C1

Regulation of Electrophysiology and Pharmacology of Voltage-Gated Potassium Channels by Ancillary Subunits Found in the Pregnant Uterus

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The electrical profile of the myometrium undergoes dramatic changes during pregnancy, as the myometrial smooth muscle changes from being largely quiescent to exhibiting well coordinated excitation-contraction coupling. Voltage-gated potassium channel currents contribute to repolarization, and therefore play a key role in determining myometrial excitability. KCNE1 is a small protein (130 amino acids) which forms a single transmembrane alpha helix. The expression of KCNE1 in the uterus is strongly and rapidly mediated by estrogen. KCNE1 does not form a functional channel by itself, but acts as an ancillary subunit to the KCNQ1 voltage-gated ion channel. KCNQ1 (KvLQT1 or Kv7.1) in the uterus is thought to contribute to repolarization of the myometrial action potential. Changes in the biophysical and pharmacological behavior of KCNQ1 by KCNE1 will likely contribute to changes in the electrical and pharmacological profile during pregnancy.

We used two-electrode voltage clamp to study the effect of KCNE1 on KCNQ1 when cloned KCNQ1 (P51787) and KCNE1 (NP_000210) were heterologously expressed in Xenopus oocytes. Oocytes were injected with 50 ng mRNA for KCNQ1, and co-injected with KCNE1 as noted, in a 1:1 ratio. We used the KCNQ1-specific open channel blocker chromanol 293B and the sodium/potassium channel blocker quinidine as pharmacological probes.

KCNE1 significantly slows KCNQ1 activation, resulting in a sigmoidal onset of activation. The KCNQ1/KCNE1 current continues to increase, even at the end of a 3 s depolarizing pulse. KCNE1 also slows deactivation, and removes voltage-dependent inactivation. When a 500 ms depolarizing pulse from -90 to +50 mV was applied with a 500 ms inter-pulse interval there was a potentiation of KCNQ1/KCNE1 current. The increase was well described by two exponentials, τ_{fast} = 1.07 ± 0.04 s, and τ_{slow} = 7.66 ± 0.43 s (n = 4). The fast time constant dominates: A_{fast}/A_{slow} = 4.78 ± 0.16 (n = 4).

There was a ~4 fold increase in the pharmacological sensitivity of KCNQ1 to Chromanol 293B when it was co-expressed with KCNE1. IC_{50} for KCNQ1 alone was 65.4 ± 1.7 μM in contrast to the IC_{50} for KCNQ1/KCNE1, which was only 15.1 ± 3.3 μM. KCNE1 had a similar effect on KCNQ1 affinity for quinidine. Application of 400 μM Quinidine reduced KCNQ1 current by 8.0 ± 4.5 % (n = 5), whereas KCNQ1/KCNE1 was reduced by 30.3 ± 4.0 % (n = 4).

These data suggest that the estrogen-dependent KCNE1 ancillary subunit strongly modulates voltage-gated KCNQ1 ion channel physiology and pharmacology. Understanding the contribution of KCNQ1 to the uterine electrical and pharmacological profile therefore requires an understanding of how KCNE1 subunits modulate KCNQ1.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C2

Myogenic regulation to intravascular pressure changes of uterine arteries isolated from non-pregnant rats

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Myogenic reactivity is an important regulatory mechanism for maintaining arterial diameter in the face of changing physical stresses. Although well-studied in many vascular beds, surprisingly little is known as to the nature of myogenicity in arteries serving the uterus, which is particularly important given the substantial haemodynamic changes occurring in this organ during pregnancy. Therefore, the aim of this study was to investigate myogenic responsiveness of pressurised uterine arteries, isolated from rats, to changes in intravascular pressure (IVP).

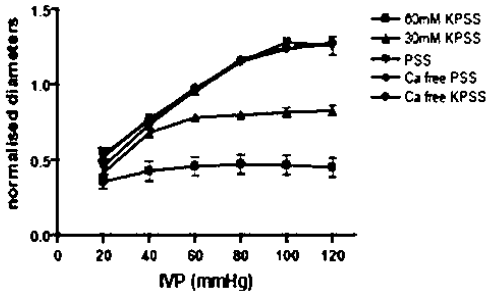
Virgin adult Sprague Dawley rats (150-250g) were humanely killed by stunning and cervical dislocation. Third order uterine arteries were dissected and mounted on an arteriograph and pressurised to 60mmHg. Arteries were superfused at 37°C with physiological salt solution (PSS), gassed with 95% air/5% CO2 and left for 90 minutes to determine the extent of myogenic tone development. The subsequent influence of stepwise (5 min) IVP changes (20-120mmHg) in 20mmHg increments was investigated in arteries with and without pre-constriction to KPSS (30mM or 60mM) or AVP at a concentration determined to produce 50% of maximal constriction to AVP. Endothelium integrity was tested by addition of 10μM Carbachol. Experiments were repeated in calcium-free PSS or KPSS.

