Available online at www.sciencedirect.com

SCIENCE DIRECT

Regulatory Peptides 126 (2005) 233–240

REGULATORY PEPTIDES

www.elsevier.com/locate/regpep

Immunocytochemical localisation of the apelin receptor, APJ, to human cardiomyocytes, vascular smooth muscle and endothelial cells

Matthias J. Kleinz[a,*], Jeremy N. Skepper[b], Anthony P. Davenport[a]

[a]*Clinical Pharmacology Unit, University of Cambridge, Level 6, Centre for Clinical Investigation, Box 110 Addenbrooke's Hospital, Cambridge, CB2 2QQ, UK*

[b]*Multi-Imaging Centre, Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK*

Received 1 July 2004; received in revised form 6 October 2004; accepted 7 October 2004
Available online 15 December 2004

Abstract

The novel G protein-coupled receptor APJ, recently paired with the proposed cognate peptide ligand apelin, mediates potent vasodilator and positive inotropic actions in rats. Radioligand binding showed apelin receptors in rat and human heart and human large conduit vessels. The specific cell types expressing the receptor, however, have not been determined. Apelin, the cognate receptor ligand, is present in endothelial cells. However, the exact pathway of endothelial apelin synthesis and secretion is not known.

We therefore investigated the cellular distribution of APJ receptor-like immunoreactivity (APJ-LI) in a range of human tissues using immunocytochemistry and fluorescent double staining confocal microscopy. The same techniques were applied to determine the intracellular localisation of apelin-like immunoreactivity (apelin-LI) in cultured human umbilical vein endothelial cells (HUVECs).

APJ-LI is present in endothelial cells, vascular smooth muscle cells and cardiomyocytes. Apelin-LI localises to secretory vesicles and the Golgi complex/endoplasmic reticulum of HUVECs. Apelin-LI does not co-localise with von Willebrand factor in Weibel-Palade bodies, suggesting synthesis of apelin via the constitutive pathway.

The proximity of receptor and ligand in the human vasculature, together with evidence for local vascular apelin synthesis, suggests an important role for APJ/apelin as a paracrine cardiovascular regulator system.

Keywords: G protein-coupled receptor; Vasodilator; Inotropic; Peptide; Secretion; Constitutive

1. Introduction

The APJ receptor, previously designated an "orphan" G protein-coupled receptor, was first cloned from a human gene by O'Dowd et al. [1] and apelin-36 was proposed as the cognate endogenous ligand [2]. Although apelin-36 was the first apelin peptide discovered, further research has identified a number of shorter forms of the APJ receptor ligand in human tissues and bovine colostrum, which are thought to be produced by posttranslational modification of the 77-amino acid prepropeptide [2,3]. Recently, however, functional assays have provided evidence that the short pyroglutamyl form of apelin, (Pyr^1)apelin-13, may represent the biologically active endogenous ligand [4–7].

Messenger RNA encoding the APJ receptor has been shown to be abundantly expressed in the central nervous system of rats and humans [1,8–10] and the receptor was associated with a role in fluid homeostasis [7,10,11]. The APJ receptor has also been proposed as an essential co-receptor to CD4 in the infection of central nervous system cells with t-tropic or dual-tropic HIV strains [9,12,13]. The main body of evidence, however, supports a role for the APJ/apelin system in the regulation of cardiovascular function. Apelin receptors have been detected in rat and human myocardium as well as in the medial layer of human coronary artery, aorta and saphenous vein using radioligand binding and (Pyr^1)apelin-13 was shown to be a potent vasoconstrictor in endothelium denuded, isolated human

* Corresponding author. Tel.: +44 1223762564; fax: +44 1223336899.
E-mail address: mk395@medschl.cam.ac.uk (M.J. Kleinz).

0167-0115/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2004.10.019

saphenous vein [14]. In rats, *in vivo* intravenous administration of apelin leads to a significant decrease in mean arterial blood pressure, a response completely abolished by co-administration of the nitric oxide synthase inhibitor L-NAME [7,10,15,16]. Comparison of mice in which the APJ receptor gene had been deleted with wild type controls revealed a lack of apelin-induced hypotensive actions in the knockout animals. This study also showed apelin-induced over-expression of endothelial nitric oxide synthase in cultured murine endothelial cells from control animals, which was abolished in endothelial cells from APJ receptor knockout mice [17]. Furthermore, apelin has been found to elicit positive inotropic effects in the isolated rat heart [18].

