BJP British Journal of Pharmacology

DOI:10.1111/bph.12022
www.brjpharmacol.org

Themed Section: Endothelin

EDITORIAL

Themed section: endothelin

Anthony P Davenport[1] and Matthias Barton[2]

[1]*Clinical Pharmacology Unit, Addenbrooke's Hospital, University of Cambridge, Cambridge, UK*
[2]*Molecular Internal Medicine, University of Zürich, Zürich, Switzerland*

Correspondence
Anthony P Davenport, Clinical Pharmacology Unit, University of Cambridge, Level 6, Centre for Clinical Investigation, Box 110, Addenbrooke's Hospital, Cambridge CB2 0QQ, UK. E-mail: apd10@medschl.cam.ac.uk

Keywords
cardiovascular system; epigenetics; GPCR; kidney; vasoactive intestinal contractor; receptor antagonist

This themed section of the *British Journal of Pharmacology* contains reviews on recent developments in endothelin research arising from the Twelfth International Conference on Endothelin (ET-12). It includes the emerging role for endothelin-2 in the cardiovascular system, ovarian development, immunology and cancer. The action of endothelin on two key targets is discussed: the paracrine or autocrine regulation of contractility and growth in the heart and the role of endothelin in renal disease. Epidemiological studies have demonstrated cardiovascular disease and circulating levels of endothelin-1 are lower in premenopausal women than in men and evidence is presented for the contribution of sex differences in responses to the peptide. Transcription is the primary level of regulation of the endothelin gene; and current research on the epigenetic regulation of the endothelin pathway, including the silencing of the *EDNRB* gene encoding the ET_B receptor during tumourigenesis, is reviewed.

LINKED ARTICLES

This article is part of a themed section on Endothelin. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2013.168.issue-1. To view the previously published paper by Dhaun *et al.* visit http://dx.doi.org/10.1111/j.1476-5381.2012.02070.x

Abbreviations

ECE, endothelin converting enzyme; ET, endothelin; PAH, pulmonary arterial hypertension; VIC, vasoactive intestinal contractor

The catalyst for this themed section was the Twelfth International Conference on Endothelin (ET-12) held 11–14th September 2011 in Cambridge, UK, hosted by the British Pharmacological Society in association with the American Physiological Society, where delegates considered new horizons for pharmacological intervention in the endothelin system and targets for the future (see Barton and Davenport, 2012). Since the discovery of endothelin-1 in 1988 (Yanagisawa *et al.*, 1988), research on this family of peptides has continued unabated with over one thousand papers appearing each year in Pubmed since 1995 and more than 25 000 papers published to date. During the ensuing two decades, it is now well established that endothelins (ETs) comprise a family of three endogenous 21-amino-acid peptides, ET-1, ET-2 and ET-3, that interact with two GPCR subtypes, ET_A and ET_B, both in Family A, the 'druggable' class of receptor. The presence of two disulphide bridges between the Cys^3–Cys^{11} and Cys^1–Cys^{15} is unique amongst the mammalian bioactive peptides. The closest in structure are the sarafotoxins, isolated from snake venom in the same year as the discovery of ET-1(Takasaki *et al.*, 1988). The blocked N-terminal amino acid is thought to confer resistance to enzymatic degradation in the plasma. Removal of ET-1 by ET_B 'clearing' receptors that mediate internalization followed by lysosomal degradation of the peptide via the carboxypeptidase cathepsin A is particularly important in terminating the actions of this peptide.

There are a number of features ET signalling pathway that are unusual compared with other peptidergic systems that continue to intrigue investigators. ET-1 remains the most powerful constrictor of smooth muscle with an unusually long-lasting action compared with other vasoactive agents such as angiotensin II. In man, ET-3 differs from ET-1 by six amino acids and is the only isoform that can distinguish between the two receptor subtypes, having a similar potency

© 2012 The Authors
British Journal of Pharmacology © 2012 The British Pharmacological Society

at the ET_B receptor as ET-1 and ET-2 but about two orders of magnitude lower affinity for the ET_A subtype. This rank order of agonist potencies provided the initial pharmacological criteria for classification of the two subtypes.

