Quantification of pulmonary haemodynamic and vascular changes in chronic hypoxia and recovery in rats

M. Eneser, G.R. Barer, H.M. Marriott and C.J. Emery

Respiratory Medicine, Medical School, University of Sheffield, Sheffield, UK

Changes in the pulmonary vasculature in human hypoxic lung disease and in rats exposed chronically to hypoxia lead to haemodynamic changes. Muscularisation of small vessels occurs internal to the external elastic lamina (EL) and narrows the lumen, increasing resistance. We compare pulmonary haemodynamics with the development of new muscularisation of small intra-acinar (resistance) vessels 22.5–30 μm and < 22.5 μm diameter in rats (n = 3–6/group) kept in normoxia (N), normobaric hypoxia (10% O₂) for 2 or 4 weeks (CH2, CH4), or 2 weeks followed by 2 weeks air recovery period (REC).

After hypoxic exposure the rats were humanely killed by pentobarbitone overdose (100 mg kg⁻¹ I.P.). Series 1: lungs were perfused with plasma substitute (38°C, basal flow 20 ml min⁻¹) to measure pulmonary artery pressure (Ppa), and pulmonary vascular resistance (PVR) (slope of Ppa/flow line, flow to measure pulmonary artery pressure (Ppa), and pulmonary atery pressure (Ppa)), which is found in all known forms of the hormone. 125I-labelled UII was prepared by the Iodogen method and incubated with antibody and cold rat UII. Antibody bound 125I-labelled UII was separated by addition of bovine γ-globulin and polyethylene glycol, centrifugation and aspiration before counting on a gamma counter.

The rat UII assay was developed from our RIA for flounder UII (Winter et al. 1999). A polyclonal antibody was raised in rabbits against the conserved sequence of the UII peptide (CFWKYC) which is found in all known forms of the hormone. 125I-labelled UII was prepared by the Iodogen method and incubated with antibody and cold rat UII. Antibody bound 125I-labelled UII was separated by addition of bovine γ-globulin and polyethylene glycol, centrifugation and aspiration before counting on a gamma counter.

In utero protein restriction was induced by feeding female Wistar rats a diet containing 9% protein compared with an isocalorific 18% protein diet for control animals, from the day of conception until birth and a standard maintenance diet thereafter. This procedure has been shown to increase offspring blood pressure by 20–30 mmHg (Sahajpal & Ashton, 2001). Plasma was collected from anaesthetised control 18% (n = 5) and hypertensive 9% (n = 5) rats at 4 weeks of age by decapitation. Plasma was also collected from conscious adult spontaneously hypertensive rats (SHR, n = 13) and control Wistar-Kyoto rats (WKY, n = 9) by decapitation.

The rat UII RIA had a detection limit of 1.6 × 10⁻¹⁵ M with an intra-assay coefficient of variation of 9.2%. The plasma UII concentration of SHR was significantly higher than that of WKY rats (SHR 20.4 ± 1.9 × 10⁻¹² M vs. WKY 11.8 ± 1.4 × 10⁻¹² M, mean ± s.e.m., P < 0.05, unpaired t test). Similarly, the UII concentration in plasma from hypertensive 9% protein rats was significantly higher than that from control 18% rats (9% 12.7 ± 3.4 × 10⁻¹² M vs. 18% 2.0 ± 0.6 × 10⁻¹² M, P < 0.05).

Circulating UII concentrations are higher in the SHR and in utero protein restriction models of hypertension by comparison with their normotensive controls. This observation lends support to the suggestion that UII may play a role in cardiovascular disease.


All procedures accord with current UK legislation.