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CLASSICAL PERSPECTIVES

Delayed-rectifier potassium currents and the control of cardiac repolarization: Noble and Tsien 40 years after

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Following activation by depolarization, all excitable cells undergo repolarizing steps to return the transmembrane potential to the initial resting level (often called the 'resting potential'). In comparison with other excitable cells, cardiac myocytes remain depolarized for substantially greater periods, generating the action potential 'plateau' (Fig. 1*A*). The unique cardiac action potential plateau permits sufficient transmembrane Ca^{2+} entry into the cell via voltage-gated Ca^{2+} channels to maintain the sarcoplasmic reticulum Ca^{2+} stores needed for effective contraction, which occurs when the sarcoplasmic reticulum releases Ca^{2+} in response to triggering by transmembrane Ca^{2+} entry initiated via cell depolarization. The plateau also keeps Na^+ channels inactivated for a substantial 'refractory period', preventing excessively rapid beating rates during cardiac arrhythmias. Cardiac repolarization is a delicate and highly regulated process, governed by sets of ion channels, pumps and exchangers, as well as autonomic nervous system modulators (Conrath & Opthof, 2006). If the repolarization process is impaired, cardiac action potentials are prolonged (Fig. 1*B*). When prolongation becomes exaggerated, voltage-dependent Ca^{2+} currents have sufficient time to recover from the inactivation that occurs at the most depolarized levels of the action potential. The consequent reactivation of plateau Ca^{2+} currents causes abnormal, arrhythmia-generating 'early after-depolarizations'. When repolarization is excessively rapid, action potentials are abbreviated (Fig. 1*C*), permitting re-entrant arrhythmias (a form of 'short-circuiting' of cardiac electrical activity; for a detailed review see Nattel *et al.* 2007).

Findings of Noble and Tsien

Forty years ago, Noble and Tsien published papers that were seminal in our understanding of cardiac repolarization (Noble & Tsien, 1969*a*,*b*). In the first, they described the detailed properties of delayed rectification, a time-dependent increase in cardiac K^+ conductance following depolarization that was first identified in 1960 (Hutter & Noble, 1960). They identified two kinetically distinct processes. Based on predominant but incomplete K^+ selectivity, the underlying currents were named 'i_{x1}' and 'i_{x2}'. The rapid component i_{x1} showed strong inward rectification (a greater ability to pass current in the inward or depolarizing direction compared to the outward, repolarizing, direction), whereas the slower component i_{x2} displayed a nearly linear conductance. Noble and Tsien suggested that each current component consists of a K^+-selective channel and a non-specific shunt, and that the only common feature of the two types of K^+ channels is their common delayed-rectifier kinetic features. Remarkably, all of their conclusions derived from mathematical considerations and graphic solutions, in a period before high-speed computer analyses. In the second paper, the authors used graphic solutions of differential equations to reconstruct the repolarization process in cardiac Purkinje fibres. They found i_{x1} and i_{x2} to be quantitatively sufficient to account for Purkinje cell repolarization, which occurred by a regenerative ('all-or-none') process. In fact, i_{x1} was sufficient to explain repolarization in Purkinje cells, although the authors speculated that in ventricular myocytes i_{x2} might contribute significantly for plateau voltages positive to 0 mV. The transient outward current (which Noble and Tsien suggested to be predominantly a K^+ current rather than a Cl^- current as generally accepted at the time) was found to participate primarily in early repolarization and the action potential notch, and to provide no detectable contribution to overall action potential duration (APD).

