Life Sciences 118 (2014) 391–396

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Modulation of endothelin receptors in the failing right ventricle of the heart and vasculature of the lung in human pulmonary arterial hypertension

Rhoda E. Kuc [a], Myrna Carlebur [a], Janet J. Maguire [a], Peiran Yang [a], Lu Long [b], Mark Toshner [b], Nicholas W. Morrell [b], Anthony P. Davenport [a,*]

[a] Clinical Pharmacology Unit, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK
[b] Department of Medicine, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK

ARTICLE INFO

Article history:
Received 1 November 2013
Accepted 11 February 2014
Available online 26 February 2014

Keywords:
Pulmonary arterial hypertension
Endothelin
FR139317
Radioligand binding
Affinity constant
Receptor density

ABSTRACT

Aims: In pulmonary arterial hypertension (PAH), increases in endothelin-1 (ET-1) contribute to elevated pulmonary vascular resistance which ultimately causes death by right ventricular (RV) heart failure. ET antagonists are effective in treating PAH but lack efficacy in treating left ventricular (LV) heart failure, where ET_A receptors are significantly increased. The aim was to quantify the density of ET_A and ET_B receptors in cardiopulmonary tissue from PAH patients and the monocrotaline (MCT) rat, which recapitulates some of the pathophysiological features, including increased RV pressure.
Main methods: Radioligand binding assays were used to quantify affinity, density and ratio of ET receptors.
Key findings: In RV from human PAH hearts, there was a significant increase in the ratio of ET_A to ET_B receptors compared with normal hearts. In the RV of the MCT rat, the ratio also changed but was reversed. In both human and rat, there was no change in LV. In human PAH lungs, ET_A receptors were significantly increased in the medial layer of small pulmonary arteries with no change detectable in MCT rat vessels.
Significance: Current treatments for PAH focus mainly on pulmonary vasodilatation. The increase in ET_A receptors in arteries provides a mechanism for the beneficial vasodilator actions of ET antagonists. The increase in the ratio of ET_A in RV also implicates changes to ET signalling although it is unclear if ET antagonism is beneficial but the results emphasise the unexploited potential for therapies that target the RV, to improve survival in patients with PAH.

Introduction

In pulmonary arterial hypertension (PAH), increases in endothelin-1 (ET-1) contribute to elevated pulmonary vascular resistance which ultimately causes death by right ventricular heart failure. PAH involves injury to the pulmonary vasculature producing elevations in pulmonary arterial pressure. As PAH progresses, chronic pressure and volume overload cause alteration of the structure of the right ventricle (RV) including hypertrophy and dilatation. As a result, the space taken up by the RV in the pericardium increases, impeding left ventricular (LV) diastolic filling, reducing LV end-diastolic volume and altering the LV contractile function (Bogaard et al., 2009). Right heart failure is the major cause of death in PAH patients. ET antagonists are effective in treating PAH (Liu et al., 2013) but in marked contrast, lack efficacy in treating left ventricular heart failure (Kelland and Webb, 2007; Kohan et al., 2012). This is surprising as the density of ET_A receptors in the LV of patients with ischaemic heart disease is significantly increased by 50%, compared with non-failing hearts (Peter and Davenport, 1996a). However measurement of receptor density in the RV from patients with PAH using radioligand binding assays has not been studied.

ET_A receptors are the principal sub-type in the medial or smooth muscle layer of human blood vessels, including large epicardial and small resistance coronary arteries within the heart where ET_A receptors mediate vasoconstriction (Maguire and Davenport, 1995; Pierre and Davenport, 1998). We have previously shown that in human large conduit vessels these are altered in cardiovascular disease including PAH (Kuc and Davenport, 2000). Davie et al. (2002) found no change in ratio of ET_A:ET_B but increased overall receptor density in smooth muscle cells from human pulmonary small resistance arteries in PAH.

ET_B receptors localise to the endothelium and cause beneficial vasodilatation by the release of endothelium derived relaxing factors,

* Corresponding author at: Clinical Pharmacology Unit, University of Cambridge, Level 6, Centre for Clinical Investigation, Box 110 Addenbrooke's Hospital, Cambridge CB2 0QQ, UK. Tel.: +44 1223 336899 (International); fax: +44 1223 762576 (International).
E-mail address: apd10@medschl.cam.ac.uk (A.P. Davenport).

http://dx.doi.org/10.1016/j.lfs.2014.02.020
0024-3205/

opposing constrictor tone. In addition, in organs such as the lungs that are rich in ET_B receptors (Bagnall et al., 2006), this sub-type functions to clear ET-1 from the plasma (Johnström et al., 2005). Two classes of ET antagonist are used clinically, mixed antagonists that block both sub-types and ET_A selective drugs. The precise molecular mechanism whereby these antagonists produce benefit in PAH is not established. In particular, the contribution of ET_B receptors to the development of this condition and the need to block this sub-type as well as the ET_A is still unclear (Vachiery and Davenport, 2009). Our aim was to compare the density of both ET receptor sub-types in surgical samples from the right and LV of hearts and lungs removed from PAH patients at the time of transplantation, in comparison with normal tissues. Secondly to measure receptor density in a widely use animal model of PAH, which recapitulates a majority of the features of the human condition including right ventricular failure (Ryan et al., 2011).

