AuPS/ASB Meeting - Adelaide 2010

Symposium: Emerging leaders in developmental physiology

Tuesday 30th November 2010 - The Gallery - 08:30

Chair: Caroline McMillen & Helena Parkington

Developmental programming following prenatal alcohol exposure: models and mechanisms of disease

K.M. Moritz, School of Biomedical Sciences The University of Queensland St Lucia 4067. (Introduced by Caroline McMillen)

Chronic prenatal exposure to high doses of alcohol can cause a range of developmental abnormalities but whether alcohol causes detrimental effects in the fetus following short term exposure or at low doses is somewhat controversial. Many women cease drinking upon pregnancy recognition but many continue to drink during pregnancy with the pattern of drinking varying from occasional drinking to routine consumption of a low amount of alcohol on a daily basis. In this study we hypothesized that the dose and timing of exposure may be critical in determining both the short and long term effects of alcohol on fetal development and outcomes in offspring.

Thus, we have used the following three rat models of alcohol exposure to explore the immediate and/or long term consequences for the fetus and offspring:

1. High dose binge (HD) in which rats are administered 1mg/kg alcohol (or saline as a control) by oral gavage on days 14 and 15 of pregnancy.
2. Chronic low dose exposure (CL) in which the dam has *ab lib* access to a liquid diet containing 6% alcohol (~15% calories derived from alcohol) or a control diet throughout pregnancy.
3. Periconceptional exposure (PC) where the dam has *ad lib* access to a liquid diet containing 12% alcohol (~30% calories derived from alcohol) from 4 days prior to mating and then for the first 4 days of pregnancy.

Maximal blood alcohol content (BAC) reached approximately 0.11%, 0.03-0.05% and 0.1% in the HD, CL, and PC models respectively. Offspring in the HD model were born growth restricted and remained small throughout lactation but experienced catch up growth after weaning and were of a similar weight to control animals in adulthood. Animals in the CL group were lighter at day 20 of pregnancy but were of similar weight to control animals throughout weaning and early adulthood. Animals in the PC were of a similar weight to controls at day 20 of pregnancy and have yet to be studied as offspring. As adults, both male and female offspring in the HD group had elevated blood pressure (BP, $p<0.001$) whilst BP was normal in the CL group. Renal function was altered in the HD offspring with males showing an increase in GFR ($p<0.001$) whilst females showed a decreased GFR ($p<0.01$). There were alterations in the ability of the CL offspring to concentrate urine following dehydration. Offspring in both the HD and CL groups had a reduced number of nephrons in the kidney (~20-30% reduction compared to controls) when examined using unbiased stereology. In the HD group there were significant changes in genes regulating branching morphogenesis in the fetal kidney whilst apoptosis was elevated in the fetal kidneys of animals in the CL and PC groups. This suggests that kidney development is susceptible to alcohol and that multiple mechanisms may contribute to the impairment in renal development and the subsequent low nephron number. The dose and timing of alcohol exposure is likely to be important in determining the subsequent risk of adult onset disease.

Intrauterine inflammation: effects on fetal lung development

T.J.M. Moss, The Ritchie Centre, Monash Institute of Medical Research. P.O. Box 5418, Clayton, VIC 3168, Australia. (Introduced by Helena Parkington)

Intrauterine infection or inflammation is common in cases of preterm birth, especially those that occur at very early gestational ages. Exposure of the fetus to prenatal infection or inflammation is independently associated with alterations in the risk of several neonatal diseases associated with prematurity. For example, evidence of exposure to infection/inflammation before birth is associated with a reduction in the risk of neonatal respiratory distress syndrome (RDS). This life threatening disease, which accounts for many neonatal deaths, is believed to be due primarily to a lack of pulmonary surfactant. The association between intrauterine inflammation and reduced risk of RDS suggests that prenatal inflammation stimulates fetal pulmonary surfactant production. In studies using sheep we have shown that experimentally induced intrauterine inflammation or infection (induced by amniotic fluid injection of lipopolysaccharide or live ureaplasmas) causes a precocious increase in pulmonary surfactant in the preterm lungs (Moss *et al.*, 2002a; Moss *et al.*, 2008) that improves preterm lung function, consistent with observations of human preterm infants. The effects of intrauterine inflammation appear to result from direct action of proinflamatory stimuli on the fetal lungs (Moss *et al.*, 2002b) rather than by systemic signals, such as stimulation of the fetal hypothalamic-pituitary-adrenal axis and activation of the classical glucocorticoid-mediated lung maturation pathway (Notsos *et al.*, 2002). These initial experiments have focused investigation of responsible mechanisms on local pulmonary factors that might be induced by inflammation and stimulate surfactant production.

