K^+ Channels and Their Role in Cardiac Arrhythmias

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The configuration of cardiac action potentials varies considerably from one region of the heart to another. These differences are caused by differential cellular expression of several types of K^+ channel genes. The channels encoded by these genes can be grouped into several classes depending on the stimulus that permits the channels to open and conduct potassium ions. K^+ channels are activated by changes in transmembrane voltage or binding of ligands. Voltage-gated channels are normally the most important players in determining the shape and duration of action potentials and include the delayed rectifiers and the transient outward potassium channels. Ligand-gated channels include those that probably have only minor roles in shaping repolarization under normal conditions but, when activated by extracellular acetylcholine or a decrease in the intracellular concentration of ATP, can substantially shorten action potential duration. Inward rectifier K^+ channels are unique in that they are basically stuck in the open state but can be blocked in a voltage-dependent manner by intracellular Mg^{2+}, Ca^{2+}, and polyamines. Other K^+ channels have been described that provide a small background leak conductance. Many of these cardiac K^+ channels have been cloned in the past decade, permitting detailed studies of the molecular basis of their function and facilitating the discovery of the molecular basis of several forms of congenital arrhythmias. Drugs that block cardiac K^+ channels and prolong action potential duration have been developed as antiarrhythmic agents. However, many of these same drugs, as well as other common medications that are structurally unrelated, can also cause long QT syndrome and induce ventricular arrhythmia. **Am J Med. 2001;110:50–59.** ©2001 by Excerpta Medica, Inc.

PHYSIOLOGICAL ROLES AND MOLECULAR BASIS OF CARDIAC VOLTAGE-GATED K^+ CHANNELS

The initial upstroke of the cardiac action potential is determined by the opening and closing of Na^+ channels. The configuration and rate of repolarization of action potentials are controlled by many types of K^+ channel currents that differ with respect to their kinetics and density in the plasma membrane (Figure 1). Initial repolarization (phase 1) is mediated by the opening of transient outward K^+ channels. This is followed by a plateau (phase 2) that is characterized by high membrane resistance resulting from the almost equal flow of outward currents through delayed rectifier K^+ channels (I_{Kr}, I_{Ks}, I_{Kur}) and inward flow of current through L-type Ca^{2+} channels. The rate of terminal repolarization (phase 3) is enhanced after the plateau phase because of the increasing conductance of the rapid delayed rectifier K^+ current (I_{Kr}) and the inward rectifier K^+ current (I_{K1}). These currents were discovered and characterized using voltage clamp techniques, which permit precise control of the membrane potential and measurement of ion currents under carefully controlled ionic conditions. Although voltage-clamp studies are useful in defining the properties of these currents, their molecular identities had to await the advancement of molecular cloning techniques. Starting with the cloning of the Shaker K^+ channel from *Drosophila* in 1987 (1), many K^+ channels have been cloned. These studies have shown that K^+ channels are formed by coassembly of four subunits (2) and are sometimes associated with auxiliary beta subunits that can modify the gating properties of the heteromultimeric channel complex. With only a few exceptions, the molecular basis of cardiac K^+ currents have been defined (Table 1). These channels can be grouped in many ways, most logically by amino acid sequence homology (3). Another classification scheme is based on biophysical characteristics of the currents determined by voltage clamp experiments. Based on function, cardiac K^+ channels can be placed into one of four categories: transient outward, delayed rectifier, inward rectifier, and leak channels. Several excellent reviews of K^+ channel diversity based on se-

