Serum FSH level was significantly reduced, P<0.05 in treated rats, the extract showed no significant effect on other hormones which were assayed. There was no significant effect on the number of implantation sites and viable fetuses but fetal weight was significantly reduced, P<0.05, treated 1.939 ± 0.021g, control 3.073 ± 0.031g. There was a significant reduction in weight gain of treated rats when compared with control, by weeks 3 and 4. All these results revealed that the aqueous extract of Magnifera indica may cause disruption of the oestrous cycle inhibiting ovulation, while reducing weight gain during pregnancy and reducing the fetal weight However,it has no significant effect on implantation activities.

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2. Raintree nutrition (1996). Tropical plant database for Mangifera indica. www.rainfree nutrition.com.

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

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PC8

## Maternal micronutrient deficiency (Cu or Zn or Vit-E) in mice associated with abnormalities in placental IGF signaling and hyperleptinemeia in offspring's

J.F. Rosario and M. Patrick Gomez

*Dept of Biochemistry, St Josephs College (Autonomous), Tiruchirappalli, Tamil Nadu, India*

TITLE ONLY

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PC9

## Attenuated responsiveness of the adrenal medulla to stress in pregnancy

A.J. Douglas[1], H. Gooding[1] and K. Pierzchala-Koziec[2]

*[1]School of Biomedical Sciences, University of Edinburgh, Edinburgh, UK and [2]Department of Animal Physiology, University of Agriculture, Cracow, Poland*

Pregnancy changes physiological responses to stress, including attenuation of the hypothalamo-pituitary-adrenal (HPA) axis. This results, at least in part, from opioid inhibition of the central noradrenergic input to the HPA axis1,2, and during the last trimester may protect the foetus from adverse effects of glucocorticoids and stabilise metabolism of the mother and foetus. Although sympathetic and adrenal medulla responses to stress are important for the fight or flight response and immediately elevate glucose availability, their responses during pregnancy are poorly understood. We studied the hypothesis that sympathetic responses are attenuated in late pregnancy. During stress enkephalin is synthesised and secreted from chromaffin cells into blood and may mediate neuroendocrine, cardiovascular and immune functions; so we also measured adrenal medulla enkephalin responses. Virgin and 21 day pregnant rats were blood sampled via a previously implanted jugular cannula before and after exposure to airpuff startle stress3 in their home cages (n=8,6, respectively); control groups were not stressed (n=7,6, respectively). Plasma samples were analysed for ACTH, adrenaline and noradrenaline by RIA. Rats were killed at 90 min and the adrenal medulla was assayed for enkephalin content4. Data are mean±S.E.M.

Airpuff startle increased ACTH secretion at 10 min in virgin but not pregnant rats as expected. Airpuff also significantly increased adrenaline secretion at 2.5 min in virgin but not pregnant rats (to 3031±933 and 1684±205 pg/ml, respectively; 3way ANOVA, interaction across treatment, group and time p<0.05); controls did not significantly change. Delta noradrenaline secretion was 2146±722 pg/ml in stressed virgins and was not significantly different in stressed pregnant rats (1816±834 pg/ml). This indicates that although adrenaline secretory responses are attenuated, peripheral noradrenergic responses remain intact.

Adrenal gland weight was not significantly altered during pregnancy (37.5±2.1 vs 38.3±1.3 mg in virgins) or by the stress exposure. Adrenal medulla enkephalin content was not significantly altered in pregnancy (7.5±1.1 vs virgins 4.9±0.5 pmol/g). Airpuff startle increased enkephalin content in virgin rats (to 11.0±1.8 pmol/g) but not in pregnant rats (to 5.5±0.6 pmol/g, 2 way ANOVA interaction group x treatment p<0.01), suggesting that enkephalin peptide processing increases in response to stress in virgin but not in pregnant rats.

In conclusion, the data show that adrenaline and medulla responses to stress are attenuated in late pregnancy but peripheral noradrenaline responses, reflecting sympathetic activity, are not. Both mechanisms may help protect the pregnancy: attenuated adrenaline responses preventing sudden fluctuations in glucose availability and heart rate and the retained sympathetic responses allowing the mother to respond appropriately to threat.