In pathophysiological conditions in humans, left ventricular expression of APJ receptor mRNA was significantly reduced in patients with idiopathic dilated cardiomyopathy[19]. In a similar patient group, the use of microarray technology identified the APJ receptor gene to be one of two genes overexpressed after implantation of a left ventricular assist device, with changes observed in circulating apelin levels in heart failure patients [20]. This may suggest a role for APJ/apelin in the pathogenesis of heart failure.

At present, the mechanism how apelin mediates both endothelium-dependent vasodilator and endothelium-independent vasoconstrictor actions has not been established. The APJ receptor has been detected in human cardiovascular tissue using receptor autoradiography [14], but the precise cellular localisation remains to be determined. Based on our report of apelin being abundantly present in endothelial cells of the human vasculature [21], we have proposed that apelin may elicit vasoconstriction through paracrine activation of APJ receptors on vascular smooth muscle. To explain the vasodilator effects we hypothesised that in the presence of an intact endothelium vasoconstriction is counterbalanced, or even overcome, by apelin-induced release of vasodilator mediators from endothelial cells. To find supportive evidence for this proposed mechanism we investigated the precise cellular distribution of the APJ receptor in human tissues and the intra-cellular localisation of apelin and APJ receptors in both *in situ* and human umbilical vein endothelial cells (HUVECs) using immunocytochemistry and fluorescent double labelling in conjunction with confocal laser scanning microscopy.

2. Materials and methods

2.1. Materials

Unless stated, all chemicals were obtained from Sigma Aldrich (Poole, UK). Rabbit anti-APJ receptor (rat) antiserum and rabbit anti-apelin-12 (rat/human) antiserum used in immunocytochemistry was obtained from Phoenix Pharmaceuticals (Belmont, CA, USA). Mouse-anti-human von Willebrand factor and mouse-anti-human smooth muscle α-actin monoclonal antibodies, secondary antibodies, rabbit-PAP-complex and horseradish-peroxidase-conjugated swine-anti-rabbit antiserum were from Dako (Glostrup, Denmark). AlexaFluor 488 conjugated goat-anti-rabbit serum and AlexaFluor 568 conjugated goat-anti-mouse serum were obtained from Molecular Probes (Leiden, The Netherlands), Vectashield mounting medium containing 4′,6′-diamino-2-phenylindole hydrochloride (DAPI) was from Vector Laboratories (Burlingame, CA, USA) and DePeX-Gurr mounting medium from BDH Laboratory Supplies (Poole, UK). The site-directed rabbit anti-APJ receptor (rat) antiserum was raised against the extreme C-terminus of the APJ receptor (amino acid residues 349–376) sequence, a region differing in only two amino acids in rat and human. Test experiments in human tissues resulted in staining patterns similar to those in rat tissue and consistent with mRNA distribution in human tissue. A BLAST-p search of publicly available human peptide libraries retrieved no other human peptides with significant sequence similarity, making non-specific cross-reactivity of the antiserum unlikely [22].

2.2. Tissue collection

2.2.1. Human tissue sections

Human tissues were obtained with local ethical approval. Left ventricular and atrial myocardium were from patients undergoing heart transplants for cardiomyopathies (n=4) or from donor hearts for which there was no suitable recipient (n=6). Saphenous veins (n=10), radial arteries (n=4) and left internal mammary arteries (n=3) were from patients undergoing coronary artery bypass graft surgery for ischaemic heart disease. Coronary arteries were obtained from patients undergoing heart transplants for ischaemic heart disease (n=2) and donor hearts not required for further transplantation (n=2). Histologically normal kidney (n=4) and lung (n=4) were from patients undergoing nephrectomy and lobectomy respectively for non-obstructive carcinoma. The histologically normal adrenal tissue (n=3) was obtained from two patients undergoing adrenalectomy for phaeochromocytoma. On collection, tissues were snap frozen in liquid nitrogen and stored at −70 °C until further use.

2.2.2. Human umbilical vein endothelial cells

HUVECs were a kind gift from Dr. Jun Wang (Department of Medicine, University of Cambridge), cultured as described previously [23] and grown until they reached subconfluency. Culture medium was aspirated and cells were stored at −70 °C until further use.

2.2.3. Rat tissue

Rat brains (n=3), hearts (n=3) and lungs (n=3) were used to initially characterise the rabbit anti-APJ receptor (rat) antiserum and as positive controls based on previous findings (Lee, 2004). Tissue was obtained from male Sprague–Dawley rats (300–350 g, Charles River, Wilmington, MA, USA) that were euthanized by CO_2 inhalation.