Am J Med. 2001;110:50–59.
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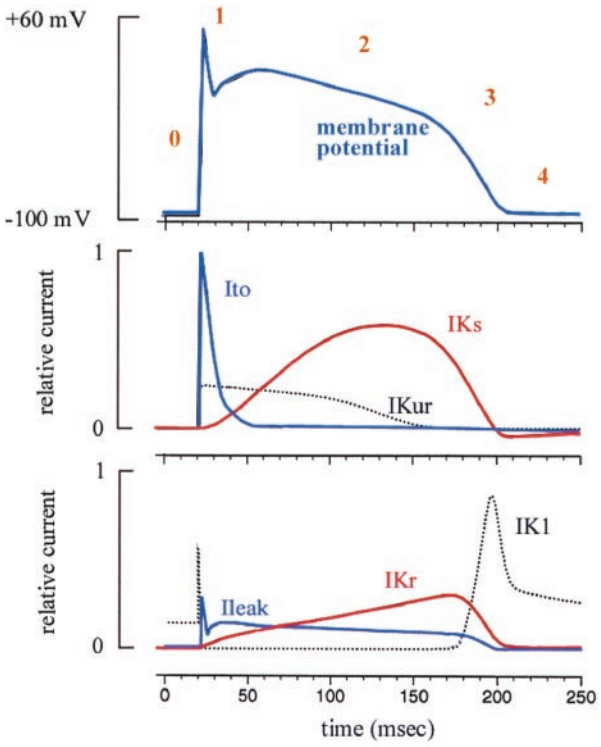

Figure 1. K^+ currents responsible for repolarization of a typical ventricular action potential. Ventricular action potential (**top**). Phase 0, rapid upstroke; phase 1, initial repolarization; phase 2, plateau; phase 3, terminal repolarization; phase 4, diastolic membrane potential. The rapid repolarization of phase 1 is the result of the contribution of the rapidly activating transient outward (I_{to}), the ultra-rapid delayed rectifier (I_{Kur}), and the leak (I_{leak}) currents (**middle and bottom**). During the plateau phase, the rapid (I_{Kr}) and slow (I_{Ks}) delayed rectifier K^+ currents as well as I_{Kur} and I_{leak} counter the depolarizing influence of L-type calcium current (not shown). I_{Kr} and the inward rectifier K^+ current (I_{K1}) provide repolarizing current during the terminal phase of the action potential [modified from (71). Reprinted with permission from the American Heart Association].

interested in a more comprehensive view of this subject (4–6).

Transient Outward K^+ Channels

Transient outward K^+ current (I_{to}) activates very rapidly in response to a rapid depolarization, such as occurs during the upstroke of the action potential. Soon after opening, these channels close, resulting in the transient nature of the net outward current (Figure 1). I_{to} is the sum of a Ca^{2+}-dependent Cl^- current and a voltage-dependent K^+ current. The K^+ current component of I_{to} is conducted by channels formed by tetrameric assembly of Kv1.4, Kv4.2, and/or Kv4.3 subunits (6). A single functional channel forms in the endoplasmic reticulum by coassembly of four identical subunits. In human atrial myocytes, the I_{to} channel is formed by coassembly of four

Table 1. The Molecular Identity of Human Cardiac K^+ Currents

Current	Cloned Channel: Alpha Subunit	Cloned Channel: Beta Subunit
Transient outward		
I_{to}	Kv4.3	
		Kv1.4
Delayed rectifier		
I_{Kur}	Kv1.5	
I_{Kr}	HERG	MiRP1
I_{Ks}	KVLQT1	minK
Inward rectifier		
I_{K1}	Kir2	
I_{KATP}	Kir6.2	SUR1
I_{KACh}	Kir3.1	Kir3.4
Leak		
		TWIK

channels, each subunit has six transmembrane domains (S1 to S6), including one domain (S4) that senses transmembrane voltage (Figure 2). The amino and carboxyl termini are located on the intracellular side of the membrane. Movement of the S4 domain in response to membrane depolarization is coupled to other regions of the protein that form the activation gate (8,9). When the activation gate is open, the channel conducts K^+ in a direction that depends on the electrochemical gradient across the plasma membrane. Immediately after depolarization, repetitive opening (activation) and closing (deactivation) of the activation gate determines how long the channel is in a conducting state. However, soon (within 10 to 100s of msecs) a portion of the amino terminus binds to a specific site near the inside of the pore region and closes the channel, a process called inactivation. Unlike the deactivated state, the inactivated state is a long-lived closed state. The channel remains closed until the membrane is repolarized to the resting potential, where channels recover from the inactivated state and again become capable of opening in response to membrane depolarization.

Delayed Rectifier K^+ Channels

The delayed rectifier K^+ current, I_K, is comprised of at least three distinct currents, I_{Kur}, I_{Kr}, and I_{Ks} that can be distinguished on the basis of kinetics of activation and pharmacologic properties (10–15). I_{Kur} activates ultrarapidly (16), I_{Kr} activates rapidly, and I_{Ks} activates very slowly. I_{Kur} is blocked by 4-aminopyridine (17,18), I_{Kr} is blocked by several antiarrhythmic agents (eg, dofetilide) (19,20), and I_{Ks} is blocked by a compound called chromanol 293B (21).