Mean arterial lumen diameters (at 60mmHg) were 152.4± 4.74μm and 156.4 ± 4.45μm (mean ± SEM) in calcium-containing and calcium-free PSS respectively. Development of myogenic tone was not observed. 60mM KPSS produced a sustained narrowing of arteries (diameter change 76.4 ± 8.5μm, n=5), whilst 30mM KPSS produced a constriction approximately half of this (diameter change 28.4 ± 5.9μm, n=5). The constriction produced by AVP (0.08-0.55 μM) was similar to that produced by 30mM KPSS (35.6 ± 4.8μm, n=8).

In calcium-free PSS and KPSS increases in IVP produced sequential increases in diameter of all arteries. The responses of arteries in calcium-containing PSS (minus constrictor) were similar. However, arteries pre-constricted with KPSS (30 or 60mM) or AVP exhibited active regulation in response to increases in IVP such that stable diameters were maintained (over 40-120mmHg) (See figure).

These results indicate that isolated uterine arteries from the non-pregnant rat, when held at an in vitro pressure of 60mmHg, do not develop spontaneous myogenic tone. Nonetheless, under the influence of pre-constrictory stimuli of varying magnitude, such vessels do exhibit prominent myogenic responsiveness to IVP changes. These observations have important implications for understanding local autoregulation in the uterine circulation.

Supported by the BHF.



The response of isolated pressurised arteries from non-pregnant rats to changes in IVP.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C3

Prokineticin-1 upregulates interleukin-8 expression in placenta

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Background: Prokineticin-1 (PK1) is a pleiotrophic peptide whose functions include tissue specific angiogenesis[1], vascular permeability[2] and haemopoiesis[3]. Although expressed in third trimester placenta, the cellular localisations of PK1 and prokineticin receptor 1 (PKR1) and signalling pathway of PK1 are not known. In addition, there is a paucity of data regarding gene upregulation by PK-1.
Aim: To characterise PK1 and PKR1 immunolocalisation, expression and signalling in human placentae.
Methods: Placentae (n=20) were collected after elective caesarean section at term (>37 weeks gestation) from women with uncomplicated pregnancies. PK1 and PKR1 were immunolocalised by standard immunohistochemical techniques. Extracellular regulated signal kinase -1/2 (ERK1/2) phosphorlyation was detected by Western blotting with signalling pathways being dissected using various inhibitors including YM25480, PP2 and AG1478, specific inhibitors of Gq, c-src and epidermal growth factor receptor (EGFR) kinase, respectively. Placental explants (n=6) were treated with 40nM PK-1 and interleukin-8 (IL-8) mRNA expression detected by taqman PCR. PK1 or PKR1 and IL-8 were colocalised using immunofluorescence and confocal microscopy.
Results: PK1 was immunolocalised to endothelium and macrophages in fetal vessels and Hofbauer cells in placental villi. In contrast, PKR1 was predominately localised in syncytial sprouts. ERK-1/2 phosphorylation in placenta was significantly upregulated (5-fold increase; $p<0.05$) following treatment with PK1 for 30 minutes. Dissection of the upstream signalling pathway by the chemical inhibitors demonstrated that PK1 induced phosphorylation of ERK-1/2 was mediated via c-src and EGFR transactivation. Treatment of placenta with PK1 for 4 hours induced a significant increase in IL-8 expression (3.26+0.45 fold increase above control; $p<0.05$). IL-8 expression in response to treatment with PK1 was inhibited following coincubation of the tissue with inhibitors of Gq, c-src, EGFR kinase or ERK1/2. Using double immunofluorescence, co-localisation/co-expression of PK-1 or PKR1 and IL-8 was demonstrated in various cellular compartments within the placenta including trophoblast and macrophages.
Conclusions: The cellular immunolocalisation of PK1 and PKR1 within placenta and upregulation of IL-8 by PK1 is supportive of PK1 being involved in placental vascular physiology. In addition, expression of PKR1 in syncytial sprouts, which characterise areas of hypoxia and immature villous formation, suggest that another role of PK1 may in mediating trophoblast differentiation in response to hypoxia. More studies are required to establish the role of PK1 in normal placental physiology and in hypoxic pre-eclamptic placentae which are characterised by increased syncytial sprouts and vaso-occlusive lesions.

