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EDITORIAL

Cardiomyocyte-specific Gq signalling and arrhythmias: novel insights from DREADD technology

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This editorial refers to 'DREADD technology reveals major impact of G_q signaling on cardiac electrophysiology' by E. Kaiser *et al.*, pp. 1052–1066.

G protein-coupled receptors (GPCRs) are the most common receptors on the surface of cardiomyocytes. GPCRs coupled to G_q play a central role in mediating the response of cardiac tissue to several hormones. Angiotensin-II (acting on the AT_1 receptor), endothelin-1 (ET_A receptor), and noradrenaline/adrenaline (alpha-adrenergic receptor) are the main hormones that share the intracellular signalling associated with Gq protein in the cardiomyocyte.

Activation of G_q-coupled receptors in the heart leads to proarrhythmic consequences (see *Figure 1*). AT1 receptors contribute to the pathogenesis of supraventricular and ventricular arrhythmias, and chronic AT_1 blockade in cardiac patients is protective against the development of atrial fibrillation and sustained ventricular arrhythmias.[1,2] Activation of α_1 receptors may contribute to adrenergic-induced arrhythmias on top of the more relevant β_1 and β_2 receptor stimulation.[3] Indeed, pharmacological inhibition of α-receptors combined with β-block provides an additional antiarrhythmic protection in patients with arrhythmic syndromes such as catecholaminergic polymorphic ventricular tachycardia (CPVT).[4] Moreover, phenylephrine, a selective α1-receptor agonist, causes acute atrial fibrillation in animal models.[5] Activation of ET_A receptors by endothelin-1 may be involved in the generation of ventricular arrhythmias in the presence of acute myocardial ischaemia.[6]

Based on pioneer studies in cardiac tissue and cells, several effectors have been proposed as mediators of the proarrhythmic activity of Gq-coupled receptors, such as Na^+/H^+ antiport for the α1-receptors.[7] However, whether arrhythmias are mediated by changes in the cardiomyocyte function or in the activity of other myocardial cells (fibroblasts and smooth muscle) remains unclear. Similarly, the beneficial effects of a number of drugs commonly employed in cardiovascular therapy to treat hypertension, such as AT_1 receptor blockers (losartan and others sartans), ET_A blockers (bosentan and others, used for pulmonary hypertension), and α1A blockers (tamsulosin and others) have been poorly explored in terms of their direct antiarrhythmic potential.

Kaiser *et al.*[8] first obtained a selective hyperactivation of the Gq pathway in the working and conduction cardiomyocytes employing a designer receptors solely activated by designer drugs (DREADD) technology. In brief, by using a combined genetic/chemical approach, an exogenous 'designer receptor' associated to a Gq-like protein (Dq protein) is introduced in the cardiomyocyte sarcolemma (and expressed both on the surface and the t-tubules). The receptor has no constitutive activity and is stimulated by the acute administration of an exogenous agonist [clozapine-N-oxide (CNO)] that exerts its effects within a few minutes. The authors show that the activation of the Gq pathway leads to ventricular and supraventricular tachyarrhythmias and degree 1 and 2 AV block, with no changes in the action potential profile. The arrhythmic events can be ascribed to a reduction in conduction velocity and the authors suggest that the effect could be mediated by the interplay between spatially co-localized Dq and connexin CX43.

Compared to previous work exploring the Gq signalling with pharmacological agonists/antagonists of Gq-coupled receptors,[1,5,6] a major advantage of this technique resides in its spatio-temporal precision. The work by Kaiser *et al.*[8] represents the first evidence of 'cardiomyocyte-specific' effects of the Gq-coupled receptors, although no functional discrimination between cardiomyocyte sub-types was envisaged (e.g. working vs. conduction myocardium, atrial vs. ventricular, or endocardial vs. epicardial myocytes).

Important insight on cardiac arrhythmias can be obtained by extending the possibility of controlling the G protein signalling *in vivo* within a specific cell type. On this respect, cell type-specific DREADD expression could by easily achieved using viral or transgenic approaches. For example, this can allow controlling the G protein cascade at sinoatrial or AV node as well as in the cardiac conduction system (e.g. Purkinje fibre) distinctly from the working myocardium. DREADD expressions through systemic delivery of viral vectors could allow challenging the hyperactivation of specific pathways also in animal models that mimic acquired or genetic cardiac diseases.

