Increases in renal angiotensinogen mRNA levels following a mixed amino acid infusion in late gestation fetal sheep

A.C. Boyce, K.J. Gibson, J. Wu and E.R. Lumbers, Dept of Physiology & Pharmacology, School of Medical Sciences, University of New South Wales, Sydney 2052, Australia.

We have previously reported that prolonged infusions of amino acids to fetal sheep in late gestation stimulated renal growth, had profound, sustained effects on fetal renal function (including increases in glomerular filtration rate, renal blood flow, a diuresis, natriuresis and increased osmolar excretion), and induced changes indicative of extracellular volume expansion (Marsh *et al.*, 1999, Marsh *et al.*, 2002). This study aimed to determine whether the fetal renin-angiotensin system was also affected when plasma amino acid levels were increased long term.

Fetal sheep were chronically catheterised under general anaesthesia induced with 1 g sodium thiopentone i.v. and maintained with 2-3% halothane in oxygen. At least 5 days after surgery, 5 fetuses aged 122 ± 1 days gestation (term ~150 days) were infused i.v. for 7 days with a mixture of alanine, glycine, proline and serine (1:1:0.6:0.6) at 0.22 mmol min^{-1} and 5 mL h^{-1}. Six control fetuses were infused with 0.15 M saline. Plasma and renal renin levels were measured as the rate of formation of angiotensin I (Ang I) when plasma or homogenates of renal cortex were incubated at 37°C and pH 7.4 with an excess of angiotensinogen (nephrectomised sheep plasma). Levels of mRNA for renin, angiotensinogen and the angiotensin receptor subtypes I and II (AT_1R and AT_2R) were measured in renal cortical homogenates by real time PCR, and expressed relative to a calibrator sample.

After 7 days of amino acid infusion, plasma concentrations of the infused amino acids had increased by between 8- and 36-fold ($P<0.05$), and kidney weights were ~28% greater than those of control fetuses ($P<0.05$). Circulating renin levels fell during the first 4 h, from 9.3 ± 2.1 (mean ± SE) ng Ang I mL^{-1} h^{-1} in control to 4.7 ± 1.5 ($P<0.05$), and remained low throughout the infusion (Day 4: 4.2 ± 2.5, n.s.; Day 7: 2.2 ± 1.1 ng mL^{-1} h^{-1}, $P<0.05$). Plasma renin levels did not change during saline infusion (baseline: 5.9 ± 1.6 ng Ang I mL^{-1} h^{-1}). Renal renin levels tended to be lower following amino acid infusion compared to control fetuses (1.1 ± 0.4 vs 2.1 ± 0.4 μg Ang I mg $protein^{-1}$ h^{-1}, n.s.). Renal renin mRNA levels were also lower (Amino acids: 3.6 ± 2.2; Saline: 10.5 ± 2.7, $P=0.075$). There was marked increase in renal angiotensinogen mRNA levels (3.6 ± 0.5 vs 1.4 ± 0.2, $P<0.005$). Renal AT_1R and AT_2R mRNA levels were not different between groups.

Prolonged increases in fetal plasma amino acid levels were therefore associated with a suppression of circulating renin levels, and tended to suppress the gene expression and levels of renin in the developing kidney. These changes were probably secondary to volume expansion. However, the stimulation of renal angiotensinogen gene expression by amino acids suggests that the renal renin-angiotensin system may have played a role in the stimulation of renal function and growth that occurred with amino acid infusion.

Marsh, A.C., Gibson, K.J. & Lumbers, E.R. (1999) *Proceedings of the Australian Society for Medical Research*, Oral 8-1.

Marsh, A.C., Gibson, K.J. & Lumbers, E.R. (2002) *Journal of Physiology* **540**, 717.