ET-2 differs from ET-1 by only two amino acids and has a similar affinity for both receptors and might appear to be redundant. *Why do we need this isoform?* ET-2 (or vasoactive intestinal contractor, the mouse analogue) remains the least studied of the ET isopeptides, and much less is known of its function and location than for ET-1 and ET-3. In this themed section, Ling *et al*. (2012) argue that it is time for reevaluation and summarize compelling evidence for expanding research in this field. Disruption of the ET_A/ET-1 pathway in genetically modified mice showed a similar lethal phenotype, dying at birth of respiratory failure secondary to severe craniofacial and cardiovascular abnormalities. This is similar to spectrum of human conditions, CATCH 22 (**C**ardiac anomaly, **A**bnormal face, **T**hymic hypoplasia, **C**left palate, **H**ypocalcemia, chromosome 22 deletions) and established the importance of this pathway in cardiovascular and craniofacial development. In ET_B or ET-3 knock-out mice, the enteric nervous system fails to develop, and animals display an aganglionic megacolon. This phenotype resembles spontaneous mutations in other animals (lethal white mutation in horses, piebald lethal in rats) and Hirschsprung's disease in man. Intriguingly, global ET-2 knock-out mouse displays a non-overlapping phenotype with either ET-1 or ET-3, with this phenotype exhibiting growth retardation and changes in energy homeostasis. The authors emphasize that ET-2 mRNA and ET-2 peptide are detected in the human cardiovascular system, including failing hearts, and its precursor big ET-2 is more abundant in the plasma than big ET-1. In addition, the review highlights some aspects of ET-2 pharmacology that are atypical as well as differences in the synthetic pathways between the isoforms. The authors review evidence for ET-2 having a pathophysiological role in heart failure, immunology and cancer. Perhaps the most exciting emerging role area is in ovarian physiology, with ET-2-mediated contraction proposed as a final signal facilitating ovulation. At present, we cannot distinguish pharmacologically between ET-1 and ET-2 and how two structurally similar peptides can exist in the same cellular compartment and signal via the same receptor but elicit different physiological responses. The answer may lie in the emerging concept of biased signalling and biased agonists, and antagonists may permit dissection the ET-1 versus ET-2 pathway (Maguire *et al*., 2012).

ET research benefited very quickly by the identification of selective ET_B peptide agonists, based on the linear (IRL-1620) or truncated (BQ3020) analogues of ET-1 or the snake venom toxin (sarafotoxin S6c). Surprisingly, a high-potency ET_A-selective agonist, still remains to be discovered. Peptide antagonists with remarkable subtype selectivity such as BQ123 or FR139317 (ET_A) and BQ788 (ET_B) have permitted the unequivocal identification of the particular subtype mediating a specific physiological or pathophysiological response, both *in vitro* and *in vivo*. Importantly, since these compounds were peptides they could be used as tool compounds for 'first in man' studies to charaterize the ET system prior to the development of small molecule antagonist. Both BQ788 and BQ123 continue to be used in acute experimental studies. Fortuitously, all three ET isoforms as well as agonists 1RL-1620 and BQ3020 possessed at least one tyrosine residue suitable for direct radiolabelling, permitting accurate measurement of key pharmacological parameters of affinity constants and receptor densities (Davenport and Maguire, 2006). ET-1 and selective ligands labelled with ^{18}F have also been developed for positron emission tomography to visualize receptors in the living animal. Activity to develop selective agonists and antagonists has been particularly intense, and over 2700 unique chemical entities have been reported as ligands or having pharmacological actions at ET receptors (European Bioinformatics Institute database ChEMBL, https://www.ebi.ac.uk/chembl/).

Thus, the endothelin system has always proved an attractive, if complex target for pharmacological intervention to block or reduce the actions of ET-1, with two distinct strategies emerging, receptor antagonists or inhibitors of ECE. The first ET antagonist to be introduced into the clinic was bosentan (Tracleer) for the treatment of pulmonary arterial hypertension (PAH) and is a mixed ET_A/ET_B antagonist blocking both receptors. This was followed by ambrisentan (Letairis, Volibris) in 2007, reported to display modest ET_A selectivity, and the more ET_A selective antagonist sitaxentan (Thelin), although this compound was withdrawn in 2010 (Kohan *et al*., 2012). Promising results with the next generation of ET antagonists such as macitentan (also a mixed ET_A/ET_B) and atrasentan (ET_A selective) are beginning appear (Kohan *et al*., 2012). While mixed ET_A/ET_B- and ET_A-selective antagonists have become established as having therapeutic benefit in PAH the relative merits of the two classes continues to be debated. Exploiting the inhibition of the enzymes of ET synthesis has not yet led to the development of a clinical compound. However, a strategy of combining inhibition of neutral endopeptidase (to increase the concentration of the beneficial vasodilator, ANP) with inhibition of ECE has led to the development of daglutril (SLV-306).