For sake of comparison, Beiert *et al.*[9] recently showed that a light-sensitive Gq-coupled receptor (melanopsin) is a powerful optogenetic tool for the investigation of spatial and temporal aspects of Gq signalling in the heart. On this respect, patterned illumination[10] could be very

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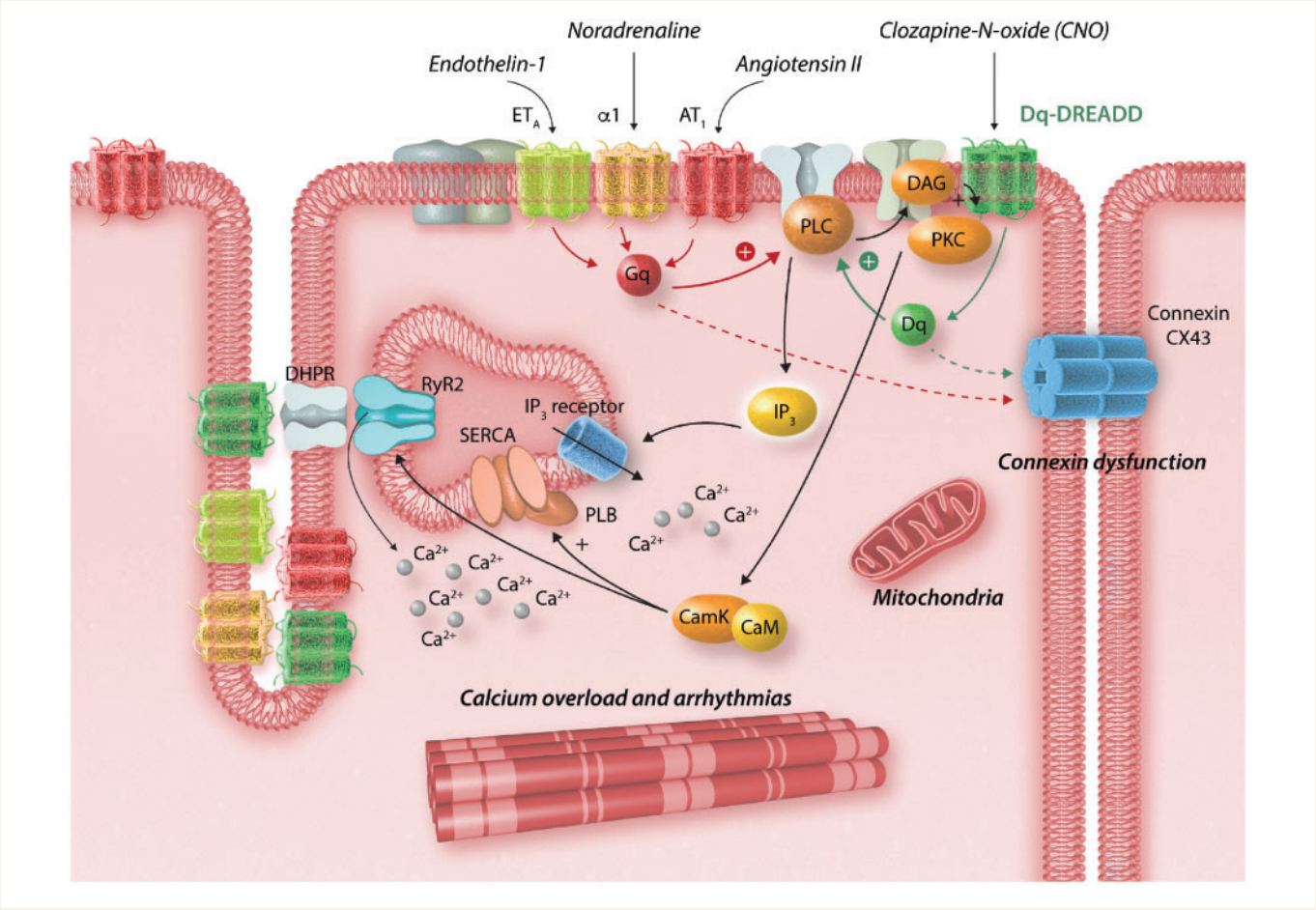

