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

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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 % O_2) 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 5–30 ml min^{-1}); lungs were then fixed with formol-saline for histology and heart ventricles weighed to give RV/LV+septum. Series 2: lungs were fixed immediately for histology. Muscularisation of small vessels was assessed by Image-Pro analysis from: (1) area of α-actin in walls after immunostaining; (2) area between external elastic lamina and new internal elastic lamina (ΔEL), after elastin staining (Gomori) (Table 1). CH4 results, not given, were in all respects similar to those of CH2.

Table 1

		N	CH2	REC
Ppa (mmHg)		14.1 ± 0.3	21.9 ± 1.1	15.9 ± 0.7***#n.s.
PVR		0.49 ± 0.01	0.63 ± 0.01	0.56 ± 0.02*#
RV/LV+s		0.25 ± 0.013	0.49 ± 0.031	0.33 ± 0.024*#
MV (actin)	< 30 μm (n)	0	30	14
Area actin	22.5–30 μm	0	88 ± 7	69 ± 6*
	<22.5 μm	0	62 ± 8	0
MV (EL)	< 30 μm (n)	4	34	11
Area ΔEL	22.5–30 μm	45, 117, 48, 41	122 ± 13	109 ± 17
	<22.5 μm	0	60 ± 11	71, 27

Means ± S.E.M. *, ***P < 0.05, 0.001, REC/CH2; #P < 0.05, REC/N. Unpaired t test. MV = total from 3 rats.

The number (n) of small muscular vessels (MV), almost absent in N rats, increased in CH. The number and amount of new muscle was reduced after air recovery REC (22.5–30 μm), being virtually lost in vessels < 22.5 μm. Haemodynamic criteria were also reduced in REC but remained greater than in N. Thus haemodynamic and structural changes were correlated in both chronic hypoxia and after air recovery.

All procedures accord with current UK legislation.

A radioimmunoassay to detect urotensin II in the plasma of rat models of hypertension

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The peptide hormone urotensin II (UII) and its receptor GPR-14 have recently been identified in mammalian species, including man. Subsequent pharmacological studies have described profound cardiovascular effects in non-human primates, suggesting a potential role in related disease states in man. To date, very little is known about the physiology of UII in the rat, the most commonly used species in the study of cardiovascular function. Accordingly, the aims of this study were firstly to develop a specific radioimmunoassay (RIA) to allow the measurement of UII in rat plasma and secondly to determine whether the circulating concentration of UII differs from control values in two models of hypertension, the spontaneously hypertensive rat and the *in utero* protein restriction model of hypertension.

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. ^{125}I-labelled UII was prepared by the Iodogen method and incubated with antibody and cold rat UII. Antibody bound ^{125}I-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 $20.4 \pm 1.9 \times 10^{-12}$ M *vs.* WKY $11.8 \pm 1.4 \times 10^{-12}$ 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 % $12.7 \pm 3.4 \times 10^{-12}$ M *vs.* 18 % $2.0 \pm 0.6 \times 10^{-12}$ 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.

Sahajpal, V. & Ashton, N. (2001). *J. Physiol.* **535.P**, 18–19*P*.

Winter, M.J. *et al.* (1999). *Gen. Comp. Endocrinol.* **114**, 249–256.

All procedures accord with current UK legislation.