A prime candidate for mediating inflammation-induced surfactant production by the preterm lung is prostaglandin E_2 and/or other arachidonic acid metabolites. Prostaglandin E_2 is a fundamental mediator of inflammation; limited available evidence indicates it can induce surfactant production in preterm lungs. Our experiments demonstrate that intrauterine inflammation induces expression of enzymes responsible for prostaglandin production in fetal lung tissue. Lung tissue analyses from these same experiments have demonstrated also that paracrine/autocrine production and/or metabolism of glucocorticoids in fetal lung tissue may occur in response to inflammation, as a result of inflammation-induced changes in expression of 11β hydroxysteroid dehydrogenase (types 1 and 2). This effect might account for at least some of the changes in fetal lung development induced by inflammation.

In order to address the role of glucocorticoid signaling in the response of the fetal lungs to inflammation, we are inducing intrauterine inflammation in transgenic pregnant mice carrying glucocorticoid receptor knockout fetuses. Consistent with our studies using sheep, intra-amniotic injection of lipopolysaccharide in wild-type mice induces large increases in surfactant protein gene expression in the preterm lungs. Demonstration of this same effect in the GR knockout mice would demonstrate this effect is independent of GR signaling.

The possibility exists that there are previously unknown mechanisms of stimulating surfactant production by the preterm lungs, which might be exploited as novel therapies for preventing respiratory distress syndrome in preterm infants. Elucidation of the effects of inflammation on the fetal lungs and other organs will allow more refined approaches to care of preterm infants exposed to inflammation *in utero*.

Moss TJ, Knox CL, Kallapur SG, Nitsos I, Theodoropoulos C, Newnham JP, Ikegami M, Jobe AH. (2008) Experimental amniotic fluid infection in sheep: Effects of Ureaplasma parvum serovars 3 and 6 on preterm or term fetal sheep. *American Journal of Obstetrics and Gynecology*. **198(1):** 122.e121-122.e128.

Moss TJM, Newnham JP, Willett KE, Kramer BW, Jobe AH, Ikegami M. (2002a) Early gestational intra-amniotic endotoxin: Lung function, surfactant, and morphometry. *American Journal of Respiratory and Critical Care Medicine* **165(6):** 805-811.

Moss TJM, Nitsos I, Kramer BW, Ikegami M, Newnham JP, Jobe AH. (2002b) Intra-amniotic endotoxin induces lung maturation by direct effects on the developing respiratory tract in preterm sheep. *American Journal of Obstetrics and Gynecology* **187(4):** 1059-1065.

Nitsos I, Moss TJM, Cock ML, Harding R, Newnham JP. (2002) Fetal responses to intra-amniotic endotoxin in sheep. *Journal of the Society for Gynecologic Investigation*. **9(2):** 80-85.

Early origins of cardiovascular disease: The heart of the matter

K. Wang,[1] K.J. Botting,[1,2] D. A. Brooks,[1] L. Zhang,[1] L. Rattanatray,[1] S. Zhang,[1] J. Duffield,[2] C. Suter,[3] I.C. McMillen[1] and J.L. Morrison ,[1] [1]Early Origins of Adult Health Research Group, School of Pharmacy and Medical Sciences, Sansom Institute of Health Research, SA 5001, Australia, [2]Discipline of Physiology, University of Adelaide, SA 5001, Australia and [3]Victor Chang Cardiac Research Institute, Lowy Packer Building, Liverpool Street, Darlinghurst, NSW 2010, Australia.

