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COMMENTARY

Pharmacogenetic diversification by alternative translation initiation: background channels to the fore: Commentary on Kisselbach *et al.*, Br J Pharmacol 171: 5182–5194

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This article is a Commentary on Kisselbach J, Seyler C, Schweizer PA, Gerstberger R, Becker R, Katus HA and Thomas D (2014). Modulation of K2P2.1 and K2P10.1 K+ channel sensitivity to carvedilol by alternative mRNA translation initiation. Br J Pharmacol 171: 5182–5194. doi: 10.1111/bph.12596

Abbreviations
ATI, alternative translation initiation; K_{2P}, two-pore domain potassium channels

This is a Commentary on an article in BJP by Kisselbach *et al.*, 2014; 171: 5182–5194. The interactions of drugs with their targets are impacted by a multitude of factors in ways that are often difficult to predict. To optimize therapeutic efficacy and minimize unwanted effects, the biology as well as the pharmacology of the system must be examined and understood. In the paper in question, Kisselbach *et al.* report novel influences on the anti-arrhythmic pharmacology of cardiac-expressed K2P channels, K2P 2.1 and K2P 10.1. These potassium-selective 'background' channels, which help to modulate cellular excitability, are shown to undergo alternative translation initiation (ATI), resulting in differing protein products. ATI is then also demonstrated to orchestrate a shift in the drug sensitivity of K2P2.1 and K2P10.1, illustrative of the complexity of native ion channel pharmacology. As studies like this inform us about the biology of current and future drug targets, the emerging challenge is to harness this knowledge to create safer drugs while maximizing therapeutic potential.

Unanticipated complexity of drug–target interactions creates a headache for those attempting to rationalize and create simple models of anti-arrhythmic 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.* (2014) are a case in point. Building on previous pioneering work demonstrating that neuronal K_{2P}2.1 potassium-selective 'background' channels (for nomenclature, see Alexander *et al.*, 2013) can become permeable to sodium ions depending on alternative translation initiation (ATI) (Thomas *et al.*,

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2008), the Thomas laboratory now shows that the 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-adrenoceptor (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 K_V channels and the inward rectifier K_{IR} 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 with their dynamic, voltage-sensing, K_V channel relatives. Unless modified by external agents, the open probability of K_{2P} 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 the 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 six subfamilies based on both primary structure and functional properties (see Alexander *et al.*, 2013). Physiological activators of the various members of this family include stimuli as diverse as intracellular and extracellular pH, heat, membrane stretch, $G_{\alpha i}$ and $G_{\alpha q}$, NO, hypoxia, hypoglycaemia, calcium, arachidonic acid, polyunsaturated fatty acids and lysophospholipids. Physiological antagonists include extracellular low pH, sumoylation, PKA and PKC, Zn^{2+}, and $G_{\alpha q}$ and $G_{\alpha s}$. Pharmacologically, K_{2P} channels exhibit a very different profile from that of other potassium channel families, being insensitive to the classic K_V channel blockers but inhibited by, for example, some local anaesthetics, and activated by the non-steroidal anti-inflammatory drug flufenamic acid and general anaesthetics 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 provide a tonic repolarizing force to oppose this. However, if these pores are closed by external stimuli, the 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, for example, mechanical stretch or pharmacological manipulation may alter the timing of cardiomyocyte action potentials and, therefore, potentially arrhythmogenesis and cardiac contractility.

This brings us back to the findings of Kisselbach *et al.* (2014). Previous 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 human ether a go-go-related gene (hERG) K_V channel, $K_V11.1$, 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 laboratories 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 the 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 non-specific 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 physi-

ology and pharmacology, we must embrace the complexity and endeavour to exploit it, rather than viewing it simply as a hurdle to overcome.

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Conflict of interest

The author has no competing interests.

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