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C4

Production of Hydrogen Sulphide in Intrauterine Tissues

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Hydrogen sulphide (H_2S) is a gasotransmitter which is produced endogenously from L-cysteine via the enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (Zhao et al., 2003). The possible role of hydrogen sulphide in reproduction has not yet been fully investigated. Sidhu et al. (2001) previously demonstrated that H_2S relaxed uterine smooth muscle in vitro. The aim of the present study was to investigate the endogenous production of H_2S in rat and human intrauterine tissues in vitro. The expression of CBS and CSE was also investigated in rat and human intrauterine tissues, using Western blotting. Basal production rate of H_2S was measured in rat and human intrauterine tissue homogenates using a standard methylene blue assay technique, involving the trapping of yielded H_2S as zinc sulphide (Zhao et al., 2003). The effects of nitric oxide (NO) and hypoxia on the endogenous production rates of H_2S were also investigated. The order of H_2S production rates (mean ± SD, n = 4) for rat tissue was: rat liver (positive control) (777 ± 165 mmol/min/g) > rat uterus (168 ± 102 mmol/min/g) > rat fetal membranes (22.3 ± 15.2 mmol/min/g) > rat placenta (11.1 ± 4.7 mmol/min/g), compared to human placenta (200 ± 103 mmol/min/g). NO significantly increased H_2S production in rat

PC17

Short-term Growth Hormone administration improves Respiratory Function in an unusual catabolic condition

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This study examined whether six days recombinant human growth hormone (rhGH) administration, 0.058 IU/kg/day, in an abstinent anabolic-androgenic steroid (AAS) using group had any respiratory, cardiovascular and biochemical effects compared with an abstinent AAS control group.

Impairment in respiratory function in adult-onset growth hormone deficiency (AO-GHD) is consequential to a reduction of respiratory muscle strength, responding to replacement therapy with rhGH (Merola et al., 1996).

RhGH significantly improves exercise tolerance in cystic fibrosis (CF) (Hutler et al., 2002) and significantly improves respiratory function in major surgery, and is more beneficial when given pre- and post-operatively than post-operatively alone (Barry et al., 1999).

Male subjects (n=48) were randomly divided, using a double blind procedure into two groups: (1): exercise control group (n=24, mean ± SD, age 32 ± 11 years; height 1.8 ± 0.06 metres); (2): rhGH using group (n=24, mean ± SD, age 32 ± 9 years; height 1.8 ± 0.07 metres). Anthropometry, peak oxygen uptake and respiratory muscle function were investigated. Respiratory measurements examined, were forced expiratory volume in one second, forced vital capacity (FEV1/FVC), MIP and maximum expiratory pressure (MEP). Cardiovascular measurements were blood pressure (BP), heart rate (HR) and rate pressure product (RPP). Biochemical analysis included; glucose, sodium, urea, creatinine, total protein, albumin, testosterone and insulin like growth factor-I (IGF-I).

FEV1/FVC (85 ± 6 vs. 82 ± 5, %), MIP (144 ± 24 vs. 129 ± 28, L), MEP (179 ± 35 vs. 157 ± 32, L), resting HR, (78 ± 11 vs. 67 ± 16, bpm) resting RPP (97 ± 14 vs. 84 ± 24, bpm.mmHg X 10-2) and IGF-I (323 ± 93 vs. 169 ± 50, ng/ml) significantly increased compared with the control group (all $P<0.05$).

