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

SA5

Suppression of neuroimmune responses at term: investigations of possible mechanisms

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It is well known that there is a general reduction in the stress response throughout pregnancy and lactation. However, in several mammals, including rats, the response to the neuroimmune stressor, lipopolysaccharide (LPS) is particularly attenuated at near term. This is manifested as a marked reduction in the febrile response in the day or so around parturition, but which recovers in the post partum period. We have carried out a series of physiological and neurochemical investigations to attempt to identify the mechanisms underlying the reduced neuroimmune response. As a number of pro-inflammatory cytokines are known to mediate LPS fever, we measured circulating cytokines using ELISAs to test the hypothesis that there was an alteration in peripheral cytokines at term. However, levels of pro-inflammatory cytokines (IL-1 β , IL-6, IFN γ , and TNF α) in the plasma taken 2h after LPS were similar at gestational day (G) 15, G22 or lactation day (L) 5. In addition to pro-inflammatory cytokines, LPS also causes synthesis and release of a variety of anti-inflammatory molecules, but neither IL-1ra and IL-10, nor the immunosuppressive hormone, corticosterone, were different at the 3 stages of reproduction. Peripheral pyrogens, including cytokines are known to cause fever by initiating the synthesis of prostaglandin E (PGE) that acts on neurons in the anterior hypothalamus/preoptic area to activate thermogenesis and reduce heat loss. To test the hypothesis that PGE synthesis or action was altered at term, we used semi-quantitative Western blots to measure levels of inducible COX-2, the rate limiting enzyme for the synthesis of PGE that is found in endothelial cells of the brain vasculature. We found that COX-2 levels are reduced at G22 compared to G15 or at L5. Pro-inflammatory cytokines activate COX-2 through a number of signaling pathways, so we explored if these were similarly altered. However, the reduced COX-2 expression was not associated with alterations in activation of transcriptional factors NF κ B, STAT 3 and STAT5 or of ERK1/2. While lipocalin-prostaglandin D2 synthase, an enzyme that could potentially produce anti-inflammatory cytokines, was generally reduced at late gestation, its further reduction at L5 dissociated the activity of this enzyme from the reduced neuroimmune response at term. Thus we concluded that the reduced fevers at term were most likely associated with a reduction in PGE synthesis in the brain, but the mechanisms responsible for the reduced COX-2 induction remain elusive.

There also appear to be changes within the brain itself that will affect febrile responses. For example, febrile responses to intraventricular application of PGE are reduced specifically at near term. This does not appear to be associated with alterations in hypothalamic levels of the prostaglandin receptor EP-3, but does appear to reflect a generalized suppression of sympathetic output at this time; activation of brown adipose tissue (which in rats is a major contributor to the elevation in body temperature), heart rate and blood pressure were all attenuated to a similar extent at term. The brain contains neurotransmitters such as arginine vasopressin (AVP) that act as antipyretics; as their levels are increased in brain during pregnancy we asked if the suppressed sympathetic output was associated with either altered receptors numbers or action of AVP. However, neither mRNA nor protein levels of the V1 AVP receptor were altered at term and central application of an AVP antagonist failed to reverse the attenuated PEG responses.

We conclude that the reduction in neuroimmune responses seen at term in the pregnant rat can be explained by a reduction in the synthesis of prostaglandins due to reduced COX-2 induction as well as a reduced effectiveness of the thermogenic outputs activated by the hypothalamic PGE. Further studies are required to identify the mechanism for these altered responses.