The amplitude of the delayed rectifier K^+ currents varies during repolarization of the action potential because

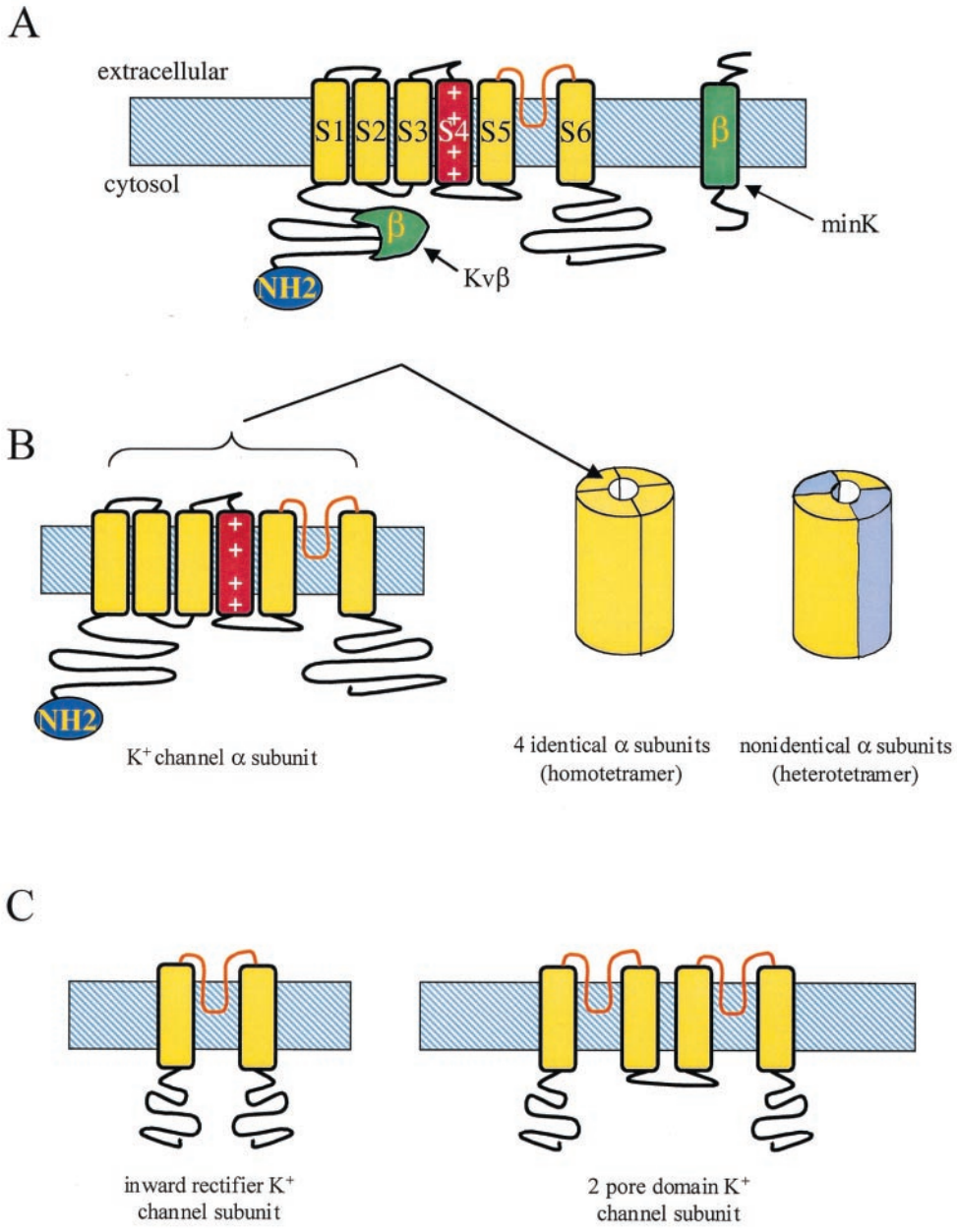

Figure 2. Proposed topology of K^+ channel subunits. **A**. Schematic representation of a voltage-gated K^+ channel alpha subunit composed of six membrane-spanning alpha helices (S1 to S6). The fourth membrane-spanning unit (S4) contains positively charged residues at approximately every third position and is the voltage sensor. The residues between S5 and S6 (shown in orange) form the ion selective pore. Auxiliary beta subunits (shown in green) modify the gating properties and protein trafficking of the pore-forming alpha subunits. $K_v$beta subunits are cytoplasmic proteins that bind to the N-terminus. MinK, a component of the I_{Ks} channel, is a membrane-spanning beta subunit. **B**. K^+ channel alpha subunits coassemble to form a tetrameric channel composed of four identical subunits (homotetramer) or nonidentical subunits (heterotetramer). **C**. Inward rectifier K^+ channels are formed by subunits containing two membrane-spanning alpha helices, separated by a pore domain. Like the six transmembrane voltage-gating ion channel, four subunits coassemble to form the inward rectifier K^+ channel. TWIK channels are a unique class of channels formed by subunits containing four membrane-spanning domains and two pore loops.

force, and relative rates of activation and inactivation (Figure 1). I_{Kur} activates extremely rapidly and does not inactivate appreciably during the time course of the action potential. I_{Kur} amplitude decreases during repolarization solely because of a decrease in electrochemical driving force. I_{Kr} amplitude increases during repolarization, reaching a peak at approximately −30