Significance of the Noble and Tsien studies

The impact of the 1969 Noble and Tsien work was enormous. A literature search with the key word sequence 'delayed-rectifier current cardiac' identified 1207 citations. The importance of their work is particularly impressive considering the tools that were available at the time, in particular dual microelectrode voltage clamp of Purkinje fibre false tendon tissue, a tedious and exacting technique, along with graphic solutions of mathematical functions. The detailed description of delayed-rectifier currents by Noble and Tsien, as well as their analysis of the role of various K^+ currents in cardiac repolarization, have withstood the test of time and been confirmed by numerous subsequent studies. The methods they pioneered, including the envelope of tails test and the study of fully activated current–voltage relations, have been applied in innumerable analyses of cardiac ionic currents. Furthermore, the delayed-rectifier currents they described have proven to be crucial regulators of cardiac physiology and pathophysiology, and key targets of pharmacological control mechanisms.

Mechanisms underlying cardiac delayed-rectifier currents

Noble and Tsien initially favoured the notion that i_{x1} and i_{x2} were carried by independent voltage-dependent K^+-selective ion channels. This idea was strongly contested, perhaps because alternative explanations of the two kinetic components existed that did not require the postulation of two different and independent currents. Indeed, many investigators argued that delayed rectification might be related to K^+ accumulation in intercellular clefts during depolarizing pulses and to artifacts of the voltage clamp methodology. The Noble laboratory itself performed a detailed experimental and computational analysis of extracellular K^+ accumulation and concluded that it might account for slow delayed rectification, but argued against any artifactual basis for i_{x1} (Brown *et al.* 1980). So controversial was the notion that delayed rectification is due to the parallel function of two distinct K^+ channels that when we presented evidence for an important role of delayed-rectifier K^+ currents in human atrium (Wang *et al.* 1993), we were forced by the reviewers to remove results arguing for distinct rapid and slow components,

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which we published later as a separate paper (Wang *et al.* 1994). The issue was finally settled by the demonstration that the rapid and slow delayed-rectifier currents corresponding to i_{x1} and i_{x2}, which came to be known as I_{Kr} and I_{Ks} following the seminal work of the Sanguinetti laboratory (Sanguinetti & Jurkiewicz, 1990), are carried by distinct channels with differing ion-channel subunit compositions: the *human ether-a-go-go related* (*HERG*) gene product for I_{Kr} *versus* the *KCNQ1*-encoded KvLQT1 α-subunit and *KCNE1*-encoded minK β-subunit for I_{Ks} (Sanguinetti *et al.* 1995, 1996; Barhanin *et al.* 1996).

Delayed-rectifier currents and cardiac arrhythmias

APD is a key determinant of re-entrant arrhythmias, and drugs that prolong APD are particularly effective in preventing re-entry. However, APD-prolonging drugs can also destabilize repolarization, causing early afterdepolarizations and associated ventricular tachyarrhythmias called torsades de pointes (TdP). Sanguinetti & Jurkiewicz (1990) discovered that clinically used APD-prolonging drugs act by selectively blocking I_{Kr}, consistent with Noble and Tsien's demonstration (1969*a*,*b*) of the crucial role of i_{x1} in cardiac repolarization. Furthermore, the molecular structure of the underlying channel encoded by the *HERG* gene particularly lends itself to drug block (Sanguinetti & Tristani-Firouzi, 2006). In fact, I_{Kr} block is so easy to achieve that many non-cardiovascular drugs have collateral I_{Kr}-blocking actions, and is so potentially dangerous that pharmaceutical companies make major efforts to avoid developing compounds with I_{Kr}-blocking properties (Finlayson *et al.* 2004). It has thus become a common practice in the pharmaceutical industry to test all proposed compounds against cells expressing HERG channels before their development is approved.