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Suppressed maternal hypothalamo-pituitary-adrenal (HPA) axis responses to cytokines in late pregnancy

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Glucocorticoids have powerful actions on metabolism and the immune system, especially when secretion is increased by stressors. In pregnancy glucocorticoid is important in fetal maturation, and in determining the onset of parturition via actions on the placenta. Protection of these fetal processes, and the mother's metabolic and immune systems, from stress levels of maternal glucocorticoid may be advantageous for a successful pregnancy. One protective mechanism is placental 11 β -hydroxysteroid dehydrogenase 2, which inactivates cortisol (human; corticosterone in rats); another is attenuation of maternal HPA axis responses to stress. Failure of these mechanisms in late pregnancy, as revealed by giving the synthetic glucocorticoid dexamethasone (which crosses the placenta), results in adverse fetal programming. Attenuated HPA axis stress responses in the last week of rat pregnancy (gestation is ca. 22 days) are seen with a mild emotional/physical stressor (e.g. forced swimming; [1]). Measurement by in situ hybridisation (ISH) of rapid gene expression changes in the hypothalamic parvocellular paraventricular nucleus (pPVN), reflecting activation of the corticotropin releasing factor (CRF)/ vasopressin (VP) neurones, shows they are less excited by stressors in late pregnancy. Since CRF and VP cause corticotropin (ACTH) release from the anterior pituitary, and

hence glucocorticoid secretion from the adrenal cortex, reduced pPVN neurone activation explains reduced secretion of ACTH and glucocorticoid. Hence adaptations in pregnancy underlying reduced HPA axis responses are in the maternal brain. Physical stressors such as chemical signals of infection, lipopolysaccharide (LPS; endotoxin) and interleukin-1 β (IL-1 β , given to conscious rats via a jugular vein cannula), stimulate strong and prolonged HPA axis activation in virgins, but HPA responses are completely suppressed in late pregnant rats [2]. Similarly, neuropeptide orexigens that signal energy need activate the HPA axis in virgin but not in late pregnant rats [3].

To investigate causes of the profound suppression of HPA axis responses to IL-1 β in late pregnancy, we tested the hypothesis that activation of an endogenous opioid mechanism in the brain prevents pPVN CRH/VP neurone responses to IL-1 β , by a local action in the pPVN, and that this opioid mechanism is induced by actions of allopregnanolone (AP), a neuroactive steroid metabolite of progesterone. Progesterone, abundant in pregnancy, is metabolised by 5 α -reductase (5 α -R) and 3 α -hydroxysteroid dehydrogenase to AP, in the brain and liver, resulting in high brain concentrations in late pregnancy [4].

If late pregnant rats are given the opioid antagonist naloxone (i.v.) a few minutes before IL-1 β , IL-1 β increases ACTH and corticosterone secretion and CRF mRNA expression in the pPVN, thus revealing an inhibitory endogenous opioid action [2]. IL-1 β acts by stimulating noradrenergic neurones in the nucleus of the tractus solitarius (NTS) in the medulla oblongata, via prostaglandins generated by activation of endothelial IL type1 receptors on local blood vessels. ISH shows a lack of stimulation by IL-1 β of cyclo-oxygenase-2 (COX-2) mRNA expression in the NTS in late pregnant rats, contrasting with stimulation of expression in virgin rats and indicating altered post-IL type1 receptor signalling in late pregnancy. Nonetheless, the number of NTS neurones expressing Fos protein (an indicator of recent neuronal activation) after i.v. IL-1 β is similarly increased (compared with vehicle injection) in virgin and late pregnant rats [2]. Similar activation of Fos expression in central amygdala neurones in virgin and late pregnant rats is confirmation that IL-1 β can signal into the brain in late pregnancy. In virgin rats activation of NTS neurones by i.v. IL-1 β leads to noradrenaline release, measured via microdialysis, in the pPVN. This is not seen in late pregnant rats, but naloxone infused into the pPVN restores local release of noradrenaline after i.v. IL-1 β [2]. These results show preterminal opioid inhibition on noradrenergic synapses in late pregnancy that blocks the stimulation, via NTS projections, of the HPA axis by i.v. IL-1 β . The source of this opioid is likely to be the NTS neurones themselves, since pro-enkephalin (PENK) A mRNA expression in the NTS is increased at the end of pregnancy [2]. Overnight treatment of late pregnant rats with finasteride, a 5 α -R antagonist, also restores HPA axis responses to i.v. IL-1 β in late pregnant rats, and conversely allopregnanolone treatment reduces HPA axis responses to IL-1 β in virgin rats, and induces PENK-A mRNA expression in the NTS selectively. Hence, increased allopregnanolone production in pregnancy, from luteal progesterone, suppresses maternal HPA axis responses to IL-1 β through up-regulation of opioid gene expression in the maternal brainstem.

