Commentary

Modulation of hERG potassium channels by a novel small molecule activator

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KCNH2-encoded hERG1 channels conduct the rapid delayed rectifier potassium current (I_{Kr}), which plays a vital role in controlling the duration of cardiac action potentials. Ever since the importance of hERG1 currents in regulating cardiac repolarisation was first demonstrated there has been considerable interest in pharmacologically manipulating these channels. In this issue, Sale *et al*. (2017), have characterised the properties of a structurally and functionally novel hERG activator, ITP-2, which dramatically increases hERG1 current amplitudes.

Long before hERG1 activators became available, selective hERG1 channel blockers were developed as Class III antiarrhythmic drugs to prolong refractory periods and treat re-entrant arrhythmias. However, post-marketing surveillance and then clinical trials revealed that selective hERG1 channel inhibitors could produce excessive prolongation of repolarisation and, thereby, be pro-arrhythmic. Subsequent work found that hERG1 is also sensitive to pharmacological inhibition by diverse non-cardiac drugs (reviewed by Vandenberg *et al*, 2012) and this is one of the main causes of drug-induced long QT syndrome (LQTS). Genetically inherited loss-of-function hERG1 mutations that reduce hERG1 channel function, through either altered kinetics or impaired trafficking, have also been linked to inherited LQTS (LQTS2) and a greatly increased risk of ventricular fibrillation and sudden death. Gain-of-function hERG1 mutations have also been identified that cause an excessive shortening of QT interval and also carry an increased risk of ventricular fibrillation and sudden death. This has led to the "Goldilocks" principle in which the magnitude and timing of repolarising hERG currents needs to be 'just right'.

The unique and complex gating properties of hERG underlie its crucial role in cardiac action potential repolarisation. hERG gating is characterised by a very rapid and voltage dependent form of inactivation, which unusually, is faster than activation gating. During the early part of the action potential, hERG current magnitude is small because the channels activate slowly and most have rapidly inactivated. However, once repolarisation starts to occur, the channels quickly recover from inactivation, resulting in a rapid increase in hERG amplitude that plays a central role in timing the end of the plateau phase of the action potential. hERG channels also deactivate slowly ensuring they remain open to contribute to final repolarisation and also oppose undesirable premature depolarisations.

Inherited LQTS can result not only from mutations to hERG1, but also a variety of other cardiac genes. Seventeen different LQTS associated genes have been identified – most of which encode ion channel subunits (Campuzano, Sarquella-Brugada, Brugada, and Brugada,

2015). LQTS is one of the most common genetic diseases and affects an estimated 1 in 5000 to 10000 people worldwide and is a particularly significant cause of sudden death among young people. hERG1 activators (or agonists) are compounds that increase the amplitude of hERG1 currents. At least in principle, hERG1 activators have therapeutic potential for reducing the risk of sudden death in LQTS patients by increasing the amount of repolarising K^+ current during the cardiac action potential (Grunnet, 2010).

The pharmaceutical industry has made tremendous progress in identifying compounds early in development that have the potential to cause the drug-induced form of LQTS. This has been facilitated by the advent of new medium throughput electrophysiology platforms that make it feasible to test large numbers of compounds for their potential to block hERG1 channels. Ironically, the identification of hERG1 activators has predominantly come from efforts to screen out hERG1 blockers.

Three main groups of compounds with the ability to increase hERG1 current amplitudes have been described based primarily on their mechanisms of function, and to a lesser extent on their putative site of action (Perry, Sanguinetti and Mitcheson, 2010). Type 1 activators enhance current primarily by profoundly slowing channel closure (deactivation). These activators bind to sites on the cytoplasmic end of the pore; at sites facing away from the conduction pathway and inner cavity of the channel. They constrain movements of the activation gate and delay closure of the channel. Type 1 activators may also reduce channel inactivation, but that is not their major mechanism of action. Type 2 activators on the other hand, predominantly exert their effects by causing large positive shifts of the voltage dependence of inactivation and by slowing the onset of inactivation, resulting in a large increase of channel open probability at depolarised potentials. Mutagenesis and molecular modelling studies suggest these activators are likely to bind to sites close to the selectivity filter on the extracellular side of the pore. They may stabilise the open conformation of the selectivity filter or alter the ability of the voltage sensor to interact with the pore and induce inactivation. The type 1 and 2 classification of activators should probably be extended to include a third type that enhance hERG1 currents by accelerating activation and shifting its voltage dependence to more negative potentials. An example is KB130015, a derivative of the hERG blocker amiodarone. KB130015 is likely to bind within the inner cavity since it competes with amiodarone and other blockers that are known to bind at this pore location. Another group of compounds that also cause negative shifts of the voltage dependence of activation are referred to as facilitators because while they enhance hERG1 currents with

