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COMMENTARY

More types than one: multiple muscarinic receptor coupled K^+ currents undergo remodelling in an experimental model of atrial fibrillation

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The common cardiac arrhythmia atrial fibrillation (AF) tends to show progression in its severity, which is associated with 'remodelling': structural and electrophysiological changes that facilitate arrhythmia induction and maintenance. In this issue of the *BJP*, Yeh and colleagues demonstrate for the first time, down-regulation of three types of muscarinic cholinergic receptor (mAChR) coupled K^+ currents ($I_{KM2,}$ I_{KM3} and I_{KM4}) and of M_2, M_3 and M_4 mAChR subtype proteins, in a canine model of atrial tachycardia (AT) induced remodelling. The I_{KM}s and their extent of AT-induced remodelling were similar in left-atrial and pulmonary vein (PV) myocytes, so remodelling of M_2–M_4 receptor-linked currents appears not to underlie the unique contribution of PVs to AF. Parasympathetic stimulation can increase susceptibility to AF; thus remodelling of M_2–M_4 receptors and K^+ currents could be adaptive in AT. Further work is warranted to determine whether or not remodelling of multiple mAChRs and currents also contributes to human AF.
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Abbreviations: AF, atrial fibrillation; AP, action potential; 4-AP, 4-aminopyridine; AT, atrial tachycardia; I_{KM2}, I_{KM3}, I_{KM4}, subtypes of muscarinic receptor-coupled K^+ current; LA, left atrium; mAChR, muscarinic acetyl-cholinergic receptor; M_2,M_3,M_4, subtypes of mAChR; PV, pulmonary vein

Atrial fibrillation (AF) is the most common cardiac arrhythmia and can have serious clinical consequences including heart failure and stroke (Chugh *et al*., 2001). Once established, AF tends to be resistant to antiarrhythmic therapy, and cardioversion to sinus-rhythm is frequently followed by reversion to AF (Waktare *et al*., 1998). Evidence from AF patients and from rapid-atrial-pacing animal models supports the notion that *AF-begets-AF*: Atrial tachycardia (AT) leads to electrical and structural remodelling in the atria that contributes to the arrhythmic substrate (Allessie *et al*., 2002; Nattel, 2002, 2005). An ectopic focus associated with the junctions of the pulmonary veins (PVs) with the left atrium may play a role in the initiation of paroxysms of AF; indeed, procedures involving catheter isolation of the PVs are used clinically to isolate the source of triggered arrhythmia (see for example, Haissaguerre *et al*., 1998; Chen *et al*., 1999). Moreover, there is evidence that vagal denervation reduces the recurrence of AF in patients receiving PV ablation (Pappone *et al*., 2004), consistent with a role for parasympathetic innervation, particularly of the PV, in arrhythmogenesis (see for example, Allessie *et al*., 1984; Coumel, 1996; Liu *et al*., 1997; Schauerte *et al*., 2000). The effectiveness of genetic I_{KACh} ablation at preventing AT has led to the suggestion that I_{KACh} might represent a therapeutic target in AF (Kovoor *et al*., 2001). However, mixed changes to $I_{K,ACh}$ in AF have been reported: while AT increases a constitutively active component of $I_{K,ACh}$, the ACh-induced current density is reduced (Dobrev *et al*., 2001, 2005; Ehrlich *et al*., 2004; Cha *et al*., 2006). As vagal stimulation is a known cause of AF, a reduction in receptor-mediated activation of $I_{K,ACh}$ may be an adaptive response, reducing the risk of arrhythmia. The major component of $I_{K,ACh}$ (called I_{KM2}) arises from M_2 mAChR activation. However, recent evidence suggests that other mAChR subtypes are present in the heart and that these couple to distinct K^+ currents: I_{KM3} being coupled to the M_3 receptor and I_{KM4} coupled to the M_4 receptor (for example, Navarro-Polanco *et al*., 1997; Shi *et al*., 1999).

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Table 1 Summary of the biophysical and pharmacological properties of mAChR-activated K^+ currents in canine left atrial and pulmonary vein cardiomyocytes, including the pharmacological protocol used for their selective activation in the study by Yeh *et al.* (2007)

Current	*Receptor*	*Voltage-dependence type*	*Physiological agonist*	*Experimental agonist*	*M_1-selective blocker (100 nM pirenzipine)*	*M_2-selective blocker (20 nM methoctramine)*	*M_3-selective blocker (2 nM 4-DAMP)*	*M_4-selective blocker (200 nM tropicamide)*
I_{KM2}	M_2	Inward rectifier	Acetylcholine	Carbachol[a]	[b]		[b]	[b]
I_{KM3}	M_3	Delayed rectifier	Acetylcholine ?	Choline	[b]	[b]		[b]
I_{KM4}	M_4	Delayed rectifier	Acetylcholine ?	4-Aminopyridine		[b]	[b]	[b]

[a]Current recorded as atropine-sensitive difference current.
[b]Included in experiments. For example, to measure I_{KM2} antagonists of M_1, M_3 and M_4 were present.
? = presumed physiological agonist.

