Pharmacogenetic diversification by alternative translation initiation: background channels to the fore

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Unanticipated complexity of drug-target interactions creates a headache for those attempting to rationalize and create simple models of antiarrhythmic action, but can also introduce opportunities for increased drug specificity, or for potentially advantageous spatial and temporal variation in drug effects. The newest findings reported by Kisselbach *et al*. in this issue are a case in point. Building upon previous pioneering work demonstrating that neuronal $K_{2P}2.1$ potassium-selective "background" channels can become permeable to sodium ions depending upon alternative translation initiation (ATI) (Thomas *et al.*, 2008), the Thomas lab now shows that ATI of $K_{2P}2.1$ and $K_{2P}10.1$, which are also expressed in the heart, can cause a fivefold shift in sensitivity to block by the β-receptor (and potassium channel) antagonist, carvedilol (Kisselbach *et al.*, 2014).

The initial cloning of the K_{2P}, or two-pore domain, potassium channels opened the floodgates for research into a surprisingly numerous and diverse family of α subunits within the already crowded and varied array of channel types that form potassium ion-selective pores (Ketchum *et al.*, 1995). These revelations also ended the decades-long search for the molecular correlates of the "background" conductance, a rather uninspiring moniker that belies the versatility and physiological importance of these channels (also unfairly referred to as "leak" channels). The K_{2P} channels are unusual among potassium channels in that they form via dimerization of two α subunits, each of which contains four transmembrane segments (in mammals), and two P-loops (or pore domains) in tandem (Goldstein *et al.*, 2001). Most other K^+ channels, including the voltage-gated Kv channels and the inward rectifier Kir channels, co-assemble post-translationally from four α subunits each bearing a single P-loop.

At first glance, K_{2P} channels might appear pedestrian compared to their dynamic, voltage-sensing, Kv channel relatives. Unless modified by external agents, K_{2P} open probability

is mostly voltage-independent, although K_{2P}9.1 and K_{2P}4.1 show mild voltage dependence to their gating (Mathie *et al.*, 2010). However, this permits K_{2P} channels to exert a major influence on the resting membrane potential, explaining in large part why excitable cells sit at membrane potentials approaching the potassium equilibrium potential (E_K) until excited.

K_{2P} channels exhibit Goldman-Hodgkin-Katz (open) rectification, therefore in symmetrical K^+ conditions (experimentally imposed high K^+ inside and outside the cell) K_{2P} channels exhibit a linear current-voltage relationship, reversing (i.e., passing the zero current point) at 0 mV. Because of the availability of ions under standard physiological conditions (low extracellular K^+, high intracellular K^+), *in vivo* K_{2P} channels pass outwardly rectifying current, with K^+ efflux at membrane potentials positive to E_K (around -80 mV) – still in compliance with Goldman, Hodgkin and Katz. This permits K_{2P} channels to exert considerable repolarizing force throughout the action potential, with current increasing with driving force, unlike the inward rectifier K^+ channels, which are blocked at an intracellular site within the pore by Mg^{2+} or polyamines at positive voltages.

What makes K_{2P} channels especially interesting is that their activity *in vivo* is exquisitely modulated by a huge spectrum of stimuli, and they therefore act as crucial conduits between cellular and external environmental signals, and membrane excitability. Human K_{2P} channels comprise a 15-member family, which can be divided into 6 subfamilies based on both primary structure and functional properties. Physiological activators of the various members of this family include stimuli as diverse as intracellular and extracellular pH, heat, membrane stretch, Gαi and q, nitric oxide, hypoxia, hypoglycemia, calcium, arachidonic acid, polyunsaturated fatty acids and lysophospholipids. Physiological antagonists include extracellular low pH, sumoylation, protein kinases A and C, Zn^{2+}, and Gαq and s.

Pharmacologically, K_{2P} channels exhibit a very different profile to that of other potassium channel families, being insensitive to the classic Kv channel blockers but inhibited by, e.g., some local anesthetics, and activated by the nonsteroidal anti-inflammatory drug flufenamic acid and general anesthetics including halothane and nitrous oxide (Duprat *et al.*, 2007; Mathie *et al.*, 2010). For excitable cells to fire action potentials, they must first reach threshold, and open K_{2P} pores providing a tonic repolarizing force to oppose this. If these pores are closed by external stimuli, however, threshold can be reached and action potentials propagated. In the brain this might mean the difference between a neurone firing versus not firing. In the heart, K_{2P} regulation by, e.g., mechanical stretch or pharmacological manipulation, may alter the timing of cardiomyocyte action potentials, and therefore potentially arrhythmogenesis and cardiac contractility.

This brings us to back to the findings of Kisselbach and colleagues (Kisselbach *et al.*, 2014). Prior research led to the observations that ATI influences K_{2P} ion selectivity (Thomas *et al.*, 2008) and block by the antidepressant, fluoxetine (Eckert *et al.*, 2011). The present article is the first to extend this pharmacological flexibility to the cardiac realm and demonstrate ion channel ATI dictating anti-arrhythmic sensitivity; a related phenomenon has been reported for the hERG Kv channel, but this involved alternative splicing of the N-terminal segment and not ATI (Abi-Gerges *et al.*, 2011). The findings are timely - research into the role of K_{2P} channels in cardiac physiology and arrhythmogenesis is in the early discovery stage, with labs such as the Thomas group leading the charge, particularly when it comes to K_{2P} channels as anti-arrhythmic targets.

The challenge now is to figure out how observations such as ATI-dependent drug sensitivity can be harnessed to the greatest therapeutic advantage. This will require elucidation

of the precise role of different K_{2P} isoforms in the heart, a fuller understanding of how, where, when and why ATI is regulated for specific channels, and how this affects their pharmacology, but it does not necessarily mandate development of more highly start-site-specific K_{2P} channel modulators. Anti-arrhythmic drug selectivity has not been a proven indicator of clinical utility or success - the widely used "class III" anti-arrhythmic amiodarone, for example, is a highly effective but "dirty" drug with actions in all four classes of the Singh-Vaughan-Williams classification.

Should the more drug-sensitive $K_{2P}2.1$ or $K_{2P}10.1$ ATI-dependent variants happen to be relatively enriched in more rapidly pacing cells, for example, this would enhance drug efficacy in the more arrhythmogenic myocyte subpopulation and could be therapeutically advantageous. This would be the case even if, overall, the drug were a relatively nonspecific one such as carvedilol (although K_{2P} sensitivity for carvedilol, as the authors acknowledge, may be outside the useful therapeutic range). An analogous phenomenon has been observed for vernakalant, which blocks several types of K^+ channel but also inhibits voltage-gated sodium channels with a state dependence that renders it relatively more efficacious in the rapidly pacing cells sustaining atrial fibrillation than in slower pacing cells (Fedida *et al.*, 2005). In any case, as studies such as the one by Kisselbach *et al* continue to increase the resolution of our picture of cardiovascular physiology and pharmacology, we must embrace the complexity and endeavour to exploit it, rather than viewing it simply as a hurdle to overcome.

Competing Interests

The author has no competing interests.

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