Noble and Tsien's descriptions of cardiac delayed-rectifier currents and their role in cardiac repolarization were the dominant publications in this field for over 25 years, with the next major development being the discovery of the molecular basis of I_{Kr} and I_{Ks} and the role of I_{Kr}/I_{Ks} subunit mutations in congenital long QT syndromes (Sanguinetti *et al.* 1995, 1996; Barhanin *et al.* 1996). These discoveries extended the Noble and Tsien concepts to the molecular level, and permitted new dimensions of understanding of the physiological and

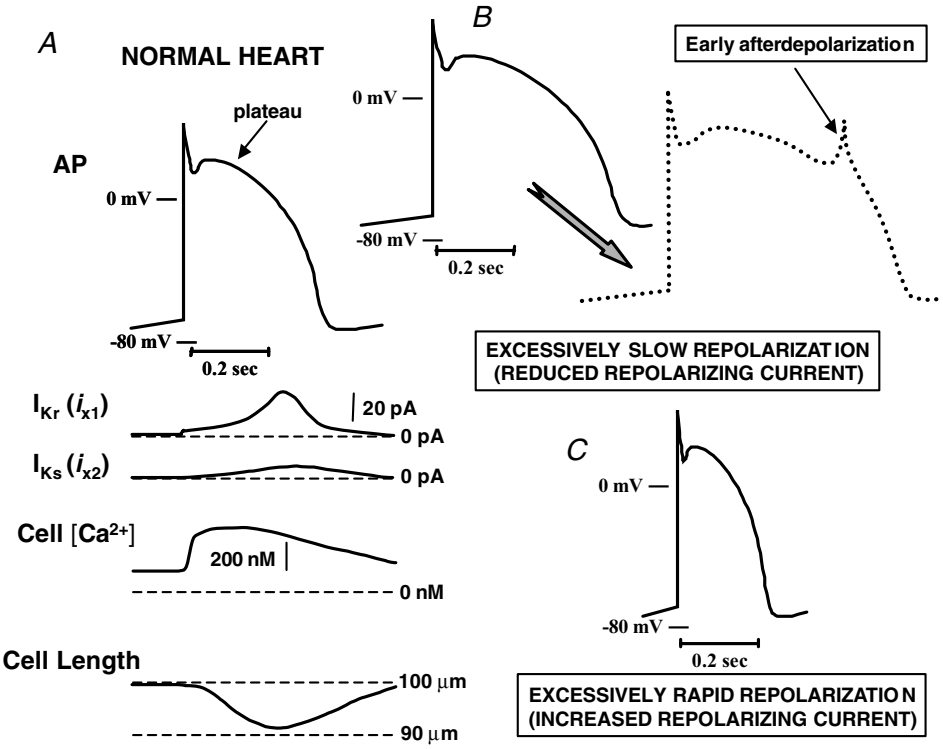

Figure 1. Schematic representation of the cardiac action potential and the consequences of repolarization abnormalities
A, the normal cardiac action potential, with corresponding delayed-rectifier currents (rapid, I_{Kr}, and slow, I_{Ks}, along with the corresponding Noble/Tsien nomenclature, i_{x1} and i_{x2}), intracellular Ca^{2+} signal and cell shortening indicating contractile function (the horizontal dashed lines indicate 0 current, 0 intracellular [Ca^{2+}] and reference cell-length values, respectively). Repolarization timing is determined largely by I_{Kr}, which is much larger than I_{Ks} over the course of the normal action potential. The relatively long cardiac action plateau allows sufficient Ca^{2+} to enter via L-type Ca^{2+} channels to maintain sarcoplasmic reticulum Ca^{2+} stores, essential for cardiac contractility. *B*, reduced repolarizing current delays repolarization and prolongs the action potential. When action potential prolongation is excessive, reactivation of Ca^{2+} currents provides enough inward current to generate early afterdepolarizations (dotted action potential). *C*, increased repolarizing current accelerates repolarization and shortens action potential duration. Action potential abbreviation reduces the refractory period, allowing re-entrant arrhythmias to emerge (for more detailed discussion, see Nattel *et al.* 2007).

pathophysiological roles of cardiac delayed rectifiers. Subsequent genetic studies in the late 1990s showed that even subtle derangements in delayed-rectifier currents can cause life-threatening arrhythmia syndromes (Priori *et al.* 1999), implying that delayed-rectifier function is finely tuned to ensure electrophysiological integrity. The notion that APD-prolonging drugs act by mimicking potentially lethal congenital arrhythmia syndromes provided sober food for thought (Nattel, 2000), and disease-induced delayed-rectifier channel subunit down-regulation was found to produce substrates for TdP (Tsuji *et al.* 2006).

