Role for apelin in human atherosclerosis and discovery of novel agonists for its receptor APJ

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APJ is a class A G-protein coupled receptor, recently paired with its endogenous ligand apelin. We have previously localised APJ and apelin to the human cardiovascular system (Kleinz *et al*., 2004; Kleinz *et al*., 2005) and shown apelin to have vasoactive and inotropic actions (Maguire *et al*., 2009). Our aims were (1) to investigate whether APJ and its ligand are altered in cardiovascular disease in man and (2) to develop pharmacological tools to further probe this system.

Firstly, a novel radioimmunoassay detected apelin-like immunoreactivity (-LI) in human large diameter blood vessels and cardiac tissue. There was a significant upregulation of apelin-LI in atherosclerotic compared to histologically normal coronary artery (73±15pg/g wet mass, n=6 vs 24±4pg/g wet mass, n=6, P<0.01 Student's *t*-test). Immunohistochemistry localised this additional apelin-LI to the atherosclerotic plaque, where it colocalised with markers for smooth muscle cells and macrophages. (Pyr^{1})apelin-13 potently constricted human endothelium denuded coronary artery (pD_2 9.1±0.6, E_{max} 19± 9% KCl, n=3).

Secondly, structure activity studies investigated the role of the C-terminal phenylalanine of apelin. Apelin-13(F13A), previously shown to be a functional antagonist in rat (Lee *et al*., 2005), competed for [Glp^{65},Nle^{75},Tyr^{77}][^{125}I]Apelin-13 binding in human left ventricle, and potently constricted human endothelium denuded saphenous vein (pD_2 10.1±0.3, E_{max} 22±4% KCl, n=4). Cleavage of the C-terminal phenylalanine from apelin by ACE2 has been assumed to be an inactivating step. However, the cleavage product (Pyr^{1})apelin-13(1-12) showed affinity for APJ in radioligand binding assays, and agonist activity in human saphenous vein *in vitro* (pD_2 9.1±0.4, E_{max} 29±9% KCl, n=5). We have developed cyclic analogues of apelin and tested these for affinity and activity at APJ. One compound (cyclo(1-6)CRPRLCHKGPMPF) (Macaluso *et al*., 2009) competed for [Glp^{65},Nle^{75},Tyr^{77}][^{125}I]Apelin-13 binding and showed agonist activity in human isolated saphenous vein (pD_2 10.5±0.2, E_{max} 22±6% KCl, n=3).

These data implicate apelin in human atherosclerosis, and increased apelin in atherosclerotic coronary artery may contribute to coronary vasospasm. Structure activity studies suggest the C-terminal phenylalanine of apelin is not essential for agonist binding or activity and ACE2 is not an inactivating enzyme in this system. Subsequent investigation of cyclic apelin analogues led to the discovery of a novel selective agonist for APJ.

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