resting level. This increase in current occurs in spite of a decrease in electrochemical driving force, because channels recover from inactivation to an open state in a voltage-dependent manner. Fast inactivation of I_{Kr} is not mediated by the amino-terminal region (22) but instead results from a mechanism believed to involve a slight constriction of the outer pore region of the channel (9). I_{Ks} activates extremely slowly and, therefore, increases in magnitude throughout the plateau phase. Only during phase 3 repolarization does I_{Ks} decrease in accordance with a decrease in electrochemical driving force for K^+.

The *Kv1.5* gene encodes subunits (613 amino acids) that coassemble to form I_{Kur} channels in most species, including myocytes of the human atrium (23). *HERG* and *KCNE2* genes encode subunits (HERG and MiRP1, respectively) that coassemble to form I_{Kr} channels (24–26). *KVLQT1* and *KCNE1* encode subunits (KvLQT1 and minK, respectively) that coassemble to form I_{Ks} channels (27,28) (Table 1). HERG (1,159 amino acids) and KvLQT1 (676 amino acids) are alpha subunits that have an overall structure similar to the Kv1 and Kv4 subunits. MinK (129 amino acids) and MiRP1 (minK-related peptide number 1, 123 amino acids) are beta subunits that have a single transmembrane domain. It is presently unclear what regions of the alpha and beta subunits interact to stabilize the heteromultimeric complex, but it is clear that this association alters the gating of the tetrameric alpha subunit channel. The change in kinetics is especially remarkable for KvLQT1, where association with minK greatly slows the rate of activation and shifts the voltage dependence of the channel opening to a more positive membrane potential (27,28). HERG-MiRP1 channels have properties more similar to I_{Kr} in native myocytes than do channels formed by coassembly of HERG alone. MiRP1 decreases the single channel conductance and accelerates the rate of HERG channel deactivation (26). Alternative splicing of *HERG* (29–31) produces a variant with a shortened amino terminus that also deactivates faster than full length HERG. Additional delayed rectifier K^+ channels are expressed in the hearts of mammals other than humans. For example, Kv2.1 subunits coassemble to form a delayed rectifier K^+ channel ($I_{K,slow}$) in the mouse heart (32).

