Pharmacology of the Apelin Receptor and Relevance to Pulmonary Arterial Hypertension

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Background: Apelin is the endogenous ligand of the class 1 G protein-coupled apelin receptor. We have previously shown that in the cardiovascular system apelin peptides mediate endothelium-dependent vasodilatation, endothelium-independent vasoconstriction, and positive inotropic effects in the heart, *in vitro* (Maguire *et al.,* 2009). A role for apelin is emerging in pulmonary arterial hypertension (PAH), a severe disease with increased pulmonary arterial pressure and right heart failure. Evidence suggested that an apelin agonist may be beneficial (Andersen *et al.,* 2011). Therefore we have synthesised an apelin analogue, MM07, which is equipotent with the endogenous apelin isoform (Pyr^{1})apelin-13 as a vasoconstrictor, and also importantly, more resistant to degradation than the native isoforms owing to its cyclised structure.

Hypothesis and Objectives: Our hypothesis was that the apelin system is altered in PAH and apelin receptor agonists might be beneficial. The first aim was to determine whether the apelin peptide and receptor are present in PAH tissue, and localise them to specific cell types in rat heart and lung. Secondly, we studied the receptor pharmacology of MM07 in comparison with two endogenous apelin isoforms, (Pyr^{1})apelin-13 and apelin-36, in a β-arrestin recruitment assay.

Experimental Approach: Peroxidase-anti-peroxidase immunocytochemistry and dual-labelling fluorescent immunocytochemistry experiments were conducted using 30µm sections of heart and lung tissues from male Sprague-Dawley rats treated with saline (n=4) or monocrotaline (MCT) (n=4), as a model of PAH. Agonist-induced β-arrestin recruitment was investigated in Chinese hamster ovary cells artificially expressing the apelin receptor using a β-galactosidase fragment complementation assay. Concentration-response curves were constructed for (Pyr^{1})apelin-13 and apelin-36 (both 10^{-11}~10^{-6}M), and MM07(10^{-9}~10^{-4}M).

Key results: Apelin-like immunoreactivity (LI) localised to the vascular endothelium, whereas apelin receptor-LI was found in the endothelium, vascular smooth muscle and myocardium of normal and MCT-treated tissues. Specific staining of apelin and its receptor appeared to be reduced in endothelium in PAH compared to healthy tissue. In the β-arrestin assay, MM07 (pD_2=5.8±0.2, n=6) was less potent than the endogenous agonists (Pyr^{1})apelin-13 (pD_2=8.5±0.09, n=15) and apelin-36 (pD_2=8.5±0.01, n=3).

Conclusions and Implications: The presence of apelin and its receptor in PAH was confirmed in rat heart and lungs, and their cell-specific localizations were determined. The data suggested down-regulation of the apelin system in PAH, which needs to be confirmed using quantitative measurements. In addition, MM07 was significantly less potent ($p<0.05$, Student's t test) as an agonist in the β-arrestin recruitment assay than the endogenous apelin peptides, although we had previously shown that these peptides were equi-effective vasoconstrictors. This suggests MM07 may be a biased agonist.

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