Materials and methods

Human heart

Surgical samples of LV and RV were obtained from PAH patients (idiopathic pulmonary artery hypertension) undergoing heart–lung transplantation and from normal controls that were not suitable for transplantation. Samples of PAH lung were obtained from patients undergoing lung transplantation and histologically normal control tissue was from patients undergoing lung lobectomy procedures. All tissues were collected with informed consent and ethical approval.

MCT-rat tissue collection

The procedures used in this study were approved by the local animal ethical committee and were performed under UK Home Office Project Licence authority; the study conformed to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Male Sprague–Dawley rats (approximately 250 g) received a single subcutaneous injection of monocrotaline (60 mg/kg) at day 0 to induce PAH (Long et al., 2013). The rats were maintained for three weeks following injection to develop muscularization of small pulmonary arteries in the lungs and right ventricular hypertrophy but without developing dilated heart failure (Long et al., 2013). Rats were euthanized by CO_2 inhalation. Organs were removed and snap-frozen in liquid nitrogen and stored at −70 °C until further use.

Competition assays

Cryostat-cut tissue sections (10 μm) were mounted onto gelatine coated microscope slides.

Competition binding assays were performed as previously described (Maguire et al., 2012a), to determine the affinities (K_D) and maximum densities (B_{MAX}) of ET_A and ET_B receptors.

Sections were incubated with 0.1 nM [^{125}I]-ET-1 (Perkin Elmer) and increasing concentrations (20 pM–10 μM) of the ET_A selective agonist FR139317 for 2 h at 23 °C. Non-specific binding (NSB) was determined using 1 μM of unlabelled ET-1. Following incubation and washing (3 × 5 min) in ice-cold Tris–HCl buffer to break the equilibrium, sections were counted in a gamma counter.

Competition curves were obtained by plotting specific binding as a percentage of total binding (binding in the absence of competitor) against the log concentration of the competing ligand. The data were analysed (see Maguire et al., 2012b) using non-linear iterative curve fitting programmes (KELL, containing EBDA and LIGAND programmes, Biosoft, Cambridge UK) to calculate K_D (affinity constant) and B_{MAX} (maximum density of receptors).

Autoradiography

For autoradiographical analysis, binding was carried as previously described (Ling et al., 2012) using assay conditions outlined above in a set of adjacent sections, to determine total [^{125}I]-ET-1 (0.1 nM) binding, non-specific binding (1 μM unlabelled ET-1) and with selective antagonists, either 0.1 μM BQ3020 or 0.1 μM FR139317 to determine ET_A and ET_B receptor distribution respectively. Adjacent sections were stained to facilitate histological identification of pulmonary vasculature. Sections were washed to break the equilibrium and apposed, together with calibrated radioactive standards, to radiation-sensitive film (Kodak BioMax MR-1, Perkin Elmer). Resulting autoradiograms were analysed by measuring diffuse integrated optical density using the Quantimet 970 image analysis system. ET-1 receptor density was measured by digitizing each autoradiographical image and regions of interest on tissue sections were delineated. Optical densities were converted to specifically bound radioligand by interpolation from standard curves and subtraction of non-specific binding in an adjacent section.

Results

Pharmacodynamic parameters in human and rat heart

In human normal hearts, competition binding revealed the expected ratio of ET_A to ET_B receptors (Fig. 1A, Peter and Davenport, 1996a). FR139317 competed biphasically for the binding of [^{125}I]-ET-1, with a two-site fit preferred over a one-site model with no significant difference in affinity constants (Table 1, K_D) between patient groups (Fig. 1). Whilst there was no significant change in receptor sub-type ratio in LV, there is a significant increase in ET_A with a concomitant decrease in ET_B receptors in the failing RV (Fig. 1B, C).

In the rat model (Fig. 1D) the expected ratio of receptor sub-types was observed in both chambers of the hearts of control rats (Peter and Davenport, 1996b). In the MCT rat, receptor density was significantly different in the RV compared with vehicle control but with ET_A down-regulation and ET_B upregulation (Fig. 1E, F). These changes led to a significant shift in relative ET_A:ET_B receptor density ratio from 73:27 in control rat RV to 51:49 in MCT-rat RV. In the LV, no significant difference in ET_A and ET_B receptor density in MCT-rat heart compared to controls was observed (Table 2).

Pharmacodynamic parameters in human PAH and MCT lung

Competition studies using whole cryostat sections in the lungs from patients with PAH compared to normal control tissues did not detect a significant difference in binding affinities (K_D) for ET_A or ET_B and no change in receptor densities (B_{MAX}) or ratio of sub-types in human PAH lungs compared with control (Fig. 2B, Table 3). In agreement, there were no changes in these parameters in MCT lungs compared with control. However, following apposition of labelled sections to radiation sensitive film, image analysis permitted the measurement of densities in discrete cell types. In the medial layer of small pulmonary arteries identified by comparison with adjacent stained sections, there was a significant increase in vascular ET_A receptors in PAH compared with control small vessels (Fig. 3A, Table 3). No equivalent changes were detected in the medial layer of MCT rat lungs compared with control (Fig. 3B, Table 3).

Discussion

Human heart with PAH

We have previously shown that ET_A receptors in the failing LV of patients with ischaemic heart disease are significantly increased by 50% (Peter and Davenport, 1996a,b). In agreement, in the failing RV of patients with PAH, there was a significant increase in the ratio of ET_A