Ma S et al. (2005) *Endocrinology* 146, 1626-1637.

Brunton PJ et al. (2005) *J Neurosci* 25, 5117-5126.

Brunton PJ et al. (2006) *Endocrinology* 147, 3737-3745.

Russell JA et al. (2006) *Neuroscience* 138, 879-889.

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SA7

Steroid control of cytokines in labor. Julia Szekeres-Bartho **Department of Medical Microbiology and Immunology,** **Medical School, Pecs University, Pecs, Hungary**

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Fifty per cent of fetal antigens are of paternal origin. There is ample evidence now that pregnancy is recognized by the maternal immune system; furthermore, the immune system reacts in a differential way resulting in successful or failed pregnancy. Due to chronic allogeneic stimulation by fetally derived antigens lymphocytes of pregnant women develop progesterone receptors (PRs). Lymphocyte PR expression is related to lymphocyte activation, independently from the hormonal environment. The percentage of PR positive lymphocytes increases throughout gestation. Recurrent abortion, spontaneous abortion, pre-term labour as well as term labour are associated with a decreased number of PR positive cells. If a sufficient concentration of progesterone is available, PR positive lymphocytes produce a 34 kDa protein, named the Progesterone Induced Blocking Factor (PIBF). PIBF affects various phases of the immune response, inhibits the release of arachidonic acid and exerts an anti-abortion effect in mice.

PIBF signals via the Jak/STAT pathway, which results in a Th2 dominant cytokine pattern. The cytokine effects of PIBF are significantly reduced in STAT6 deficient cells. PIBF activates STAT6 and inhibits the phosphorylation of STAT4. Activation of the STAT6 pathway depends on ligation of the IL-4 receptor, thus it seems plausible that the IL-4R is involved in PIBF-induced STAT6 activation. Though PIBF does not bind to IL-4R, blocking of the latter abolishes PIBF-induced STAT6 activation, whereas blocking of the IL-13 receptor has no effect. Furthermore, PIBF fails to signal in IL-4R knock down cells. The requirement of the IL-4R for PIBF signaling could be due to the lack of intracellular domains in the PIBF receptor, therefore the possibility of the PIBF receptor being a GPI anchored protein was considered. Digesting the GPI-anchor with Phosphatidylinositol-specific Phospholipase C (PI-PLC) abolished the STAT6 activating effect of PIBF, while that of IL-4 remained intact. These data suggest the existence of a novel type of IL-4R, composed of the IL-4R and the GPI-anchored PIBF receptor. Upon ligation the GPI-anchored PIBF receptor combines with the alpha chain of the IL-4 receptor, and induces Jak1 phosphorylation, which in turn activates STAT6.

In urine samples from healthy pregnant women PIBF concentrations continuously increase up to the 37th week of gestation and start to decline thereafter, to fall to a minimum value during labour. PIBF concentrations in urine of healthy pregnant women are significantly higher than in those of non-pregnant

individuals or women at risk for premature pregnancy termination.

In vitro data suggest a correlation between the rate of progesterone receptor expression as well as PIBF production and the success or failure of pregnancy, but provide no direct evidence for their role in maintaining gestation. To test the biological significance of our findings, we used animal systems. In vivo studies revealed that: a) The anti-abortive effect of the PIBF in vivo is manifested via inducing a Th2 dominant cytokine pattern and keeping the NK activity at a low level: b) A proper stimulation of the maternal immune system is required for the operation of the progesterone-dependent immunomodulatory pathway: c) Neutralization of endogenous PIBF results in pregnancy termination. These data allow the conclusion that the operation of progesterone-dependent immunomodulation contributes to maintaining normal gestation.