Inward Rectifier K^+ Channels

In most cardiac cells, the inward rectifier K^+ current, I_{K1}, largely determines the resting membrane potential. The channels that conduct this current are open at all voltages. However, K^+ is preferentially allowed to conduct in the inward direction (from the extracellular space to the cytosol). The conductance of outward current becomes progressively less as the membrane potential is made more positive than approximately −95 mV, the equilibrium potential for K^+ (Figure 1). A voltage-dependent channel pore causes low outward conductance (33–35). The pacemaker cells of the sinoatrial and atrioventricular node do not express these inward rectifier K^+ channels, and therefore the maximum diastolic potential is more depolarized than nonpacing cells of the atria or ventricles. Atrial pacemaker cells have another type of inward rectifier K^+ current (I_{KACh}) that is activated by binding of acetylcholine to m2 muscarinic receptors located on the surface of the cell membrane. M2 receptor binding activates a G protein that in turn increases the probability of K^+ channel opening (36). Activation of I_{KACh} slows the spontaneous firing rate of pacemaker cells and shortens action potential duration. Most cardiac myocytes also express an inward rectifier K^+ channel that is inhibited by cytosolic ATP (37). The current conducted by these channels (I_{KATP}) is activated under conditions of metabolic stress that reduce intracellular ATP and can lead to pronounced shortening of the action potential (38).

Coassembly of Kir2.1, Kir2.2, Kir2.3, or Kir2.4 subunits form channels that underlie I_{K1} (5). These subunits are smaller than the voltage-gated transient outward or delayed rectifier K^+ channel subunits. For example, human Kir2.1 subunits are composed of 427 amino acids. The proposed membrane topology of Kir2 channels is similar to all the other inward rectifier K^+ channels, having two putative transmembrane domains linked by a single pore loop (39) (Figure 2). Analysis of the conduction properties of channels constructed by tandem multimers consisting of three or four Kir2.1 subunits suggest that subunits coassemble to form a tetrameric channel complex similar to the voltage-gated K^+ channels. It is presently unclear if the channels form as homo- or heteromultimeric complexes. However, based on conductance measurements of single channel currents, there is ample evidence that multiple types of inward rectifier K^+ channels are present in myocytes. K_{Ach} channels are formed by coassembly of two Kir3.1 and two 3.4 subunits into a tetrameric complex (40). Kir3.1 and 3.4 subunits have 501 and 419 amino acids, respectively. K_{ATP} channels are formed by coassembly of four Kir6.1 subunits and four sulfonylurea receptor (SUR) subunits (41). Human Kir6.1 and SUR1 subunits have 424 and 1,581 amino acids, respectively. Kir3 and Kir6 subunits have the same overall structure as Kir2 subunits, having two transmembrane domains flanking a pore region (Figure 2C).

Leak K^+ Channels

Most cells have a very small background K^+ conductance that contributes to maintenance of the resting potential and repolarization of the action potential. In the heart this conductance may be the result of a weakly inward rectifying K^+ channel, TWIK-1 (42) or Kcnk3 (43). The TWIK channel is 336 amino acids in length with four transmembrane domains. This channel has an unusual

