Online supplementary data

Materials and Methods

Table 1. Antibodies used for immunohistochemistry, immunofluorescence and Western blotting

Target	Antibody	Clone	Epitope location	Supplier	Use
ET-A	AER-001A	rabbit polyclonal	intracellular c-terminus	Alomone Labs	IH
ET-A	53D	rabbit polyclonal		A.Davenport (Univ. Cambridge)	IH
ET-A	ab12977	rabbit polyclonal	extracellular 2nd loop	Abcam plc	IH, IF
ETA	Ab76259	rabbit polyclonal	intracellular c-terminal	Abcam plc	WB
ETB	AER-002B	rabbit polyclonal	intracellular i3 loop	Alomone Labs	IH
ETB	51D	rabbit polyclonal		A.Davenport (Univ. Cambridge)	IH
ETB	ab12980	rabbit polyclonal	extracellular n-terminal	Abcam plc	IH, IF
ETB	ab39960	rabbit polyclonal	peptide ET-B residue 1-100	Abcam plc	WB
eNOS	sc654	rabbit polyclonal	c-terminus	Santa Cruz Biotech	IH
CD31	1A10	mouse monoclonal	Extracellular domain	Zymed	IH, IF

Semiquantitative analysis of immunohistochemical staining

Each anatomical region of the vessel wall was scored separately. Scoring was: - 0 for unlabelled, 0.5 for patchy labelling, 1 for uniform weak labelling and 2 for uniformly strong labelling of the media and intima, including proliferative cell layers, and for the endothelium.

Western blotting of endothelial cell extracts

Protein samples were separated using a 7.5% salt SDS-PAGE and then transferred electrophoretically to nitrocellulose membranes (Amersham Biosciences, Little Chalfont, UK). Blots were washed in Tris buffered saline containing 0.1% Triton X-100 (TTBS) and blocked with 5% milk in TTBS, before being incubated overnight at 4°C with the ET-A

(Abcam 76259, 1:1000) or ET-B (Abcam 39960 1:1000) receptor antibody. After several washes, blots were incubated for 1hr at room temperature with porcine anti-rabbit peroxidase (1:1000; Dako), followed by several washes in blocking buffer. All primary and secondary antibodies were diluted in 1% milk in TTBS. Blots were processed and developed using the ECL Plus chemiluminescent immunoblot detection system (GE Healthcare). For beta actin staining, blots were stripped with stripping buffer (2% SDS and 7μl/ml β-mercaptoethanol in 0.05M Tris pH 6.8) for 30 min at 55^0C and re-probed with mouse monoclonal anti-β-actin (Sigma AC15 diluted at 1:10,000) for 30 minutes at room temperature to assess loading density. Protein content was assessed by densitometry using NIH Image software and IP band densities, of normal and IPAH endothelial cell extracts, normalized to those of β-actin, within each blot were compared.