Cardiac action of the first G protein biased small molecule apelin agonist

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Pulmonary arterial hypertension (PAH) has a poor prognosis and is associated with pulmonary vasoconstriction, right ventricular hypertrophy and right heart failure. Current therapies aim to reduce vasoconstriction but do not benefit the heart and more efficacious treatments are required.

The peptide apelin signals through the apelin receptor to produce vasodilatation and cardiac inotropy, while its expression is decreased in PAH. Importantly, the apelin receptor is not down-regulated and infusion of apelin is beneficial in animal models[1]. As a peptide, apelin is not an optimal drug-like molecule owing to its lack of bioavailability, limited half-life and rapid internalisation of the receptor through β-arrestin signalling. In PAH, we hypothesise that a G protein biased small molecule apelin agonist could replace the missing endogenous peptide to produce vasodilatation and protect against cardiac remodelling of the right ventricle without receptor desensitisation. We characterised, *in vitro* and *in vivo*, the pharmacology of a novel small molecule agonist, CMF-019, demonstrating G protein bias at the apelin receptor.

In competition radioligand binding experiments in heart homogenates CMF-019 bound to human, rat and mouse apelin receptor with high affinity (pK_i= 8.58±0.04, 8.49±0.04 and 8.71±0.06 respectively). In cell-based functional assays, whereas CMF-019 showed similar potency for the $G_{i±i}$ pathway to the endogenous agonist [Pyr[1]]apelin-13 (pD_2=10.00±0.13 n=11/4 vs pD_2=9.34±0.15 n=8/4 respectively), in β-arrestin (pD_2=6.65±0.15 n=13/4 vs pD_2=8.65±0.10 n=12/4) and internalisation (pD_2=6.16±0.21 n=6/2 vs pD_2=9.28±0.10 n=6/2) assays it was much less potent. Results are expressed as mean±sem and for cell-based assays, n-values are given as the number of replicates/number of experiments. Bias analysis was performed using the methodology of van der Westhuizen *et al.* (2014)[2] and bias factors of ~400 for signalling through the $G_{i±i}$ compared to the β-arrestin pathway and ~5800 compared to receptor internalisation were obtained.

Normotensive male Sprague-Dawley rats (273 ± 6g) were induced and maintained under anaesthesia with inhaled isoflurane (3% and 1.5% respectively) carried by oxygen (1.5l/min) and a pressure-volume catheter placed in the left ventricle to measure cardiac parameters. Intravenously injected CMF-019 (2500µg) caused a significant increase in cardiac contractility (dP/dt_{Max}, 833±152mmHg/s n=9) compared to saline (88.7±94.4mmHg/s n=3) ($p<0.001$, student's t-test).

CMF-019 is the first biased small molecule identified at the apelin receptor and displays activity *in vivo*. This molecule provides evidence that biased agonism can be developed in small molecules and provides a basis for the rational design of new biased apelin receptor agonists for the treatment of cardiovascular conditions such as PAH.

1. Yang P, Maguire JJ, Davenport AP. Apelin, Elabela/Toddler, and biased agonists as novel therapeutic agents in the cardiovascular system. *Trends Pharmacol Sci* 2015;36:560-567.
2. van der Westhuizen ET, Breton B, Christopoulos A, Bouvier M. Quantification of ligand bias for clinically relevant beta2-adrenergic receptor ligands: implications for drug taxonomy. Mol Pharmacol. 2014;492-509.