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PC10

## Dynamic region-dependent changes in oxytocin receptor expression in the rat brain at parturition

S.L. Meddle[1], V. Bishop[1], E. Gkoumassi[1], F.W. van Leeuwen[2] and A.J. Douglas[1]

*[1]School of Biomedical Sciences, University of Edinburgh, Edinburgh, UK and [2]Netherlands Institute for Brain Research, Amsterdam, UK*

Centrally oxytocin plays a pivotal role in parturition, facilitating its own release centrally and peripherally and effecting maternal behaviour. Since quality of social and maternal behaviours depends upon pattern of oxytocin receptor (OTR) distribution1, we investigated the expression and activation of oxytocin-receptive neurones perinatally. OTR mRNA expression was quantified in dioestrus virgin, 21 and 22 day pregnant (pre-parturition), parturient and postpartum (<12h) rats (n=10,8,7,9,9,

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.

We thank The Wellcome Trust & BBSRC for financial support.

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PC11

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

L. Gambling[1], A. Czopek[1], H.S. Andersen[1], K.S. Srai[3], Z. Krejpcio[2] and H.J. McArdle[1]

*[1]Vascular Health, Rowett Research Institute, Aberdeen, UK, [2]Department of Hygiene and Human Nutrition, Agricultural University, Poznan, Poland and [3]Department of Molecular Biology, Royal Free Hospital, London, UK*

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**

L.A. McCallum[1], I.A. Greenwood[2] and R.M. Tribe[1]

*[1]Maternal and Fetal Research Unit, Division of Reproduction and Endocrinology, St Thomas' Hospital, King's College London, London, UK and [2]Division of Basic Medical Sciences, St George's University of London, London, UK*

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

PC15

## Allopregnanolone acts centrally to restrain oxytocin responses to interleukin-1β in pregnancy

P.J. Brunton, J. Bales and J.A. Russell

*Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK*

In the late pregnant rat stimulated oxytocin secretion is inhibited, preserving the expanded neurohypophysial oxytocin stores for birth and minimising the risk of preterm labour. In virgin, but not pregnant rats, systemic administration of the cytokine interleukin-1β (IL-1β), which mimics infection, increases oxytocin secretion and the firing rate of supraoptic nucleus (SON) oxytocin neurons (1). Blood and brain levels of the neuroactive steroid allopregnanolone (AP; progesterone metabolite) increase during pregnancy and AP acts on $GABA_A$ receptors on oxytocin neurons, to enhance inhibitory transmission (2). Here we tested whether AP is the pregnancy-related factor responsible for restraining oxytocin responses to IL-1β in pregnant rats, and whether its actions are central. Jugular vein cannulae were implanted under halothane anaesthesia 5 days prior to the experiments. Oxytocin secretion following IL-1β (500ng/kg i.v.) was measured (by radioimmunoassay) in plasma from: a) virgin and pregnant (day 21) Sprague Dawley rats treated with vehicle (oil) or finasteride (FIN, 25mg/kg s.c.; 5α-reductase inhibitor to block AP production) 20h and 2h before IL-1β. b) Pregnant rats given FIN and AP/oil; and virgins given AP (3mg & 1mg/kg s.c.) or oil pre-treatment (20h and 2h before IL-1β). To investigate central SON oxytocin neuron responses, perfuse-fixed brain sections from pregnant rats treated with oil/FIN + vehicle/IL-1β, and virgin rats treated with oil/AP + vehicle/IL-1β, were processed for Fos (indicator of neuronal activation) and oxytocin immunoreactivity. Results: IL-1β significantly increased oxytocin secretion (3.4-fold; $p<0.001$, two-way repeated measures ANOVA; n=7) and Fos expression in SON oxytocin neurons (4.4-fold; $p<0.001$, two-way ANOVA; n=8) in virgin, but not pregnant rats (1.2-fold for secretion and Fos; n.s., n=7). FIN had no further effect on oxytocin secretion in virgins, but significantly restored an oxytocin response to IL-1β in the pregnant rats (3.3-fold; $p<0.001$, two-way repeated measures ANOVA; n=6), while AP significantly reduced the oxytocin response to IL-1β in virgins by 50% ($p<0.001$, two-way repeated measures ANOVA; n=6) and reversed the effect of FIN in pregnant rats. Consistent with the secretion data, FIN significantly increased IL-1β-induced Fos expression in identified SON oxytocin neurones of pregnant rats (2.4-fold; $p<0.005$, two-way ANOVA; n=6), while AP significantly reduced the number of SON oxytocin neurones activated by IL-1β by 48% ($p<0.05$, two-way ANOVA; n=6). Thus, AP acts centrally to restrain oxytocin secretory responses to IL-1β in pregnancy. This mechanism will serve to reduce the likelihood of preterm labour and prevent depletion of posterior pituitary oxytocin stores, required for parturition.