Cardiovascular disease currently affects over 3 million Australians. Reduced growth in fetal life together with accelerated growth in childhood results in an increased risk of hypertension and ~50% greater risk of coronary heart disease in adult life. It is unclear why changes in growth patterns in early life lead to a vulnerability to cardiovascular disease. Left ventricular hypertrophy is the strongest predictor of progressive heart disease and poor cardiovascular outcomes in adult life. Pathological ventricular hypertrophy begins as an adaptive response to increase cardiac pump function. However, if this response is prolonged it can lead to dilated cardiomyopathy, heart failure and sudden death. We propose that the mechanisms that support the growth of the heart when substrate supply is restricted during fetal life are the same as those which are recruited to induce pathological hypertrophy in later life. This may explain the vulnerability of the heart to the development of cardiovascular disease as the heart ages and is required to undergo hypertrophy in response to ischemic heart disease or hypertension in order to maintain cardiac output. Insulin-like growth factor 1 (IGF1) has been implicated in the initiation of ventricular hypertrophy. In a range of experimental models, IGF1 acts *via* the IGF1 receptor (IGF1R) both *in vivo* and *in vitro* to increase the size of cardiomyocytes. Recently it has been shown *in vitro* that when the IGF1R signalling pathway is blocked, addition of IGF2 results in an increase in the size of cardiomyocytes. This suggests that IGF2 may act to stimulate heart cell growth through the IGF2 receptor (IGF2R), which is interesting as the IGF2R has traditionally been viewed as a receptor which acts to clear IGF2, rather than as a receptor which is part of a ligand mediated growth pathway.

The adaptation of the fetal heart to a period of reduced substrate supply and decreased body growth has critical consequences for heart health in later life because at birth, the human heart contains most of the cardiomyocytes it will have for life. The growth of the heart in early development initially occurs through the division and hence proliferation of mononucleated cardiomyocytes which then undergo differentiation to form binucleated cardiomyocytes. These cells are unable to divide and heart growth then predominantly occurs through an increase in the size of the binucleated cardiomyocytes (hypertrophy). In a sheep model of intrauterine growth restriction (IUGR), induced by restriction of placental growth, we have investigated the proliferation and growth of cardiomyocytes and the pattern of differentiation of mononucleated to binucleated cardiomyocytes in the fetal heart. We have found that heart mass was maintained relative to fetal body mass, but that there was a relative increase in the size of binucleated cardiomyocytes in the heart of the IUGR sheep fetus. In addition, the low birth weight lamb has increased relative left ventricular weight at 21d of age. We propose that in response to a poor substrate supply in the fetus, the IGF1R signalling pathway plays a protective role in the heart through its anti-apoptotic and angiogenic actions. We also suggest that once up-regulated, the IGF2R signalling pathway mediates cardiomyocyte hypertrophy. This is a novel and significant hypothesis as it places the IGF2R, rather than the IGF1R, signalling pathway as a key mechanism underlying the changes in heart cell growth *in utero* which may contribute to a later vulnerability of the heart to pathological hypertrophy.

Maternal diets rich in fat programme obesity, hypertension and altered sympathetic nervous system activity in adult offspring

J.A. Armitage,[1,2] L.J. Prior,[2] S.L. Burke[2] and G.A. Head,[2] [1]Department of Anatomy and Developmental Biology, Building 76 Monash University, Wellington Road, Clayton, VIC 3800, Australia and [2]Neuropharmacology Laboratory, Baker IDI Heart and Diabetes Institute, PO Box 6492 St Kilda Road Central, Melbourne, VIC 8008, Australia. (Introduced by Janna Morrison)

The prevalence of obesity and related disease are rising rapidly worldwide. The majority of Australian adults are overweight or obese and the cardiovascular and metabolic consequences are predicted to have high financial and social costs. Adult risk factors, genetic predisposition and socioeconomic factors all contribute to the development of obesity and obesity related hypertension however there is compelling evidence that the early life environment also contributes to disease progression. This process is termed "developmental programming" and it is hypothesized that maternal dietary imbalance in pregnancy results in fetal and neonatal adaptations including redistribution of blood flow, altered organogenesis and growth in response to altered nutritional availability later in life (Barker 2001; Armitage, Taylor & Poston, 2005). Maternal obesity, maternal essential fatty acid deprivation (Weisinger *et al.*, 2001), or high fat consumption in pregnancy can programme obesity and hypertension in the offspring (Khan *et al.*, 2003). This programmed obesity related hypertension has been associated with dysfunction of the peripheral vascular, metabolic and renal systems (Armitage *et al.*, 2004). We hypothesized that the hypertension and obesity seen in offspring of fat fed mothers was associated with elevated sympathetic nerve activity and altered hypothalamic responses to peripheral appetite controlling peptides and hormones including leptin and ghrelin, and developed an animal model to test the hypothesis.