The kidney is exquisitely sensitive to the unwanted vasoconstrictor actions of ET-1 largely mediated by ET_A receptors, whereas ET_B responsible beneficial anti-natriuretic and vasodilator actions. As Dhaun *et al*. (2012) discuss in their review, chronic kidney disease remains a major but challenging target for therapeutic intervention by ET antagonists with ET having a number of pathophysiological actions in addition to unwanted vasoconstriction. They consider the distribution of ET receptors and ET action on the glomerulus, the functional unit of filtration and its constituent cells, podocytes, mesangial and endothelial, particularly in the context of cell proliferation, inflammation and fibrosis. This information is essential in understanding the potential impact of mixed ET_A/ET_B- versus ET_A-selective antagonists or whether strategies to reduce over-expression of ET-1 via ECE inhibition would be more effective. The authors review research on animal models of renal disease and the more limited information from clinical studies with ET antagonists, including their own pioneering research, and conclude that most of the adverse actions are via the ET_A subtype; but more studies in laboratory and clinic are needed to establish ET as a valid target in the treatment of chronic kidney disease.

One of the key questions in ET research remains unresolved. ET antagonists have shown benefit for the treatment of PAH, which is characterized by right ventricular heart failure and is the most common cause of death in patients

with this condition. However, ET antagonists have failed in the treatment of left ventricular heart failure. Why are ET antagonists beneficial in one patient group with right heart failure but not those with left? The heart is the first organ to develop in the embryo, and vessels must be patent within the third week to circulate blood and maintain perfusion of the developing embryo. The human heart has a high density of ET receptors, and the peptide is generated by endocardial endothelial cells as well as endothelial cells lining the coronary circulation. As Drawnell *et al*. (2012) indicate, the ET-1/ET_A pathway is crucial from the development of the aortic arc in the embryo and subsequently critical in the adult heart for myocyte survival and preventing loss during aging – a crucial peptide from cradle to (almost) grave. ET-1 modulates cardiac contractility in normal physiology, a potent inotropic response with comparable in magnitude with that generated by catecholamines and appears universal across species. In pathophysiology, ET-1 has been established to function in remodelling of the heart, particularly maladaptive cardiac hypertrophy, and the authors review associated downstream signalling pathways. The review concludes with speculation as to why these trials may have failed.

PAH may be more prevalent in women than in men, but the reason for this is unknown. Also, women have lower endothelin levels than men and oestrogen therapy reduces circulating levels of endothelin (Polderman *et al*., 1993). Conversely, as emphasized by David Pollock and colleagues, hypertension and chronic kidney disease are more common in men compared with premenopausal women. One explanation for gender differences may reside in the role of sex hormones, but clearly in the modulating the ET system, this is complex. In this thought-provoking review, Kittikulsuth *et al*. (2012) highlight some of the molecular mechanisms that underlying sex differences in animal models, which include higher levels of ET-1 and greater sensitivity to vasoconstriction in male rats, amplifying the pathophysiogical effects of the peptide mainly by the action of testosterone. Remarkably, ET_A receptors have a previously unsuspected action – at least in experimental animals – in producing adverse effects in males, whereas females are protected by an increase in ET_B function. The review emphasizes the importance of considering potential sex differences in experimental designs with previous research difficult to interpret because the sex of the animal was not recorded. The challenge will be whether these sex differences can be exploited to improve the efficacy of treatment.

What are the new drug targets for the future? We are familiar with changes in phenotype that can be induced by the environment on genetically identical individuals, resulting in extremes exemplified by the honeybee queen versus the workers. Molecular mechanisms of epigenetics underlying these changes are now being unravelled and increasingly recognized as a powerful force in all organisms and importantly may yield new strategies for drug treatment. One definition of epigenetics is 'heritable changes in phenotype through mechanisms other than changes in DNA sequence', which implies that epigenetic changes will be preserved when cells divide and therefore have a profound role in normal development and disease processes. There are a number of physiological processes thought to mediate epigenetic regulation, including DNA methylation and histone modification. The latter process involves post-translational covalent modification of histone proteins by a range of writers, erasers and readers, which modulates the ability of associated DNA to be transcribed. The histone code is read by specific families of proteins such as bromodomains. The latter is of pharmacological significance because of the recent discovery of small molecule inhibitors, which selectively modulate gene expression (Prinjha *et al*., 2012). Brian Cain and colleagues emphasize that epigenetics in the endothelin pathway is of particular importance (Welch *et al*., 2012). The prevailing consensus is that transcription is the primary level of ET-1 regulation of the gene EDN1 by histone modifications and DNA methylation. This provides a mechanism for tissue-specific production of ET-1 to enable the biologically diverse actions of the peptide. Importantly, the authors have examined whole genome databases and concluded that in addition to the ET-1 gene, there is evidence for epigenetic regulation of the ET_B gene (EDNRB), and they highlight the clearest example for altering ET-1 signalling by the silencing of the EDNRB gene by DNA methylation during development of tumors, resulting in the down-regulation of the receptor. Under these conditions, the beneficial effects of ET-1 in being removed by ET_B clearing receptors and promotion of apoptosis would be reduced or lost, suggesting the ET_B receptor system would be a target for epigenetic drugs or ET_B agonists. This might be specific to the type of tumour as ET may be the cause of some tumour types including melanomas and oligodendrogliomas (see Bagnato *et al*., 2011).