Body mass index (27.7 ± 3.1 vs. 27.5 ± 3, kg.m-2), fat-free mass index (22.3 ± 1.9 vs. 21.9 ± 1.9, kg.m-2), peak oxygen uptake (45.4 ± 9.9 vs. 41.8 ± 9.8, ml.kg-1.min-1), MIP (144 ± 24 vs. 131 ± 30, L), MEP (179 ± 35 vs. 165 ± 36, L), IGF-I (323 ± 93 vs. 159 ± 54, ng/ml) and serum sodium (141.8 ± 2.5 vs. 140.6 ± 2.6, mmol/L) significantly increased, whilst body fat (19 ± 6 vs. 20 ± 6, %), total protein (73.1 ± 4.5 vs. 75.7 ± 4.9, mmol/L) and albumin (42.5 ± 4 vs. 44.4 ± 4, mmol/L), significantly decreased within the GH group (all $P<0.017$).

The findings of this study indicated that short term high dose rhGH increased aerobic performance and respiratory muscle strength in former AAS users, but may have an adverse effect on the cardiovascular system, as evidenced by the increase in resting rate pressure product.

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PC18

Effect of maternal low protein and folic acid supplementation on interscapular fat mass and uncoupling protein (UCP)-1 expression in brown adipose tissue of resultant offspring

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Folic acid supplementation to the mother during pregnancy can prevent significant congenital malformations. However, the extent to which it can impact upon other metabolic processes is unknown. Previous studies, using a rat model, have demonstrated that consuming a maternal low protein (MLP) diet plus/minus folic acid supplementation has differential effects upon resting blood pressure (Torrens et al., 2006). However, the extent to which this diet may affect offspring adiposity after weaning is currently unknown. The present study, therefore, aimed to examine the effects of a MLP diet with/without a folate supplementation on interscapular (comprising of brown (BAT) and white (WAT)) fat mass and UCP-1 abundance in BAT.

Fifteen virgin female Wistar rats (180-220g) were mated and randomly assigned to one of four feeding groups: control diet (CP: containing180g casein/kg, 1mg folic acid/kg; n=4), low protein (MLP: containing 90g casein/kg diet, 1mg folic acid/kg; n=3), control protein with folate supplementation (CPF: 180g casein/kg, 5mg folic acid/kg; n=4), or low protein with folate supplementation (MLPF: containing 90g casein/kg diet, 5mg folic acid/kg; n=4). All offspring were weaned at 21 days of age onto standard laboratory chow. One male and one female pup from each dam were culled at 13 weeks of age, and interscapular adipose tissue weight recorded. All procedures accorded with current UK legislation. Mitochondrial fractions were prepared from BAT and UCP-1 abundance determined. Results are given as means ± SEM and expressed as a percentage of a reference sample. Statistical differences ($p<0.05$) were determined using a General Linear Model test.

Neither MLP nor folate supplementation affected offspring body weight at 13 weeks of age. However, females were lighter than males in all four diet groups (Figure 1; $p<0.05$) and possessed slightly more BAT relative to body weight ($p=0.06$) but only in the folate supplemented group. Folic acid supplementation significantly increased relative interscapular fat mass (CP: 2.6±0.3,

respectively) by in situ hybridisation using a 35S-labelled riboprobe; data are mean±S.E.M and each brain region was analysed by 1 way ANOVA and posthoc tests. The highest OTR mRNA expression was in the supraoptic (SON) and paraventricular (PVN) nuclei. Pre-parturition OTR mRNA expression was increased only in the SON compared to virgins (43333±3889 vs. 30556±1111 pixels per cell, $p<0.01$), suggesting oxytocin control of the input from the uterus during labour. During parturition peak OTR mRNA expression was observed in the SON, A2/C2 and A1/C1 brainstem regions, medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST), olfactory bulbs and medial amygdala (increase above virgin=45,82,68,119,55,79 & 46% respectively; all $p<0.05$). Parturition increased OTR mRNA expression vs. pre-parturition only in the olfactory bulb and amygdala (increase=34 & 21% respectively, $p<0.05$), reflecting a rapid response to birth stimuli. Within 12h postpartum, OTR mRNA expression decreased and was not significantly different from virgins in all regions. OTR mRNA expression in the PVN and lateral septum did not alter perinatally.

Further virgin, 21day pregnant and parturient (n=6,6,8, respectively) rats were perfused-fixed and their brains processed by double immunocytochemistry for Fos and OTR. The number of Fos-positive OTR neurones was significantly increased during parturition but not before, especially in the SON (63.5±8.8 vs virgin 9.8±3.2 cells per region); for A2/C2 and A1/C1 brainstem regions, MPOA, BNST and medial amygdala Fos-positive OTR neurones per region were 3.3,12.0,11.4,15.3 & >22 fold above virgins, respectively ($p<0.05$). Fos and OTR co-expression in the parvocellular PVN and lateral septum was not significantly changed during parturition. So, selected OTR expressing neurones in selected brain regions are activated during birth and play a role in mediating behaviour.