weak depolarisations they inhibit currents elicited by stronger depolarisations. Facillitators also appear to exert their actions from the intracellular side of the channel, often compete with pore channel blockers and are less effective when inner cavity sites are mutated. All of these effects are consistent with a binding site within the inner cavity ITP-2, the compound characterised by Sale *et al.* (2017) in this issue of the *BJP,* is interesting because it is more potent than most previously described activators (EC_{50} of 1 µM) and because its mechanism of action is distinct from the different types of activators described above, combining properties of the last two groups with some further unique features of its own. During conventional two step I-V protocols, ITP-2 enhances hERG1 currents during the first test pulse at all potentials from -60 to +60 mV, but surprisingly the tail currents measured upon repolarisation to -65mV are profoundly inhibited following most test pulses. The mechanism for the enhanced currents during the test pulses is likely to be a combination of attenuated inactivation and increased activation. Both gating processes have shifts in the voltage and time dependent kinetics that would lead to an increase of current amplitudes during the test pulses. The mechanism for the attenuated tail currents is much less clear. There is no change of reversal potential and so an intriguing possibility that warrants further investigation is that the compound has a second binding site that leads to channel block. There are several pieces of evidence that support this conclusion, including that the concentration response relationship is bell-shaped (with less activation at 10 µM than at 3 µM) and that for several mutants that disrupt inactivation gating the current activation is absent, but the inhibitory effect remains.   What is clear is that despite the attenuated currents at negative potentials, the overall effect of ITP-2 during a cardiac action potential is a substantial increase of current during a cardiac ventricular action potential that would be expected to shorten repolarisation times. However it should be noted that during late repolarisation and post-repolarisation phases, ITP-2 may actually reduce hERG1 channel currents, increasing vulnerability to unwanted premature depolarisations. This is the opposite outcome of what would be aimed for in developing an antiarrhythmic drug.

Another very interesting finding of this study is that heteromeric hERG1a/1b channels are much less sensitive to the ITP-2 activator effect than homomeric hERG1a channels. From a therapeutic potential this is important because there is clear evidence that native cardiac tissues express both the full length hERG1a transcript and the shorter hERG1b variant. Functional data demonstrate that heteromeric hERG1a/1b channels mediate cardiac I_{Kr}. From a pharmacological perspective, ITP-2 is one of a small, but steadily increasing, number of

compounds that show differential effects to hERG1b containing channels. Understanding the molecular basis for this could be very beneficial in a number of clinical settings, including the treatment of hERG1b containing cancers or forms of hERG-associated LQTS where a complex situation could arise because of the presence of both wild-type and mutant hERG1a/1b subunits. The only structural difference between 1a and 1b is the amino terminus, which interacts with the pore and voltage sensing domains from the intracellular side of the membrane and is unique and much smaller in the hERG1b variant. The amino acid sequences of the transmembrane domains are identical. The authors show that ITP-2 mediates its effects on hERG1a from the extracellular side of the membrane and thus it seems likely that the amino terminus has an allosteric effect on the ITP-2 activation binding site.

The paper by Sale *et al*. (2017) contains a wealth of information and mechanistic insight, but clearly further investigations of this fascinating compound are merited. We do not yet know its selectivity for hERG1 relative to other ion channels. Investigations of its antiarrhythmic potential in cardiac preparations are also required. Understanding more about where it binds and how it modulates hERG1 activity could be useful for the development of higher efficacy I_{Kr} activators with an improved set of properties for treating cardiac arrhythmias.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan et al., 2016), and are permanently archived in the Voltage gated ion channel chapter of the Concise Guide to PHARMACOLOGY 2015/16 (Alexander et al., 2015).

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