I_{KACh} at the whim of a capricious M2R

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A prominent feature of the cells that constitute excitable tissues is their capacity to undergo sudden changes in their membrane potential. Some membrane proteins have evolved exquisite structures, voltage-sensing domains, to transduce these changes into conformational rearrangements that modulate their functions and properties. Voltage-gated ion channels are certainly the most represented and studied form of voltage-dependent proteins. Indeed, cellular excitability is heavily dependent on their selective ionic conductance as well as their ability to respond to the membrane potential with changes in their open probability. Voltage sensitivity, however, is not a prerogative of ion-conducting proteins and, not surprisingly, other plasma membrane proteins have been found to possess intrinsic voltage-sensing properties, so that their mode of operation is fine-tuned by the cell membrane potential. An outstanding example is the G-protein-coupled muscarinic receptor (M2R), recently found to possess intrinsic voltage dependence which causes its affinity for acetylcholine to decrease at depolarized membrane potentials (Ben Chaim *et al.* 2003, 2006). While the physiological implications of this modulatory effect of the membrane potential are still under investigation, interesting new data prelude to exciting novel developments. In a recent issue of *The Journal of Physiology*, two back-to-back manuscripts (Moreno-Galindo *et al.* 2011; Navarro-Polanco *et al.* 2011) are shedding light on the molecular mechanism by which M2R ligand sensitivity varies with membrane potential and its implication to an aspect of the parasympathetic regulation of heart function.

M2R does not possess the typical voltage-sensing structure as found in voltage-gated channels; nevertheless, it undergoes a conformational rearrangement when the membrane potential changes. A series of experiments by Navarro-Polanco and colleagues demonstrate that depolarization-induced structural rearrangements in M2R decrease the affinity for acetylcholine but augment the potency of another agonist, pilocarpine. That is, the membrane potential can shift the M2R affinity for the two agonists in an opposite direction, suggesting that membrane depolarization induces a structural change in the ligand binding pocket.

Capitalizing on these findings, the authors of the companion manuscript take advantage of the differential effect of membrane depolarization on acetylcholine and pilocarpine affinity to provide a mechanistic interpretation for the somehow puzzling kinetic behaviour of the acetylcholine-activated potassium current in the atrial myocytes (I_{KACh}). In the right atrium of the heart, within the sinoatrial node, G-protein-gated inwardly rectifying potassium channel subunits GIRK1/Kir3.1 and GIRK4/Kir3.4 form heterotetrameric channels which are specifically activated by a direct interaction with G$\beta\gamma$ subunits. Acetylcholine released during vagus nerve activity activates M2R resulting in the dissociation of the Gα subunit and the G$\beta\gamma$ complex. G$\beta\gamma$ binding to GIRK channels activates the hyperpolarizing potassium current I_{KACh}, decreasing the rate of diastolic depolarization and consequently slowing down the heart rate. A mysterious feature of I_{KACh} is its slow development at hyperpolarized potentials, known also as relaxation gating, first described more than 30 years ago in rabbit sinoatrial node cells (Noma & Trautwein, 1978) and more recently attributed to the voltage-dependent changes in cytosolic [Ca^{2+}] affecting the activity of proteins regulating G-protein signalling (RGS) (Ishii *et al.* 2001).

The papers by Navarro-Polanco, Moreno Galindo and colleagues present evidence for an alternative mechanism underlying I_{KAch} relaxation gating, attributing its behaviour to the voltage dependence of M2R ligand affinity. At hyperpolarized potentials, M2R binds acetylcholine with higher affinity than at depolarized voltages, resulting in an increased level of intracellular G$\beta\gamma$ which activates I_{KACh} and regulates its timecourse, giving rise to the characteristic 'relaxation kinetics'. As the affinity of M2R for acetylcholine is lower at positive potentials, membrane depolarization resulted in deactivation of I_{KACh}. In contrast, when acetylcholine was substituted by pilocarpine, I_{KACh} was activated by membrane depolarization and deactivated at negative potentials, reflecting the voltage dependence of the potency of pilocarpine in activating M2 receptors. Thus, significant evidence is provided that I_{KACh} relaxation gating is indeed dominated by the voltage dependence of M2R ligand affinity. Furthermore, these studies offer an elegant demonstration of how an intrinsically voltage-independent conductance acquires voltage dependence by coupling its activation pathway to the voltage dependence of M2R ligand affinity.

The physiological implications of these findings are intriguing and warrant further exploration of the physiopathological regulation of cellular excitability by voltage-dependent G-protein-coupled receptor (GPCR) operation. I_{KACh} is increasingly gaining interest as a therapeutic target for atrial fibrillation (AF) (Ehrlich & Nattel, 2009), the prevalent form of tachyarrhythmia in humans. Atrial activation of I_{KACh} by vagal nerve stimulation or cholinergic agonists accelerates the repolarization phase of the action potential, shortening its duration and increasing the heterogeneity of the refractory period, thus providing a substrate that can promote the occurrence of sustained AF. This scenario can be exacerbated in the setting of atrial tachycardia electrical remodeling, which is associated with an increase in inward rectifier K^+ currents, including the constitutively active acetylcholine-dependent K^+ current ($I_{KACh,c}$).

Further investigations are anticipated to establish the physiological scope of this intriguing signalling mechanism. It would be particularly interesting to see computational modelling of the atrium as well as *in vivo* studies focused on the voltage-dependent properties of M2R, to reveal the role and the relevance of these observations in shaping atrial excitability

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and the heart rate. While the work of Navarro-Polanco, Moreno Galindo and colleagues will undoubtedly inspire new studies to better understand GPCR function, it is also likely to influence drug development, to take further advantage of M2Rs as therapeutic targets, exploiting their peculiar voltage-dependent properties, a venture of particular importance in light of the contribution of parasympathetic stimulation to atrial fibrillation and heart disease.

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