Thus, as in the uterus, there are dynamic changes in oxytocin receptor expressing cells at parturition. Responses are region-dependent, altering the pattern of oxytocin receptor distribution in the brain perinatally. Increased expression and activation of OTR neurones reflects the crucial role they play in orchestrating birth and maternal behaviour; altered patterns may shape quality of behaviour.

Olazabal DE and Young LJ. (2006) Horm Behav 49:681-7.

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PC11

Fetal iron status regulates maternal iron metabolism during pregnancy in the rat

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Iron metabolism during pregnancy is heavily biased towards maintaining the fetal supply, even at the extent of inducing severe anaemia in the mother. In this study we examined the effect of iron (Fe) deficiency and supplementation on the hierarchy of Fe supply and the gene expression of proteins involved in Fe metabolism.

Female Hooded Lister rats were fed a control diet for 2 weeks following weaning, then a diet with control (50mg/kg) or deficient (7.5mg/kg) Fe content. Four weeks later, they were mated with males of the same strain. Following mating, the dams continued on the deficient diet or were given an Fe supplemented (150mg/kg) diet during either half of pregnancy. A control group were maintained on a normal Fe diet throughout the experiment. The dams were killed by exsanguination under terminal anaesthesia, at either day (D) 0.5, 12.5 or 21.5 of gestation and tissues and blood samples collected. Samples were also collected from fetuses, killed by Schedule 1 method, at D21.5. All animal procedures were conducted in accordance with the UK animals (Scientific Procedures) act. All data are expressed as mean ± s.e.m, n=8 per time point and treatment.

Maternal liver Fe levels were already lower in deficient animals at the start of pregnancy, and never recovered, irrespective of treatment. Haematocrit (Hct; 39±0.5%) was maintained in control and deficient dams until D12.5 but dropped to 28.6±0.4% ($p<0.05$) in the deficient dams by D21.5. In the fetus, in contrast, fetal liver Fe was returned to normal (1.3±0.06 mg/g dry wt) by supplementation in the second half of pregnancy, and Hct (35±0.6%) followed the same pattern. The data show, therefore, that fetal Hct and liver stores are restored at the expense of both maternal Hct and liver stores.

Placental transferrin receptor (TfR) expression was higher in deficient animals and in those supplemented in the first half of gestation only. As expected, levels correlated closely with fetal liver Fe levels ($p<0.0001$). The data suggest that hepcidin from the fetal liver mediates this signalling, since there is significant correlation between the two parameters ($p<0.001$). There was a linear relationship between maternal liver Fe levels, maternal liver TfR and hepcidin mRNA. However, there was a significantly greater interaction between them and fetal liver Fe. This was best described for TfR by a "broken stick" mode ($p<0.001$), with the break occurring at about 1.2mg/g dry wt. This is particularly exciting, since it suggests that the fetal liver is communicating Fe status to the maternal liver, and regulating metabolism through some, as yet unidentified, mechanism. In summary, the data show that the fetus has a remarkable capacity to accumulate Fe at the expense of the mother, and does so by manipulating Fe stores, haematocrit and the genes of Fe metabolism.

This work is supported by SEERAD and the European Union.

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PC12

Regulation of KCNQ and KCNE Gene Expression in Non-pregnant Mouse Myometrium During the Oestrous Cycle

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Background: KCNQ genes encode for the pore forming α subunits of K_v channels. The KCNQ gene family comprises 5 mem-

bers (KCNQ1-5), with KCNQ1 expressed predominantly in cardiomyocytes and KCNQ2-5 localised to neurones where they contribute to the resting membrane conductance. The expression products of KCNQ genes exhibit a range of phenotypes due to the formation of heteromultimers within each family and the association with auxiliary (β) subunits encoded by the KCNE gene family, which modulate channel function and pharmacology. There are only a few reports of ion channels encoded by KCNQ in smooth muscle cells and a paucity of information concerning their role in uterine smooth muscle (myometrium). The aim of the present study is to determine the expression profiles of KCNQ and KCNE genes in myometrial tissue from non pregnant mice during the oestrous cycle.