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SA8

Cytokines and myometrial intracellular signalling

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During human pregnancy the smooth muscle of the uterus, the myometrium, is relatively quiescent until the onset of contractile activity associated with labour. The cascade of events precipitating human labour remains unclear, but it is proposed that the myometrium becomes primed to contract at term by the activation of a complex array of genes encoding for proteins which include cyclo-oxygenase-II (COX-2), the oxytocin receptor and calcium regulatory proteins (TRPC isoforms and sarcoplasmic reticulum calcium ATPases). Several concordant stimuli ('physiological' inflammation, maternal and foetal endocrine signals and uterine stretch) have been implicated in this process and are proposed to drive the integration of uterine contractile activity and labour. In support of a central role for inflammatory cytokines, IL-1 β , IL-8 and IL-6 have been found in myometrial tissue taken in late pregnancy, and raised concentrations are reported in amniotic fluid during human term and preterm labour. Experimental models of preterm labour have also demonstrated that introduction of bacterial products or cytokines into the amniotic cavity of pregnant animals leads to cytokine synthesis, up-regulation of Toll-like receptors and premature uterine contraction.

This presentation will discuss how the inflammatory mediator IL-1 β modulates uterine excitability. It is well known that IL-1 β can stimulate myometrial prostaglandin synthesis, but our studies demonstrate for the first time that IL-1 β can also enhance calcium signalling events. IL-1 β treatment of human myometrial cells induces spontaneous calcium oscillations and increases resting calcium concentrations in parallel with an augmentation of store-dependent calcium entry and a substantial increase in TRPC3 protein expression. Interestingly, IL-1 β treatment does not alter expression patterns of any other TrpC isoforms suggesting that TRPC3 is differentially regulated. IL-1 β -treated

smooth muscle cells also exhibit augmented calcium responses to a diacylglycerol analogue (OAG), a prominent activator of TrpC3 channels. These data implicate TRPC3 channels as key mediators of the IL-1 β enhancement of myometrial smooth muscle calcium signalling, and provide a plausible mechanism by which uterine excitability may be augmented in term and preterm labour. This cell model may also prove useful as an endogenous 'overexpression' system with which to explore the function, regulation and role of TrpC3 proteins.

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Prolactin and the neuroendocrine adaptations of the maternal brain

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Levels of lactogenic hormones, prolactin from the maternal anterior pituitary and/or the closely related placental lactogen, are elevated during pregnancy and lactation. While these hormones are well established to have an critical role in mammary development and lactogenesis, they also exert important actions in the brain. Prolactin receptors are expressed in the choroid plexus and in several hypothalamic nuclei, and we have shown that levels of expression increase during pregnancy and lactation. Prolactin is known to influence a variety of hypothalamic functions, including regulation tuberoinfundibular dopamine (TIDA) neurons, stress responses, appetite and food intake, and fertility (1). Many of these prolactin-sensitive functions appear to change during pregnancy in a manner consistent with the influence of prolactin. Two specific examples have been examined to evaluate the role of prolactin in mediating neuroendocrine adaptation in the maternal brain: a) Decreased sensitivity of TIDA neurons to prolactin, leading to decreased secretion of dopamine and subsequent hyperprolactinaemia. b) Increased food intake and the development of leptin-resistance during pregnancy. Both changes are important maternal adaptations to pregnancy, providing high prolactin for mammary development and maternal behaviour, and increased energy storage to meet the metabolic demands of lactation, respectively.

Prolactin acts directly on prolactin receptors on TIDA neurons inducing phosphorylation of STAT5b and activation of tyrosine hydroxylase (TH, the rate limiting enzyme responsible for dopamine synthesis) and an increase in TH mRNA expression. We have measured mRNA for the long form of the prolactin receptor on TIDA neurons by in situ hybridisation and this does not change during pregnancy or lactation (2), although there is an increase in expression of met-enkephalin in prolactin-responsive TIDA neurons. Prolactin-induced phosphorylation of STAT5b in TIDA neurons is suppressed during lactation, associated with a prolactin- or suckling-dependent increase in mRNA for several endogenous inhibitors of STAT pathways (CIS, SOCS1