Unravelling the role of ETs in pathophysiological conditions continues to be an active area for research including cardiac hypertrophy and fibrosis, pre-eclampsia, pain, glaucoma, ischemic stroke, diabetes, cirrhosis, pulmonary and experimental arterial hypertension. Cancer represents a tantalizing prospect for one of the first applications of a GPCR antagonist to treat certain types of tumors and particularly metastasis of cancer cells (Kohan *et al*., 2012). Over a dozen clinical trials using ET compounds are currently recruiting in new areas such as asthma, porto-pulmonary hypertension, polycystic ovary syndrome, where there is an unmet clinical need (http://clinicaltrials.gov/ct2/). ET research continues into its third decade in robust health, and we look forward to ET-13 in Tokyo, Japan (http://www.endothelin-conferences.org/Conferences/tokyo2013/).

Acknowledgement

This work was supported in part by grants to APD from the British Heart Foundation, (Grant numbers PS/02/001 and PG/09/050/27734.), Wellcome Trust (268427 085686/2/08), Pulmonary Hypertension Association and the NIHR Cambridge Biomedical Research Centre.

Conflict of interest

None.

References

Bagnato A, Loizidou M, Pflug BR, Curwen J, Growcott J (2011). Role of the endothelin axis and its antagonists in the treatment of cancer. Br J Pharmacol 163: 220–233.

Barton M, Davenport AP (2012). The twelfth international conference on endothelin (ET-12), Cambridge 2011. Life Sci 91: 462–465.

Davenport AP, Maguire JJ (2006). Endothelin. Handb Exp Pharmacol 176: 295–329.

Dhaun N, Webb DJ, Kluth DC (2012). Endothelin-1 and the kidney – beyond BP. Br J Pharmacol 167: 720–731.

Drawnell FM, Archer CR, Roderick HL (2012). The role of the paracrine/autocrine mediator endothelin-1 in regulation of cardiac contractility and growth ET-1 in the heart. Br J Pharmacol 168: 296–317.

Kittikulsuth W, Sullivan JC, Pollock DM (2012). ET-1 actions in the kidney: evidence for sex differences. Br J Pharmacol 168: 318–326.

Kohan DE, Cleland JG, Rubin LJ, Theodorescu D, Barton M (2012). Clinical trials with endothelin receptor antagonists: what went wrong and where can we improve. Life Sci 91: 528–539.

Ling L, Maguire JJ, Davenport AP (2012). Endothelin-2, the forgotten isoform: emerging role in the cardiovascular system, ovarian development, immunology and cancer. Br J Pharmacol 168: 283–295.

Maguire JJ, Kuc RE, Pell VR, Green A, Brown M, Kumar S *et al*. (2012). Comparison of human ET(A) and ET(B) receptor signalling via G-protein and β-arrestin pathways. Life Sci 91: 544–549.

Polderman KH, Stehouwer CD, van Kamp GJ, Dekker GA, Verheugt FW, Gooren LJ (1993). Influence of sex hormones on plasma endothelin levels. Ann Intern Med 118: 429–432.

Prinjha RK, Witherington J, Lee K (2012). Place your BETs: the therapeutic potential of bromodomains. Trends Pharmacol Sci 33: 146–153.

Takasaki C, Tamiya N, Bdolah A, Wollberg Z, Kochva E (1988). Sarafotoxins S6: several isotoxins from Atractaspis engaddensis (burrowing asp) venom that affect the heart. Toxicon 26: 543–548.

Welch AK, Jacobs ME, Wingo CS, Cain BD (2012). Early progress in epigenetic regulation of endothelin pathway genes. Br J Pharmacol 168: 327–334.

Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y *et al*. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332: 411–415.