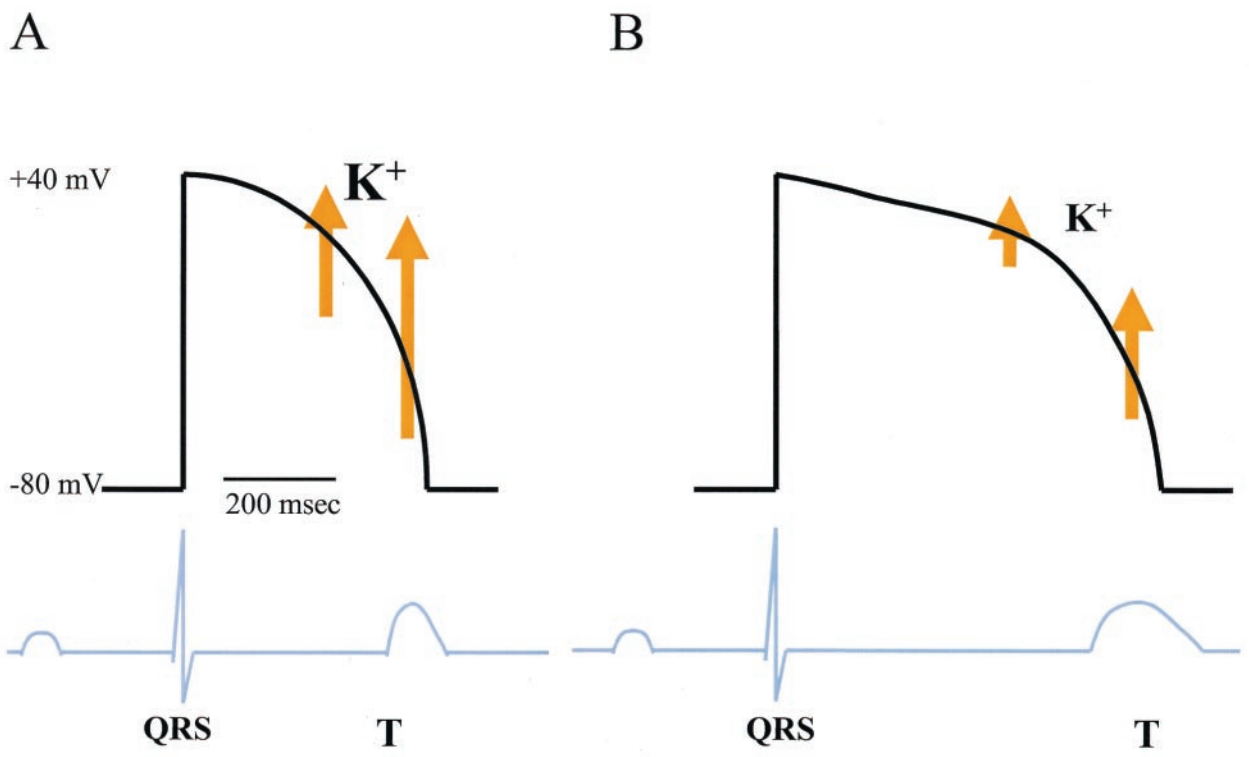

Figure 3. The role of K^+ channels in mediating phase 3 repolarization of the cardiac action potential. **A**. Yellow arrows represent K^+ efflux through the rapid (I_{Kr}) and slow (I_{Ks}) delayed rectifier K^+ channels. Outward movement of positively charged K^+ hyperpolarizes the cell membrane and terminates the action potential. The surface electrocardiogram is depicted below. The QRS corresponds to the rapid upstroke of the action potential. The T wave represents the change in membrane potential associated with repolarization. **B**. Mutations in the genes encoding subunits of the I_{Kr} and I_{Ks} channels reduce the amount of repolarizing current available during the terminal phase of the cardiac action potential. Decreased repolarizing current prolongs the action potential, which is reflected on the surface electrocardiogram as prolongation of the QT interval.

is like two Kir channels that are connected in tandem (Figure 2).

LONG QT SYNDROME CAUSED BY MUTATIONS IN GENES ENCODING SUBUNITS OF CARDIAC RAPID AND SLOW DELAYED RECTIFIER K^+ CHANNELS

Long QT syndrome (LQTS) is a disorder of ventricular repolarization that predisposes affected individuals to cardiac arrhythmias and sudden death. The most common form of LQTS is acquired, caused by medications that block cardiac K^+ channels, such as certain antiarrhythmic drugs, antihistamines, and antibiotics, and is exacerbated by bradycardia and hypokalemia (44). LQTS can also be inherited as an autosomal dominant (Romano-Ward syndrome) or recessive (Jervell and Lange-Nielsen syndrome) disorder (45). The more severely affected individuals can have intermittent syncope de pointes, characterized by a sinusoidal twisting of the QRS axis around the isoelectric line of the electrocardiogram (46). Spatial dispersion of ventricular repolarization and an alteration in the predominance of two ectopic foci have been postulated to be the underlying cause of torsades de pointes (47). Sudden cardiac death can occur if torsades de pointes arrhythmia degenerates into ventricular fibrillation.

Mark Keating and colleagues used a positional cloning approach to discover one gene (*KVLQT1*) and a candidate gene approach to identify three other genes (*SCN5A*, *HERG*, *KCNE1*, *KCNE2*) that cause LQTS (48–52). *SCN5A* encodes the cardiac sodium (I_{Na}) channel and so will not be discussed further in this review. As discussed above, the *HERG* and *KCNE2* genes encode subunits that form the I_{Kr} channel, and *KVLQT1* and *KCNE1* genes encode subunits that form the I_{Ks} channel. Mutations in any of these K^+ channel subunits cause a decreased outward K^+ current during the plateau phase of the cardiac action potential, delayed ventricular repolarization, and an increased QT interval (Figure 3). It is clear that envi-

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