Female New-Zealand White rabbits were fed either a control (3.5% fat) or high fat diet (HFD, 13.5% fat) for 3 weeks prior to mating and throughout gestation and lactation. After weaning, all offspring were fed a calorie controlled 3.5% fat diet. At 4 months of age all rabbits were instrumented with intracerebroventricular (icv) cannulae and renal nerve electrodes under isoflurane anaesthesia (Prior *et al.*, 2010). The central ear artery was catheterized and arterial pressure, heart rate and sympathetic nerve activity recorded under basal conditions and in response to a stressful stimulus; a jet of air blowing at 100 L/min was directed at the face for 10 minutes. The cardiovascular and renal sympathetic nerve responses to increasing doses of leptin (recombinant murine leptin Peprotech USA, 5-100ng delivered icv) and ghrelin (human ghrelin Auspep, Melbourne 1-5 nMol, icv) on separate days. Body weight was similar between groups, however HFD offspring (n = 9) had heavier visceral white adipose tissue compared with control offspring (n = 8, $p < 0.05$). Rabbits from fat fed mothers demonstrated greater mean arterial pressure (+7%, $p < 0.05$) tacchycardia (+11%, $p < 0.05$) and elevated renal sympathetic activity (+17%, $p < 0.05$) compared with control offspring. The cardiovascular and sympathetic responses to acute stress were similar between groups. Interestingly, although icv administration of ghrelin reduced the pressor, tachycardic and RSNA response to stress in both groups, this reduction in stress responses was abrogated in offspring of fat fed rabbits, compared with controls ($p < 0.05$).

Maternal high fat feeding during pregnancy and suckling results in the development of obesity related hypertension in the offspring. Our results indicate that elevated renal sympathetic activity is associated with the hypertension and that perturbations of the leptin and ghrelin systems in the hypothalamus may underlie the phenotype. Further understanding of which hypothalamic nuclei are affected are the subject of ongoing work.

Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L. (2004) *Journal of Physiology* **561(2):** 355-377.

Armitage JA, Taylor PD, Poston L. (2005). *Journal of Physiology* **565(1):** 3-8.

Barker DJ. (2001). *British Medical Bulletin* **60**: 69-88.

Khan IY, Taylor PD, Dekou V, Seed PT, Lakasing L, Graham D, Dominiczak AF, Hanson MA, Poston L. (2003). *Hypertension* **41(1):** 168-175.

Prior LJ, Eikelis N, Armitage JA, Davern PJ, Burke SL, Montani JP, Barzel B, Head GA. (2010.) *Hypertension* **55(4):** 862-868.

Weisinger HS, Armitage JA, Sinclair AJ, Vingrys AJ, Burns PL, Weisinger RS. (2001). *Nature Medicine* **7(3):** 258-259.

Early life environments and programming of the vascular phenotype

M. Tare, Department of Physiology, Monash University, VIC 3800, Australia.

Disturbances in the early life environment can have life long repercussions on cardiovascular health. Perturbations during critical times in development including fetal and early postnatal life can influence arterial structure and function, predisposing to cardiovascular disease. We have studied the effects of a variety of early life challenges on vascular function. Challenges investigated include vitamin D deficiency, several different models of intrauterine growth restriction, prenatal glucocorticoid or alcohol exposure, and the lactational environment. The striking finding to emerge from this work is that the nature of vascular dysfunction exhibits regional heterogeneity. Vascular mechanisms that are targeted include endothelial function, neuromuscular transmission, smooth muscle reactivity and wall stiffness. Of these mechanisms, we have found that a change in wall stiffness is generally the most consistent indicator that there has been an exposure to an early life insult. Intrauterine growth restriction causes stiffening of the coronary arteries of the fetal sheep. Alcohol exposure in the fetal sheep causes stiffening of arteries across the body. Some insults can also give rise to functionally opposing responses in different vascular beds. For instance, maternal alcohol intake results in endothelial vasodilator dysfunction in coronary arteries and hyperfunction in the mesenteric arteries of fetal sheep. Changes in vascular function that persist into adulthood increase the risk of cardiovascular disease. Seven year old sheep exposed to two days of prenatal glucocorticoids early in pregnancy had significantly increased coronary artery stiffness. Aged rats exposed to vitamin D deficiency during early life had persistent alterations in renal artery function including altered wall stiffness and augmented neurovascular constriction. The early postnatal environment can also influence vascular function. Changes in the lactational environment can either rescue or aggravate disturbances in vascular function caused by prenatal insults. Although not tested in all models, there appears to be sexual dimorphism in the nature and extent of vascular dysfunction, with males tending to have worse outcomes than females. In conclusion, a variety of early life insults can induce adaptations in the developing vasculature that may cause lasting alterations in function. Persistence of vascular dysfunction into adulthood will increase the risk of cardiovascular disease.