One element that remained mysterious following the 1969 Noble and Tsien papers was the functional role of the slow component (i_{x2} or I_{Ks}), which appeared to be insignificant for Purkinje cell repolarization. A clue to I_{Ks} function came from studies in the early 1980s, which revealed that delayed-rectifier K^+ current is very sensitive to adrenergic stimulation (Kass & Wiegers, 1982), along with the subsequent demonstration that adrenergically induced current augmentation is due to highly selective regulation of I_{Ks} (Sanguinetti & Jurkiewicz, 1990). In fact, both inward L-type Ca^{2+} current (I_{CaL}) and outward I_{Ks} have similar sensitivities to adrenergic stimulation (Kass & Wiegers, 1982). Thus, when I_{CaL} is enhanced by adrenergic agonists to strengthen cardiac contraction, I_{Ks} also increases and prevents potentially dangerous repolarization delays, a response that can be unmasked by I_{Ks} blockade (Han *et al.* 2001). This phenomenon explains why arrhythmic attacks are typically precipitated by stressful situations in congenital long QT syndromes due to I_{Ks} dysfunction, and can be prevented by β-adrenoceptor blockade (Schwartz *et al.* 2001).

In the late 1990s, observations of variable occurrence of TdP in congenital and acquired long QT syndromes led to a crucial advance in understanding K^+ current control of repolarization, the notion of 'repolarization reserve' (Roden, 1998). Repolarization reserve represents the ability of remaining K^+ currents to increase when one K^+ current is suppressed, thus minimizing APD prolongation. I_{Ks} is an important contributor to repolarization reserve, because interventions that reduce outward current prolong and elevate the action-potential plateau, allowing I_{Ks} to activate more extensively. When I_{Ks} is suppressed, excessive APD prolongation in response to repolarization stress becomes more likely (Jost *et al.* 2005). Recent work suggests feedback regulation of I_{Ks} expression, indicating that delayed-rectifier channel subunit expression may be regulated to control repolarization (Xiao *et al.* 2008).

Until recently, arrhythmia-inducing delayed-rectifier current abnormality was equated with loss of function. However, over the past few years it has become clear that gain-of-function delayed-rectifier current mutations also predispose to serious ventricular arrhythmogenesis (Bellocq *et al.* 2004; Brugada *et al.* 2004), presumably by increasing the risk of re-entrant arrhythmias. Similarly, gain-of-function delayed-rectifier current mutations can lead to atrial fibrillation (Chen *et al.* 2003; Brugada *et al.* 2004).

Quantitative analysis of repolarization and therapeutic approaches

An important element introduced by Noble & Tsien (1969*b*) was the rigorous quantitative analysis of ionic current determinants of repolarization. The subsequent development of high-speed computational systems allowed for detailed exploration of the mechanisms governing repolarization and arrhythmogenesis. The sophisticated Luo-Rudy model developed in the mid-1990s permitted extensive analysis of the role of delayed-rectifier currents, the dynamic mechanisms governing their function and the consequences of their dysfunction (Zeng *et al.* 1995; Viswanathan & Rudy, 2000). Similar approaches were subsequently developed to study the control of atrial repolarization and its role in atrial arrhythmias (Courtemanche *et al.* 1998; Nygren *et al.* 1998). The application of complex computational models promises to improve the development of anti-arrhythmic drug therapy targeting repolarization (Courtemanche *et al.* 1999; Noble, 2008).

Conclusions

Back-to-back papers by Noble & Tsien in 1969 provided extensive and detailed characterizations of cardiac delayed-rectifier K^+ currents and their role in repolarization. These groundbreaking studies had a major impact on the understanding of cardiac physiology and their influence resounds strongly to the present day.

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