Methods: The oestrous cycle of c57/BL6 mice was monitored by daily vaginal smearing and uterine horns dissected at the different stages of the cycle: diestrous (n=5), proestrous (n=5), oestrous (n=5), metestrous (n=5). Total RNA was extracted using Trizol (Invitrogen) and cDNA synthesised with Superscript III (Invitrogen). RT-PCR and qRT-PCR for KCNQ1-5 and KCNE1-5 were performed using tRNA from myometrium and heart/brain (positive controls). qRT-PCR data was quantified using a standard curve and expressed relative to β-Actin. Non-pregnant mouse myometrial strips were used for preliminary in vitro tension measurement (n=3).

Results: All of the KCNQ and KCNE isoforms studied were detected in mouse myometrium by RT-PCR but expression levels appeared to vary during the oestrous cycle. qRT-PCR confirmed that KCNE3 was significantly down-regulated in the metestrous group (n= 5, p=0.01), whereas KCNE4 was significantly up-regulated in proestrous group (n = 5, p=0.01), compared to the diestrous group (n =5). Tension studies in vitro indicated that XE991 (1-3 mM, KCNQ channel inhibitor) and retigabine (10μM, KCNQ channel opener) enhanced and attenuated mouse myometrial contractility respectively.

Conclusions: We have comprehensively demonstrated the presence of KCNQ and KCNE isoforms in mouse myometrium, which coupled with the preliminary in vitro tension data, suggests a role for the KCNQ channels in the control of uterine contractility. KCNE mRNA expression appears to be regulated in preference to that of KCNQ. The loss of KCNE3, the presence of which promotes KCNQ1 opening, and an increase in KCNE4 mRNA, which inhibits KCNQ1, suggests that regulation of these accessory subunits is an important mechanism for regulating uterine contractility (and hence receptivity) during different stages of the oestrous cycle.

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PC13

Hypertension in offspring of iron deficient rats, is the kidney involved? - gene expression study

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Inappropriate nutrition during gestation can result in increased risk of diseases such as hypertension in the adult. In our rat model, the possibility that the kidney may play an important role is given credence by the fact that the pups born to Fe deficient mothers, as well as developing hypertension, have smaller kidneys at birth (Gambling et al., 2003; Gambling et al., 2004). In order to test whether the two phenomena are linked, we have examined the expression of genes involved in kidney development and function, specifically vasculogenesis/angiogenesis, apoptosis and cell proliferation.

Female Rowett Hooded Lister rats were fed diets with 2 different Fe contents (50 mg Fe/kg and 7.5 mg Fe/kg diet) before and during pregnancy. The dams were killed by exsanguination under terminal anaesthesia at either day 21.5 (D21)of gestation or within 12 hours of giving birth. Fetuses were delivered by caesarean section and killed by a schedule 1 method. Neonates were killed within 12 hours of birth by decapitation. Fetal and neonatal kidneys were collected and processed for mRNA extraction. All experiments were approved by Home office and carried out according to UK Animals (Scientific Procedure) Act, 1986. Using quantitative real time RT-PCR and SYBR Green, we found no difference in gene expression of the markers of vasculogenesis, angiopoietin 1 and 2, vascular endothelial growth factor A and C or haeme oxygenase 1 and 2. Markers of apoptosis, Bcl2 and Bax, showed no differences in expression in either fetal or neonatal kidneys. Cell proliferation markers, p21 and p27, also were not significantly different between the two dietary groups or either time point. In contrast, at D21, renin gene expression was increased in pups from deficient mothers (relative expression. mRNA/18s mRNA; Fe deficient 43.11 ± 3.9, control 30 ± 2.33, p=0.02). This difference disappeared at birth. Expression of other components of the renin-angiotensin system was not affected by maternal diet either in fetal or neonatal samples. In rats, kidney development continues after birth, but little is known concerning the period where it is most vulnerable to nutritional stress. Changes in expression of renin in our model suggest that there could be a window earlier in kidney organogenesis which is more sensitive to maternal influence. Clearly, this requires further investigation.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC14

Comparison of the effects of dose-dependent zinc supplementation of pregnant rats on cognitive behavior and memory function in their offsprings

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TITLE ONLY

PC24

Placental materno-fetal transfer of leucine by amino acid exchangers and by non-exchange mechanisms