Brunton PJ *et al* (2006). *Eur J Neurosci* **23**, 1241-1247.

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This work was supported by the BBSRC.

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PC16

## Liver-placental signalling - development of an in vitro model

J.A. Duff, L. Gambling and H.J. McArdle

*Rowett Research Institute, Aberdeen, UK*

Iron (Fe) is one of the key nutrients transported by the placenta during gestation, required for many biological processes. Iron deficiency is disturbingly common and is even more prevalent in pregnant women, as the requirements for Fe increase due to the demands of the growing baby.

Iron is absorbed mainly through the duodenum and is stored in the liver until required. We have shown, using dietary approaches, that the fetus accumulates Fe at the expense of the mother, maintaining normal iron levels even when she is seriously anaemic, but how this is accomplished is not well understood. The fetal requirements for Fe are regulated through the fetal liver and the placenta, and in this presentation, we demonstrate a model that we are using to investigate how the interaction takes place. The model may also be of value to others investigating the interactions between the fetal liver and the placenta.

The approach involves co-culturing BeWo (b30 subclone) cells with HepG2 cells. The BeWo cells are grown on polycarbonate 0.4 μm pore inserts with HepG2 cells on the plate itself. The HepG2 cells on the base represent fetal liver cells and the basolateral side of the placenta while the compartment on top of the b30 cells represents the apical side of the placenta. The cells were cultured together for 7 days and during this time the transepithelial resistance (TEER) of the b30 cells was measured daily. During this time, TEER levels increased to about $175 \pm 3\ \Omega$ (n= 7). There was no significant difference between plates with or without HepG2 cells in the basal layer

At day 7, $^{59}$Fe-Tf (1 μM, 0.5 $\mu Ci.ml^{-1}$) was added to the apical side of the placental cells. At increasing time intervals up to 8 h, 0.25 mL aliquots were removed from the basolateral medium, and were replaced by new medium. The aliquots removed were counted for the appearance of $^{59}$Fe. Active transport was demonstrated by comparing the differences between cells incubated at 4 °C and 37 °C. Transfer of $^{59}$Fe across the BeWo cell layer was markedly greater in plates grown without HepG2 cells ($3.86 \pm 0.6$ pmol/min/filter) than those with ($1.56 \pm 0.19$ pmol/min/filter, $p = 0.004$, $n = 6$). The difference could not be accounted for by accumulation of $^{59}$Fe in the HepG2 cells but suggest that the presence of the liver cells in the basal layer inhibits the uptake and transfer of $^{59}$Fe by the BeWo cells. At this stage, there are many possible explanations, but we are testing the hypothesis that the liver cells signal their normal iron status, to the placental cells, and are indicating a reduced Fe requirement. This is a very exciting possibility, since, if correct, it suggests that we can study liver and placental interactions in vitro in a model which is relatively easy to manipulate when compared to in vivo studies in animals.

This work was supported by SEERAD and the EU (FOOD-CT-2005-007036)

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