While remodelling of receptor-activated I_{KM2} with AT pacing has been established, until now there has been no comparable information for M_3 or M_4-linked currents. Furthermore, despite the importance of the PVs in AF, there is little information in the literature on mAChR-activated currents in PV cardiomyocytes. It is against this background that Yeh and colleagues have investigated the different mAChR subtypes and their coupled K^+ currents in left atrial (LA) and PV myocytes (see pages 1021–1032) of this volume of the journal).

Yeh *et al.* (2007) have employed a canine model of AT remodelling that produced changes to refractoriness and increased the duration of burst pacing-induced AF episodes. Western blotting revealed that the densities of M_2, M_3 and M_4 mAChR proteins were decreased by ~50% in membrane samples from AT-paced dogs. The respective effects of AT-pacing on I_{KM2}, I_{KM3} and I_{KM4} were evaluated pharmacologically, applying agonists of each receptor in the presence of inhibitors of other mAChR subtypes (see Table 1). I_{KM2} was found to be decreased by ~60% compared to controls in both atrial and PV cells from AT-paced dogs, with current densities similar between the two cell types in each group. I_{KM3} and I_{KM4} were each decreased by ~50% without concomitant changes in current kinetics and, as for I_{KM2}, both currents exhibited similar densities in atrial and PV cells in each group. However, no changes were seen in agonist sensitivity of the currents activated by each receptor type (which is notable given that distinct intracellular-signalling pathways may couple the various receptors to their respective currents (Shi *et al.*, 1999, 2004b)).

Thus far, two conclusions can be drawn: first, I_{KM2}, I_{KM3} and I_{KM4} are all present in canine LA and PV myocytes and undergo AT-induced remodelling. Second, the particular sensitivity of the PVs to ACh-induced arrhythmias cannot be accounted by differential remodelling of these receptors/currents. It is tempting to draw a third conclusion based on the broadly similar changes to receptor density and current magnitudes that the changes to each of the currents resulted from a decrease in their respective receptors. However, downregulation of G-protein and/or ion channel subunits could also be involved in the effect, a possibility that is as yet difficult to investigate since it is not clear which K^+-channel subunits underlie I_{KM3} and I_{KM4}, although work from another group showing sensitivity of all mAChR-activated current components by tertiapin-Q (a $G\beta\gamma$-gated K^+-channel blocker), is consistent with the involvement of similar channel subunits in all three components (Benavides-Haro *et al.*, 2003).

What, then, of the functional consequences for atrial electrophysiology of the downregulation of the different muscarinic receptor activated K^+ currents? I_{KM2} has been relatively well-studied, but there is little information on the roles of I_{KM3} and I_{KM4}, which both show a differing voltage dependence (delayed-outward-rectification) to that of the inwardly rectifying I_{KM2}. This is a challenging question, given the lack of completely-selective, high-affinity agonists for each muscarinic receptor-type. Nevertheless, Yeh *et al.* (2007) have addressed the issue. First, they measured currents activated by ACh in the absence of mAChR blockers, in the presence of M_2 receptor blockade and then with M_2 blockade + M_3 and M_4 antagonists, with results concordant with a M_3 and M_4-activated outward current component. Second, they assessed the effects of ACh on atrial action potential (AP)-duration in the presence of M_2 receptor activation and then with M_3 and M_4-receptors blockade. With M_2-receptors blocked, ACh produced a modest (~10%) reduction in AP-duration at 90% repolarization, which was antagonized by M_3 and M_4 receptor blockade. Both sets of observations are consistent with I_{KM3} and I_{KM4} being able to contribute to ACh-mediated atrial AP-shortening in this species. It is feasible, therefore, that downregulation of I_{KM3} and I_{KM4} as well as of I_{KM2} may contribute to the adaptive changes that counter the otherwise proarrhythmic effects of vagal-stimulation in AF.

The intriguing observations in this comprehensive study (Yeh *et al.*, 2007) raise a number of questions, including: (1) whether or not I_{KM3} and I_{KM4} show similar functional expression in human atrium and PVs, as seen in dog and (2) what might the role of these currents be in the response of the human atrium to physiological/pathophysiological levels of vagal stimulation? In addition, it is interesting to note that distinct remodelling of the three I_{KM}s reported here occurs in experimental congestive heart failure (Shi *et al.*, 2004a). Since AF can arise from a number of different aetiologies, it would be of interest to determine whether or not similar remodelling of the three I_{KMs} to that reported here occurs in other AF aetiologies/experimental models (of hypertension, for example (Kistler *et al.*, 2006; Choisy *et al.*,

2007), as this is a major risk factor for AF). Ultimately, will it be possible to intervene therapeutically with agents targeted selectively towards the different mAChR-subtypes? While the answer to that lies in the future; the study of Yeh *et al*. (2007) provides an important demonstration that three mAChR-activated K^+-currents, and not one, may be players in AF.

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