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Objectives: The mechanisms mediating amino acid transport across the basal membrane and out of the placental syncytiotrophoblast into the fetal circulation are not well understood. Our previous data indicate that amino acid exchangers mediate serine transport into the fetoplacental circulation in exchange for alanine, serine, leucine, threonine, tryptophan and glutamine but not for glutamate. This study characterises amino acid stimulation of leucine transfer into the fetoplacental circulation.
Methods: Human placentas (n = 5) were collected within 30 minutes of delivery and an intact cotyledon was perfused with a modified Earl's bicarbonate buffer. The maternal arterial circulation was perfused with 50 μmol/l L-leucine & glycine, 0.6 μmol/l ^{14}C-leucine and 20 μmol/l ^{3}H-glycine. Amino acid [12.5 μmol] boluses were administered to the fetal side inflow perfusate. ^{14}C-leucine and ^{3}H-glycine were measured in maternal and fetal venous samples by dual label liquid scintillation counting. Data (mean ± SEM) were expressed as area under the curve (AUC) and analysed by one-way ANOVA.
Results: In the absence of amino acids in the fetal circulation (which are required for exchange) leucine, but not glycine, was transferred to the fetal circulation. Following fetal arterial boluses of specific amino acids transfer of leucine, but not glycine, increased, indicating that transport by exchange was taking place. Leucine exchanged for 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH; a System L substrate), leucine or tryptophan but not serine, glycine, threonine, glutamate or lysine.
Conclusion: This study demonstrated that in the perfused human placenta leucine was transported into the fetal circulation by exchange and non-exchange mechanisms and glycine was not actively transported into the fetal circulation. None of the known amino acid transporters are thought to mediate leucine efflux across the basal membrane except for exchangers. It is therefore unclear what is mediating the non-exchange mediated transport.

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Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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The Influence of the Pre- and Postnatal Hypercholesterolemia on the Development of Cardiovascular Dysfunction in Adult Mouse Offspring

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A high fat diet leads to hypercholesterolemia and predisposes the individual to developing cardiovascular disease (CVD). We hypothesised that the mother's diet before and during pregnancy and lactation can also influence predisposition to CVD in offspring fed a hypercholesterolemic diet. We therefore examined the effects of feeding a high fat-high cholesterol diet on cardiovascular function in female mouse offspring from mothers fed a hypercholesterolemic diet during pregnancy and lactation.
Female C57BL/6 mice were fed either a high fat-high cholesterol diet (HF; 45% kcal fat) or standard chow (C; 21% kcal fat) from weaning through pregnancy and lactation. Weaned female offspring from each group were then fed either a HF or C diets to adulthood. Body weight, blood pressure, plasma cholesterol and C-reactive protein levels (a marker of CVD) were measured at 36 weeks post-weaning. Histology of the liver was also performed. Data were expressed as mean ± SEM and analysed by ANOVA followed by post-hoc test.
At 36 weeks post weaning the offspring from high fat fed mothers that were then fed a high fat diet (HF-HF) or a chow diet (HF-C), and offspring from chow fed mothers fed a high fat diet (C-HF) had significantly elevated bodyweight (gm; HF-HF 34.7±0.3; C-HF 34.6±0.4; HF-C 29.6±0.2 vs. C-C 20.6±0.1; $p<0.001$), systolic BP (mmHg; HF-HF 139±0.1; C-HF 151.6±2.0; HF-C 146.2±2.3 vs. C-C 104.7±0.1; $p<0.001$), plasma cholesterol (mml/L; HF-HF 3.2± 0.4; C-HF 3.0± 0.3; HF-C 2.7±0.2 vs. C-C 1.8±0.1; $p<0.001$) and plasma CRP levels (mml/L; HF-HF 11.9±0.2; C-HF 12.8±0.4 vs. C-C 3.8±0.1; $p<0.001$) compared to C-C offspring. Liver histology also showed lipid vacuoles within hepatocytes in the HF-HF, HF-C & C-HF but not the C-C offspring.
We conclude that as expected feeding a HF diet induces CVD risk factors. For blood pressure feeding the dam a HF diet was not protective, as previously reported in a rat model (Khan et al. 2004). Interestingly, blood pressure and cholesterol were also elevated in offspring of the HF-fed dams even when fed C. Our results may have implications for understanding the effects of the 'nutritional transition' to higher dietary intake of energy and fat which lead to increased cardiovascular disease in many societies.
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