Figure 1 G_q signalling and arrhythmias. Figure depicting the proposed mechanisms underlying the induction of arrhythmias upon activation of G_q-linked GPCRs. Activation of ET_A, α_1, or AT_1 receptors on the cardiomyocyte surface leads to activation of G_q proteins that in turn activate phospholipase-C (PLC). PLC hydrolyses phosphatidylinositol triphosphate (PI_3) to inositol triphosphate (IP_3) + diacylglycerol (DAG). IP_3 activates IP_3 receptors on the sarcoplasmic reticulum, which lead to enhanced release of Ca^{2+} from the SR, while DAG activates protein-kinase C (PKC), which in turn phosphorylates several targets including L-type Ca-channels (DHPR), which ultimately contribute to intracellular Ca-overload. Moreover, sustained increase of diastolic [Ca^{2+}] leads to activation of Ca-calmodulin kinase II (CAMK) that further aggravates Ca-overload and facilitates spontaneous diastolic openings of the Ryanodine receptor (RYR2). The increased rate of spontaneous diastolic Ca release and Ca-waves cause delayed after depolarizations (DADs, i.e. spontaneous depolarizations occurring during the diastolic period), caused by diastolic activation of electrogenic Na–Ca-exchange. DADs may cause premature action potentials and may initiate arrhythmias. Moreover, either through a direct action of G_q or via PKC-mediated phosphorylation, Cx43 connexin assemblies are made dysfunctional, thus reducing myocardial conduction speed and increasing the risk of re-entry.

useful in performing confined receptor activation across single cells or specific tissue regions. This could overcome limitations of standard delivery of pharmacological agonists where global diffusion prevent sharp localizations. However, as light needs to be very close to the target tissue or organ, optogenetic studies *in vivo* often need to be performed in open-chest approaches, with enormous limitations for the application in awake and freely moving animals, with no possibilities to study the effects of the chronic Gq pathway activation.

To date, in addition to Gq DREADDs, other signalling cascades have been selectively interrogated in neuroscience studies by means of CNO-activated DREADDs (Gi, Gs, and β-arrestin),[11] and could be of interest for the cardiovascular field. For instance, DREADD-mediated activation of G_i signalling could help elucidating whether the alternative response or diverse sublocalization of β2-adrenergic receptors are involved in the pathogenesis of Takotsubo cardiomyopathy,[12,13] and whether it involves a localized enhancement of G_i signalling in apical cardiomyocytes due to high levels of circulating epinephrine. The modulatory proteins β-arrestins contribute to desensitization of many GPCR (e.g. β-adrenergic and AT1) and initiate alternate signalling pathways, often involving nuclear expression regulation.[14] Selective cardiomyocyte-specific activation of β-arrestin may be useful to clarify the extent by which the effects of GPCRs depend on G protein independent pathways and to demonstrate whether a selective modulation of β-arrestin pathway may serve as a therapeutic strategy in cardiac diseases.[15] DREADD-mediated activation of β-arrestin and G_s in cardiomyocytes may help defining which of the downstream effects of β-receptors are dependent on each of the two alternative pathways.

In the era of emerging chemogenetics where DREADDs appear as an extremely promising technology to study selective intracellular signalling in the cardiomyocytes, a few limitations could be envisaged. Among them, the difficulty to obtain a compartmentalized response (e.g. receptors located in the surface sarcolemma vs. t-tubules) or to reproduce

some pleiotropic responses of the naïve receptors. To date, this technology cannot re-create all the downstream effects of GPCRs, in particular those that are not dependent on G-proteins. Notably, several long-term effects of AT-1 receptor activation (cardiac hypertrophy and rhythm disturbances) are independent on Gq signalling and may rely on alternate pathways.

Conflict of interest: none declared.

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